ISSCR

INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

12TH ANNUAL MEETING

VANCOUVER, CANADA

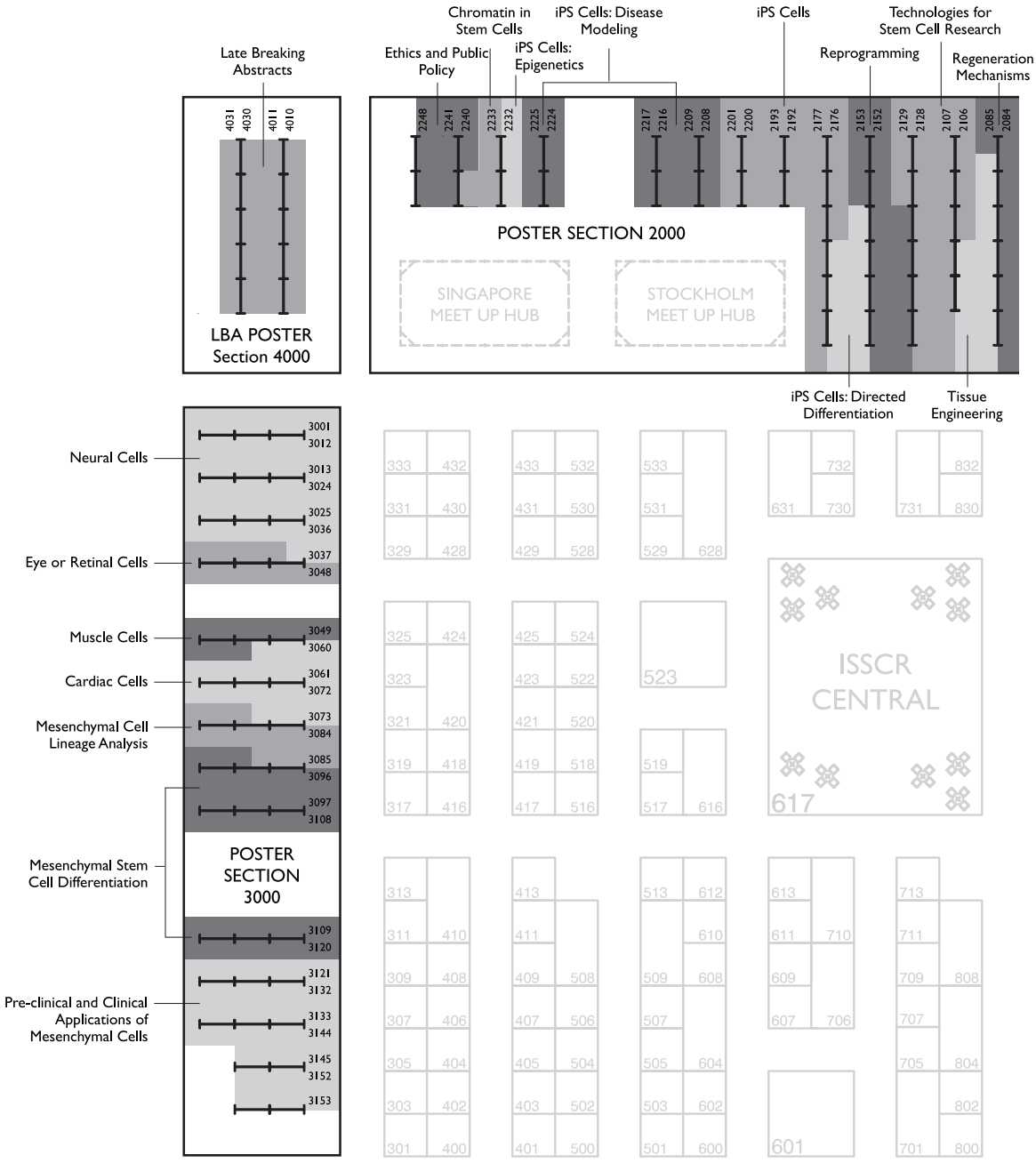
JUNE 18-21 2014

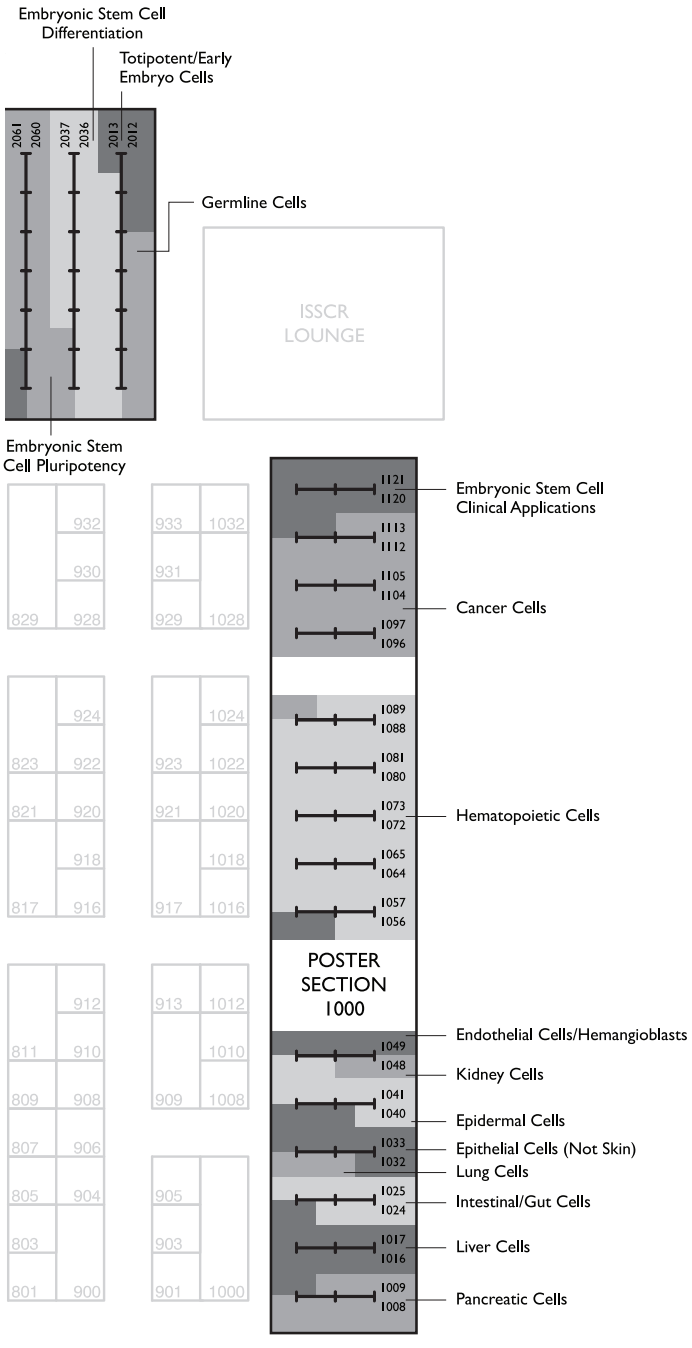
CO-SPONSORED BY

STEMCELL™ TECHNOLOGIES

POSTER ABSTRACTS

POSTER FLOOR PLAN





POSTER BOARDS BY TOPIC

POSTERS 1001-1122

POSTERS 2001-2246

POSTERS 3001-3037

POSTERS 4001-4034

TABLE OF CONTENTS

WEDNESDAY, JUNE 18

THURSDAY, JUNE 19

Tourism Vancouver / Science World British Columbia

FRIDAY, JUNE 20

JUNE 18, 2014

WEDNESDAY POSTER PRESENTATIONS

6:30 - 7:30 ODD numbered posters presented
7:30 - 8:30 EVEN numbered posters presented

PANCREATIC CELLS

W-1008
ENDOTHELIAL CELLS AND VASCULAR BASEMENT MEMBRANE COMPONENTS ENHANCE IN VITRO DIFFERENTIATION AND FUNCTIONAL MATURATION OF PANCREATIC BETA CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Talavera-Adame, Dodanim[1], Woolcott, Orison O.[2], Arumugaswami, Vaithilingaraja[3], Svendsen, Soshana P.[3], Chaiboonma, Kira L.[3], Svendsen, Clive[3], Dafoe, Donald C.[1]
[1]Surgery and Regenerative Medicine Institute, Cedars Sinai Medical Center, Los Angeles, CA, USA, [2]Diabetes and Obesity Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA, [3]Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Pancreatic beta cells derived from human induced pluripotent stem cells (hiPSCs) are promising source of cells to treat Type 1 diabetes. During embryogenesis, endoderm cells require inductive signals from surrounding endothelial cells (ECs) to give rise to functional insulin-producing pancreatic beta cells. We hypothesize that ECs and vascular basement membrane components provide essential signals for beta-cell differentiation and maturation in vitro. Embryoid bodies (EBs) from hiPSCs were cultured alone (control) or together with ECs in collagen I, IV, and laminin I gels and treated with growth factors. After 20 days, EBs with EC and EBs control were harvested to obtain a single cell suspension and mature beta-cell marker expression was analyzed in these cells by ICC, FACS, and qRT-PCR. After cell transferring, human C-peptide and cellular secretory ability were evaluated by ELISA and quinacrine fluorescence respectively in static cultures. Dynamic insulin release was evaluated by perifusion assay. Derived cells were then transplanted under the kidney capsule of SCID mice to evaluate function in vivo. Grafted kidneys were analyzed by IHC. Higher expression of mature beta-cell markers (proinsulin, GLUT1, PDX-1, Nkx6.1, GKS, SUR1, Kir6.2, PC1/3, PC2, and UCN3) was found in cells co-cultured with ECs in comparison to controls. About 70 % of these cells were positive for proinsulin in contrast to 11% found in controls. Significant reduction in quinacrine fluorescence, threefold increase in human C-peptide, and dynamic insulin release (up to 5-10 pmol/L) were found in co-cultured cells after a glucose challenge in contrast to much lower response in controls. Mice grafted with cells from co-cultures showed higher blood levels of human C-peptide after a glucose challenge in comparison to controls (150 ± 10 vs. 1.36 ± 6.7, $P<0.05$). After streptozotocin treatment, these mice maintained normoglycemia in contrast to hyperglycemia found in controls transplanted with cells from EBs cultured alone. IHC analysis showed insulin positive cells at 120 days postransplantation only in kidneys grafted with cells derived from co-cultures. These results strongly suggest that ECs and vascular membrane components are critical for adequate differentiation and functional maturation of pancreatic beta cells derived from hiPSCs.

W-1009
CELL AGGREGATION OPTIMIZES THE DIFFERENTIATION INTO PANCREATIC BUD-LIKE PROGENITOR CELLS FROM HUMAN ESCS AND IPSCS

Toyoda, Taro, Mae, Shin-Ichi, Tanaka, Hiromi, Kondo, Yasushi, Michinori, Funato, Hosokawa, Yoshiya, Sudo, Tomomi, Yamanaka, Shinya, Osafune, Kenji
Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan

The establishment of stable and efficient methods to generate pancreatic β-cells from human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) remains a major issue towards the development of regenerative medicine strategies for diabetes. The pancreatic progenitor cells generated from hESCs/iPSCs have been shown to differentiate into mature pancreatic cells *in vivo*, indicating that the cell type is at the diverging point in generating functional β-cells. However, previously published differentiation protocols for pancreatic bud-like cells ($PDX1^+NKX6.1^+$), the earliest pancreas-committed cells, are inefficient due to incomplete understanding of key differentiation factors. The purpose of this study is to identify novel factors that facilitate the differentiation to $PDX1^+NKX6.1^+$ cells. We differentiated a hESC line, KhES3, into pancreatic lineages with a stepwise protocol recapitulating developmental processes in two-dimensional culture. We found that $PDX1^+NKX6.1^+$ cells were specifically localized at posterior foregut cell clusters ($PDX1^+$), which reminded us that higher cell density might be beneficial for the differentiation of the pancreatic bud cells. When the dissociated $PDX1^+$ cells were re-seeded at various cell densities in monolayer culture format, the induction rate of $PDX1^+NKX6.1^+$ cells was correlated with cell density (4-9%). Supporting this result, the dissociated $PDX1^+$ cells were aggregated at various sizes in suspension culture, resulting in a markedly and constantly higher $PDX1^+NKX6.1^+$ cells rate (~40%), regardless of cell aggregate's size. In cellular aggregates, a pancreatic bud-specific gene *PTF1A* started to be expressed concomitantly with *NKX6.1* expression. The positive effects of the cell aggregation culture on the differentiation of pancreatic bud cells were reproduced in multiple human iPSC lines: 585A1, 604B1, 692D2, 648B1 and 409B2. The aggregates containing $PDX1^+NKX6.1^+$ cells were successfully engrafted in epididymal fat pad and renal subcapsular spaces of immunocompromised mice and developed into pancreatic epithelial-like structures by 30 days after transplantation. Moreover, human C-peptide, representing insulin secretion, was detected in mice blood after a long term *in vivo* maturation (>5 months). Taken together, these results suggest that culture with higher cell density or three-dimensional aggregation culture is crucial for the differentiation of pancreas-committed progenitor cells from hESC/iPSC. Our findings may be applied in the development of hESC/iPSC-based cell therapy for diabetes.

W-1010
A POISED ENHANCER LANDSCAPE IS INDICATIVE OF DEVELOPMENTAL COMPETENCE DURING ENDODERMAL LINEAGE DIVERSIFICATION OF HUMAN EMBRYONIC STEM CELLS

Wang, Allen[1], Yue, Feng[2], Li, Yan[2], Xie, Ruiyu[1], Harper, Thomas[1], Patel, Nisha A.[1], Muth, Kayla[1], Palmer, Jeffrey[1], Raum, Jeffrey[3], Stoffers, Doris A.[3], Ren, Bing[1], Sander, Maike[1]
[1]University of California San Diego, La Jolla, CA, USA, [2]Ludwig Institute for Cancer Research, La Jolla, CA, USA, [3]University of Pennsylvania, Philadelphia, PA, USA

The emergence of distinct lineages during embryonic development

relies on the ability of progenitors to appropriately respond to external signaling and activate lineage specific gene expression. This cellular property is referred to as developmental or transcriptional competence. Although this has been well observed throughout the development of multiple cell lineages, the molecular mechanism remains elusive. It is speculated that epigenetic changes in chromatin could play a role in this process. Here we show that during endodermal lineage diversification, epigenetic priming of lineage-specific enhancers signifies developmental competence. Chromatin mapping of enhancer related histone modifications during pancreatic and hepatic differentiation of human embryonic stem cells revealed an en masse acquisition of poised enhancer chromatin in early uncommitted endodermal intermediates. We further show that this poised enhancer landscape is significantly enriched for enhancers specific to descendant foregut lineages such as the liver and pancreas. Through subsequent experimentation, we observed that the acquisition of this poised enhancer state predicts the ability of endodermal intermediates to respond to lineage inductive signals. Further analysis revealed that these lineage-specific enhancers are first recognized by transcription factors involved in chromatin priming, while subsequent recruitment of lineage-inductive transcription factors leads to enhancer and target gene activation. Together, our results identify the acquisition of a poised chromatin state at enhancers as a mechanism by which endodermal progenitor cells gain the competence to rapidly activate lineage-specific genes in response to inductive signals.

W-1011
EGF AND NICOTINAMIDE ARE ESSENTIAL FACTORS IN A SERUM-FREE COLONY ASSAY FOR PROGENITOR CELLS FROM ADULT MURINE PANCREAS

Wedeken, Lena, Jin, Liang, Feng, Tao, Ku, Hsun (Teresa)
Beckman Research Institute of City of Hope, Duarte, CA, USA

Adult pancreatic stem and progenitor cells are a potential source of insulin-producing beta cells for cell replacement therapy of type 1 diabetes. However, it proved difficult to identify these progenitor cells and analyze their properties, in part due to a lack of an analytical tool. We recently developed a colony assay that allows quantitative and functional analysis of adult murine pancreatic progenitor-like cells in culture. Dissociated single cells from adult pancreas are plated into a semisolid medium containing methylcellulose (a viscous material), Matrigel (for extracellular matrix components), conditioned media, fetal calf serum and growth factors (nicotinamide, exendin4, activin beta and vascular endothelial growth factor). The semisolid medium restricts cell movement yet permits a cell to self-renew, proliferate, differentiate and form a colony of cells. Using this assay, we found that adult murine pancreatic progenitor-like cells are able to give rise to cystic colonies containing ductal-, acinar- and endocrine-like cells. The single cell that initiates and forms a colony is therefore termed a pancreatic colony-forming unit (PCFU). However, the undefined components in the conditioned media and serum may complicate the examination of molecular mechanisms that govern the self-renewal and differentiation of these progenitor-like cells. We therefore set out to establish a better-defined, serum-free culture condition for our colony assay. Here we report that replacing the conditioned media and fetal calf serum with epidermal growth factor (EGF), R-Spondin1, Noggin and Serum Replacement (Invitrogen) in our otherwise unchanged assay allowed formation of colonies expressing markers of all pancreatic lineages. Omission of EGF or nicotinamide abrogated colony formation, suggesting the survival and/or proliferation of the PCFUs are dependent on these factors. EGF and nicotinamide were sufficient for long-term expansion of PCFUs, but media containing all growth factors tested were found to generate a higher number of PCFUs. Treatment with different mitogen-activated protein kinase inhibitors showed that MEK kinases, but not p38 are crucial for colony formation. In conclusion, this better-defined culture condition is an improvement of our existing pancreatic colony assay and we started to identify signaling pathways that are essential for colony formation. Together this will allow for studying mechanisms of self-renewal and differentiation of adult pancreatic progenitor-like cells towards endocrine beta cells in future studies.

LIVER CELLS

W-1012
MOUSE LIVER REPOPULATION WITH HEPATOCYTES GENERATED FROM HUMAN FIBROBLASTS

Zhu, Saiyong[1], Rezvani, Milad[2], Harbell, Jack[2], Mattis, Aras[2], Willenbring, Holger[2], Ding, Sheng[1]
[1]Gladstone Institutes, San Francisco, CA, USA, [2]University of California, San Francisco The Liver Center, San Francisco, CA, USA

Human induced pluripotent stem cells (iPSCs) promise to revolutionize research and therapy of liver diseases by providing a source of hepatocytes for autologous cell therapy and disease modeling. However, although progress has been made in advancing the differentiation of iPSCs into hepatocytes (iPSC-Heps) in vitro, cells that replicate the ability of human primary adult hepatocytes (aHeps) to proliferate extensively in vivo have not been reported. This deficiency has not only hampered efforts to recreate human liver diseases in mice, it has also cast doubt on the potential of iPSC-Heps for liver cell therapy. The reason is that extensive post-transplant expansion is needed to establish and sustain a therapeutically effective liver cell mass in patients, a lesson learned from clinical trials of aHep transplantation. As a solution to this problem, we report generation of human fibroblast-derived hepatocytes capable of extensive proliferation, as evidenced by significant liver repopulation of mice. Unlike current protocols for deriving hepatocytes from human fibroblasts, ours did not generate iPSCs, but shortcut reprogramming to pluripotency to generate an induced multipotent progenitor cell (iMPC) stage from which endoderm progenitor cells (iMPC-EPCs) and subsequently hepatocytes (iMPC-Heps) could be efficiently differentiated. For this, we identified combinations of small molecules that aided endoderm fate choice and hepatocyte differentiation without compromising proliferation. After transplantation into an immune-deficient mouse model of human liver failure, iMPC-Heps were able to engraft and proliferate, and acquired levels of hepatocyte function similar to aHeps. Unfractionated iMPC-Heps did not form tumors, most likely because they never entered a pluripotent state. Our results establish the feasibility of significant liver repopulation of mice with human hepatocytes generated in vitro, which removes a long-standing roadblock on the path to autologous liver cell therapy.

W-1013

DUAL-COLOR FLUORESCENT IMAGING OF DEVELOPMENTAL SHIFT FROM CYP3A7-EXPRESSING FOETAL HEPATIC CELLS TO CYP3A4-EXPRESSING ADULT HEPATOCYTES IN HUMAN HEPATIC CARCINOMA HEPARG

Tada, Masako[1], Saori, Tsuji[2], Kawamura, Fumihiko[3], Kubiura, Musashi[3], Hayashi, Ayaka[3], Ohbayashi, Tatsuya[4], Kazuki, Yasuhiro[1], Oshimura, Mitsuo[1]

[1]Chromosome Engineering Research Center, Tottori University, Yonago, Tottori, Japan, [2]Bio Frontier Project Promotion Section Organization for Tottori Industrial Promotion, Yonago, Tottori, Japan, [3]Division of Molecular Genetics and Biofunction, Department of Biomedical Science, Graduate School of Medical Science, Tottori University, Yonago, Tottori, Japan, [4]Division of Laboratory Animal Science, Research Center for Bioscience and Technology, Tottori University, Yonago, Tottori, Japan

Human adult hepatocytes are required for cell-based assays to evaluate the potential risk of drug-drug interactions caused by transcriptional induction of P450 enzymes in early-phase drug discovery and development. Such an assay requires cells that can be grown vigorously and quickly, that respond adequately to compounds, and, most importantly, that can be similar to human functional adult hepatocytes expressing CYP3A4, a major cytochrome P450 enzyme. However, CYP3A7 is preferentially expressed in premature foetal hepatoblasts and major hepatic carcinoma cell lines. The human hepatocellular carcinoma cell line HepaRG possesses a high self-renewal capacity and can differentiate into hepatic cells similar to human adult hepatocytes in vitro. To provide a real-time evaluation system for quality assurance of hepatic differentiation into CYP3A4-expressing cells from premature hepatic precursors in real time, we created transgenic HepaRG cells by replacing the protein-coding regions of human CYP3A4 and CYP3A7 with enhanced green fluorescent protein (EGFP) and Dared reporters, respectively, in a bacterial artificial chromosome (BAC) vector that included whole regulatory elements. The intensity of DsRed fluorescence was initially high during the proliferation of transgenic HepaRG cells. However, EGFP fluorescence intensity increased and DsRed fluorescence was extinguished within a few days after differentiation. During the course of cell differentiation, cells co-expressing DsRed and EGFP were not detected, showing that CYP3A7-positive HepaRG cells might be reprogrammed into CYP3A7-negative bipotent hepatic progenitors such as adult hepatic stem cells. In near future, this modified BAC can be introduced into any human stem cells such as induced pluripotent stem (iPS) cells or stimulus-triggered acquisition of pluripotency (STAP) cells to create the BAC-transgenic cells, in which EGFP can be used as a quality assurance marker for identifying functional hepatocytes derived from stem cells without the need for many time-consuming steps.

W-1014

COMPARISON OF CAPACITY FOR DRUG METABOLISM BETWEEN GENETICALLY MATCHED HUMAN HEPATOCYTES AND IPS DERIVED HEPATOCYTE LIKE CELLS

Takayama, Kazuo[1], Morisaki, Yuta[1], Ohtaka, Manami[2], Nishimura, Ken[3], Nakanishi, Mahito[2], Tachibana, Masashi[1], Sakurai, Fuminori[1], Kawabata, Kenji[4], Mizuguchi, Hiroyuki[1]

[1]Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Japan, [2]Research Center for Stem Cell Engineering,National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan, [3]Faculty of Medicine of Tsukuba, University of Tsukuba, Ushiku, Ibaraki, Japan, [4]National Institute of Biomedical Innovation, Osaka, Japan

Human induced pluripotent stem (iPS) cell-derived hepatocyte-like cells (HLCs) are expected to be applied for human hepatotoxicity screening. However, the drug metabolism capacity of the HLCs was still lower than that of primary human hepatocytes (PHHs). We suspected that this issue might be due to either a low hepatocyte differentiation efficacy or poor metabolizer genotypes of the human iPS cells. In this study, drug metabolism capacities were compared in genetically matched PHHs and HLCs. To generate the HLCs whose genetic background is perfectly matched with PHHs, we first generated human iPS cells from PHHs, and PHH-derived iPS (PHH-iPS) cells were differentiated into the HLCs. We examined the expression levels of pluripotent markers to confirm that the generation of human iPS cells from PHHs was successfully performed. In addition, the drug metabolism capacities of the HLCs, which were differentiated from PHH-iPS cells, were compared to those of parent PHHs. PHHs were successfully reprogrammed into human iPS cells, which were positive for pluripotent markers (such as NANOG, OCT4, SSEA4, and Tra1-81). The expression and activity levels of drug metabolism enzymes (such as cytochrome P450 1A2, 2C9, 3A4) in PHH-iPS-derived HLCs were approximately half of those in genetically matched PHH, were cultured for 48 hours after the cells were plated. To the best of our knowledge, this is the first work which has compared the hepatic drug metabolism capacity in genetically matched PHHs and HLCs. Our findings would be informative for understanding current status and the clarifying problems in the field of hepatocyte differentiation from human iPS cells.

W-1015

USING HUMAN INDUCED PLURIPOTENT STEM CELLS TO MODEL LIVER DISEASE ASSOCIATED WITH CLASSIC MUTATIONS OF ALPHA-1 ANTITRYPSIN

Taketani, Tamara N.

University of California, San Diego, La Jolla, CA, USA

A major obstacle to the development of new therapies is the poor understanding of how genetic modifiers alter the onset and outcome of various diseases. A classic example is AAT deficiency, a metabolic liver disease in which the mutant gene and its product are known, but where clinical progression and outcome are extremely variable and thought to be influenced by genetic modifiers. Despite being the leading genetic cause of liver disease in children, mutations of AAT occur infrequently when compared to sporadic liver diseases. The relatively low incidence of AAT deficiency makes it impossible to obtain insight into the genetic factors that may affect progression of disease from genome-wide association studies (GWAS). The study of hepatocytes derived from AAT mutant human induced pluripotent stem cells (hIPSC) may overcome this limitation by identifying cellular phenotypes that correlate with clinical severity of disease in existing AAT patients. For this purpose, we have generated hIPSC lines from existing AAT patients

(ZZ) with variable degrees of liver disease, including those without evidence of liver damage and those who have suffered a more aggressive course leading to end stage liver disease. We are using control and AAT hIPSC-derived hepatocyte like cells (HLCs) to probe the hypothesis that the significant heterogeneity seen in disease progression due to AAT ZZ mutations is related to genetically determined variability of fundamental biological hepatocyte processes involved in cellular disposal, stress response, and cell survival pathways, including proteasomal degradation, ER stress, autophagy, and apoptosis. Prior data obtained in mouse and cell line models has shown that autophagy may act as a primary route of intracellular degradation of mutant AAT protein. Although traditionally regarded as a cellular adaptive process triggered by nutrient deprivation, autophagy in hepatocytes may also provide an important hepatoprotective mechanism. Our preliminary results show that HLCs derived from AAT mutant patients with no evidence of liver disease (AAT NLD) have increased activation of autophagy at baseline compared to AAT mutants with severe liver disease (AAT LD). Furthermore, AAT hepatocytes from patients with severe liver disease show a weaker autophagy induction response upon serum deprivation. Our data supports a role for autophagy as a potential modifier in the pathobiology of AAT related liver disease, and opens the way for mechanistic studies involving this and other basic biological pathways that may modulate hepatic injury in AAT. Our studies can impact the way we approach AAT deficiency: 1) by developing predictive diagnostics through discovery of biomarkers that identify patients at risk for severe liver disease, and 2) by promoting therapeutic candidate discovery through validation of new or existing therapeutic targets in live human hepatocytes.

W-1016
HEPATOCYTE METAPLASIA IS A SOURCE OF OVAL CELL HETEROGENEITY IN MICE

Tarlow, Branden D.1, Pelz, Carl[1], Haft, Annelise[1], Wakefield, Leslie[1], Finegold, Milton J.[2], **Grompe, Markus[1]**
[1]Oregon Health and Sciences University, Portland, OR, USA, [2]Baylor College of Medicine, Houston, TX, USA

Oval cells are heterogeneous proliferations of duct-like cells observed in diverse liver injuries and can differentiate into hepatocytes or cholangiocytes in liver regeneration. We recently found that Sox9-derived oval cells rarely contribute to hepatocyte regeneration in mouse liver injury models. Additional reports indicate that both hepatocytes and ductal Sox9+ ductal cells are sources of oval cells. Yet the functional significance of hepatocyte-derived oval cells and their capacity for hepatocytic-differentiation remains unknown. Methods: To specifically and efficiently track the progeny of adult hepatocytes we generated hepatocyte-chimeras by transplanting fluorescent-marked or unmarked Sox9-CreERT2-reporter hepatocytes into juvenile Fah-/- mice. To promote oval cell activation, mice were given 0.1% DDC diet for 5-to-8 weeks. Antibodies to Osteopontin or Sox9 was used identify oval cells by immunofluorescence. The MIC1-1C3 (MPdi1) surface marker was used for FACS isolation. The ductal identity of sorted cells was assessed by RNAseq and transmission electron microscopy. FACS purity was assessed by qPCR. For functional analysis, cells were seeded into a 3-dimensional Egf/Rspo1/Wnt culture system or differentiated towards a hepatic fate with dexamethasone, Il-6, and Osm-containing media. Hepatocyte-differentiation potential was also assessed by serial portal vein transplantation of limiting numbers of FACS sorted cells in to Fah-/- mice. Results: Combined transplantation and Sox9-CreERT2-based lineage tracing agreed with other reports that injured hepatocytes, specifically in the periportal zone, undergo metaplasia. With prolonged liver injury, hepatocytes were a significant source of Sox9+ Opn+ oval cells in murine oval cell injury. Hepatocyte-derived oval cells were primarily diploid and highly proliferative. Remarkably, hepatocyte-derived oval cells were indistinguishable from cholangiocyte-derived ductal cells in terms of Hnf1b, Sox9, and Osteopontin expression, but they expressed lower levels of other ductal markers like Ck19 and EpCAM (n=5 mice). Electron microscopy indicated that hepatocyte-derived oval cells had scant cytoplasm but retained nuclear morphology organelle features more similar to hepatocytes than cholangiocytes. In vitro, hepatocyte-derived oval cells were 10-fold more efficient at inducing albumin mRNA from baseline (n=3) but could not form hollow spheroids in a matrigel-based organoid assay (n=6). Upon transplantation hepatocyte-derived cells unregulated expression of hepatocyte marker Fah and formed large hepatocyte-like nodules in recipient mice. Conclusions: Hepatocytes undergo metaplasia and to reversibly acquire some features of ductal cells in response to injury. Oval cells of different cellular origins exhibit profound functional differences. The ability of periportal hepatocytes to adopt a ductal phenotype may facilitate the avoidance of stimuli specifically toxic to hepatocytes. Further work is required to determine whether hepatocyte-derived oval cells are a physiologically important reservoir of hepatocyte precursors in vivo.

W-1017
NEW MEDIUM TO SELECT HUMAN PRIMARY HEPATOCYTES FROM CO-CULTURE WITH HUMAN INDUCED PLURIPOTENT STEM CELLS

Tomizawa, Minoru[1], Fuminobu, Shinozaki[2], Motoyoshi, Yasufumi[3], Sugiyama, Takao[4], Yamamoto, Shigenori[5], Sueishi, Makoto[4]
[1]Department of Gastroenterology, [2]Department of Radiology, [3]Department of Neurology, [4]Department of Rheumatology, [5]Department of Pediatrics, National Hospital Organization Shimoshizu Hospital, Yotsukaido City, Japan

Background: Tumorigenicity is an associated risk for transplantation of hepatocytes differentiated from human induced pluripotent stem (hiPS) cells. A method is required to eliminate hiPS cells from the hepatocytes that are transplanted. Glucose and arginine are essential for cells to survive. Hepatocytes express galactokinase and ornithine transcarbamylase (OTC), enzymes involved in the gluconeogenesis and urea cycles, which convert galactose to glucose and ornithine to arginine, respectively. However, hiPS cells do not express these enzymes and, therefore, are not expected to survive in a medium containing galactose and ornithine but lacking glucose and arginine. Materials and Methods: A real-time quantitative polymerase chain reaction (PCR) was performed to analyze the expression of galactokinase 1 (GALK)1, GALK2, and ornithine carbamyltransferase. The hiPS cell line 201B7 (Riken Cell Bank, Tsukuba, Japan) was maintained in ReproFF (Reprocell, Yokohama, Japan). Hepatocyte selection medium (HSM) was made from powder based on Leibovitz's 15 medium. HSM contains galactose and ornithine but not glucose and arginine. After culturing in HSM, 201B7 cells were subjected to hematoxylin and eosin (H and E) staining and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) to prepare them for microscopic analysis. To assess their pluripotency in HSM, 201B7 cells were immunostained with antibodies to Nanog, SSEA4, and TRA-1-60. Primary human hepatocytes (Lonza, Walkersville, MD) were cultured in HSM with or without hiPS cells. Finally, human primary hepatocytes and hiPS cells were cultured on collagen-coated plates where the medium was changed to HSM. Results: The expression levels of GALK1, GALK2, and OTC 201B7 were 22.2% ± 5.0% (average ± standard deviation), 14.2% ± 1.1%, 1.2% ± 0.2%, and 8.4% ± 0.7%, respectively, compared with those in adult liver cells. The 201B7 cell population declined when cultured in HSM and completely disappeared after 3 days. The nuclei of cultured cells were condensed or

fragmented, thereby suggesting apoptosis. TUNEL staining confirmed that the cells had undergone apoptosis. Immunostaining results for Nanog, SSEA-4, and TRA-1-60 were positive in 201B7 cells cultured in HSM for 2 days. Primary human hepatocytes survived in HSM when either cultured alone or co-cultured with 201B7 cells. Conclusion: Therefore, HSM is an ideal medium for eliminating hiPS cells, thereby purifying hepatocytes without inducing damage.

W-1018
CULTURE OF A CGMP COMPATIBLE HESC LINE UNDER XENO FREE AND FEEDER FREE CONDITIONS USING A BIOREACTOR FOR SCALE UP AND SUBSEQUENT DIFFERENTIATION TO HEPATOCYTES USING CGMP COMPATIBLE CONDITIONS

Tschudy Seney, Benjamin[1], Hasan, Ahmad[1], Sheu, Jonathan[2], Ha, Hoang[1], Gao, Wei[1], Bauer, Gerhard[2], Duan, Yuyou[1], Zern, Mark[1]
[1]Internal Medicine, Institute for Regenerative Cures, University of California Davis Medical Center, Sacramento, CA, USA, [2]GMP Facility, Institute for Regenerative Cures, University of California Davis Medical Center, Sacramento, CA, USA

The elimination of all animal materials for culture and differentiation of hESC is critical for development of suitable conditions which can support large-scale cell production in cGMP-compliant facilities for future hESC-based therapies. We have obtained a clinical grade hESC line from BioTime (ESI017) and have developed such conditions for effectively differentiating this line. We tested many human extracellular matrix proteins and chemically-defined media and determined that the best feeder-free, xeno-free conditions were the combination of TeSR-E8 and rhvitronectin. This combination was capable of supporting hESC expansion for at least 35 passages, as well as maintaining a normal karyotype, normal expression of pluripotent genes and the capacity to form teratomas in mice. The morphology was tight colonies with small homogenous cells which contain practically no differentiation. These hESC have generated high quality definitive endoderm (DE), and were further differentiated to hepatocytes employing feeder-free and xeno-free conditions with function that was similar to those generated with our standard protocol which includes animal products and MEF. For example, ELISAs showed that 1 million hESC-derived hepatocytes (hEH) secreted 90 ug of albumin. In addition, the cells have shown the ability to exhibit a full complement of metabolic functions. The results of metabolic profiling indicated that the major metabolic pathways were the same in both hEH and primary hepatocytes, that all of the metabolites showed similar kinetic increases over time, and that the cells demonstrated the same LC/MS fingerprints. In addition, hEH responded to inducers of CYP450s in a manner similar to primary hepatocytes. To our knowledge, this is the first report that hepatocytes were generated from a GMP-compatible hESC line employing xeno-free and feeder-free conditions. To develop the best scale-up strategies, we have employed the Quantum hollow fiber cell expansion system to culture ESI017 by adapting the feeder-free, xeno-free hESC plate culture conditions in our certified GMP facility. In this system, cell culturing is an automated process, and each step, from cell seeding through harvesting is programmable and traceable. In our first six day run, we obtained a ten-fold increase in hESC number, with 99% of the cells positive for SSEA4 and Tra1-81, and qPCR revealed that the expression of SOX2, OCT4 and NANOG was essentially the same as levels obtained by culturing the hESC on plates. The cells maintain normal karyotyping, and the capacity to form teratomas. The cells were then differentiated to DE and further differentiated to hepatocytes on tissue culture plates using our xeno-free, feeder-free conditions. Even at an early stage (day 16, to date), ELISAs showed that high levels of albumin were secreted into the medium. qPCR results demonstrated that albumin expression increased 2.5 fold, ASGPR 2.7 fold, CYP3A4 19.5 fold, CYP2C9 5.2 fold, CYP2C19 7.3 fold, UGT1A1 4.6 fold, UGT1A3 4.7 fold, UGT1A6 4.5 fold, and UGT2B7 4.9 fold at day 16 when compared to levels in ESI017 grown on MEF, indicating that differentiation to hepatocytes appears to be better than with our standard protocol. This bioreactor system offers the opportunity to readily perform scale-up cultures in the 10e10 cell number range for hESCs as well as to differentiate cells under controlled conditions, thus providing the opportunity to manufacture, with one system, enough cells for planned early phase clinical trials.

W-1019
FUNCTIONAL 3D CO-CULTURE MODEL OF HEPATOCYTES AND ENDOTHELIAL CELLS ON ACELLULAR HUMAN AMNIOTIC MATRIX

Yuan, Jie, Huang, Jieqiong, Li, Xueyang, Li, Weihong, Lu, Xin, Zhang, Haiyan
Department of Cell Biology, Capital Medical University, Beijing, China

Culturing hepatocyte-like cells that are generated from various types of human stem cells in three-dimensional (3D) enable them for more predictive in vitro hepatic phenotypes, and then suitable for screening hepatotoxic compounds, testing metabolic functions, and even liver bioengineering and regeneration. The selection of the biomaterials used for scaffolding is a critical step in 3D culture. Since the specific components of extracellular matrix and its properties of physical, the amniotic membrane (AM) is considered an important and excellent candidate source for native scaffolding material. In this study,we illustrate the utility of the acellular human amniotic matrix (AHAM) as a matrix for 3D culture of hepatocytes alone or co-culture with human umbilical vein endothelial cells (HUVECs), and assessment of hepatocellular structure and function in this platform. Owing to our long-term interest in human fetal hepatic progenitor cells (hFHPCs), we explored the behavior of hepatocyte-like cells (HLCs) derived from hFHPCs on AHAM. The AHAM was de-epithelialized by 0.38% EDTA-0.25% trypsin, manufactured into freeze-drying construction, and then for seeding cells. Results showed that whether cultured alone or co-cultured with HUVECs (as 1:1 in ratio) in vitro, HLCs performed self re-organization into a 3D structure loaded by AHAM after 3 days. HE stain showed that HLCs attached on AHAM tightly, and TEM displayed the intercellular junction including tight junction and gap junction. Immunocytochemistry indicated the expression of bile duct surface-specific marker, MRP2, on the apical pore of HLCs when cultured on AHAM, while co-cultured with HUVECs, the 3D co-culture model became the reticulate structure formed between HLCs and HUVEC. Further analysis of function showed that the production of ALB on co-cultured group has two times higher than the group of HLCs cultured alone, urea production follow the same tendency. CYP1A1 enzyme activity evaluated by EROD was also higher in co-cultured HLCs with HUVECs group than HLCs alone. In conclusion, we demonstrate that the AHAM maintain the efficient generation of highly differentiated HLCs in vitro. Additionally, co-cultured with HUVEC, the differentiated hepatic functions of HLCs will be further enhanced. This result showed reflects good biocompatibility of the AHAM and provides evidence that co-cultured system can be useful for generating functional 3D structure of hepatocytes, and hence may serve as a potential candidate for artificial implantable liver systems. This work was supported by Grants of the National Natural Science Foundation of China (No. 31171310).

W-1020
IDENTIFYING PRECANCEROUS SUBPOPULATION OF HEPATIC OVAL CELLS TO ENHANCE THE THERAPY FOR HEPATOCELLULAR CARCINOMA

Zheng, Yunwen, Tsuchida, Tomonori, Shimao, Taiki, Li, Bin, Takebe, Takanori, Zhang, Ran-Ran, Sakurai, Yu, Taniguchi, Hideki
Yokohama City University, Yokohama, Japan

Hepatocellular carcinoma (HCC) is a malignant tumor with a generally poor prognosis and a high rate of recurrence. HCC usually arises in the setting of chronic liver diseases, and long-lasting premalignant conditions precede cancer development. A promising therapeutic approach is to eliminate precancerous cells, which are potential cancer stem cells and prevent further malignant transformation. In this study, we identified a subpopulation of oval cell, in a rat liver carcinogenesis model, which were enriched in the $CD133^{+}CD44^{+}CD45^{-}HIS49^{-}$ cells, are precancerous cells. Prospective isolation of the precancerous cells using flow cytometry identified stem cell properties such as the ability to expand clonally and differentiate into bi-lineage cell types. Furthermore, an acyclic retinoid, which was recently shown to improve overall survival after HCC resection, directly inhibited the extensive expansion of the isolated precancerous cells in vitro and decreased the emergence of the precancerous cells and their progeny in vivo. Long-term follow-up after the acyclic retinoid treatment confirmed reduction in precancerous changes, ultimately resulting in suppression of HCC development. These findings, together with data from recent clinical trials showing marked reduction in intrahepatic recurrence, suggest that $CD133^{+}CD44^{+}CD45^{-}HIS49^{-}$ cells should be precancerous progenitors in heterogeneous population of oval cells in HCC developing. Given recent advances in diagnostic techniques and the establishment of surveillance programs, the targeting of precancerous cells may have a huge impact on preventative cancer therapies.

INTESTINAL / GUT CELLS

W-1022
CULTURE OF INTESTINAL STEM CELLS IN SERUM FREE CONDITIONS

Yao, Chao-Ling, Mohamed, Mahmoud
Yuan Ze University, Chung-Li City, Taiwan

Intestinal stem cells (ISCs) are located at the bottom of the intestinal crypts. Additionally, intestinal stem cells have the ability of differentiation into transit amplifying cells which in turn will give rise to all the mature epithelial cells. Recently, intestinal stem cells have taken a great attention as a promising stem cell therapy for many intestinal diseases such as small bowel syndrome. In addition, many challenges were facing the study of ISCs because of the lack of definitive markers and definitive isolation and culture methods. Thus, developing a definitive culture system and serum-free medium of intestinal stem cells will be promising in the clinical applications and may help in the small intestine tissue engineering. In the present study, we are showing ISCs isolation from mouse small intestine. Furthermore, we have selected five significant growth factors which could enhance ISC proliferation in vitro and replace the serum components. Moreover, our optimum growth conditions could maintain the ISC growth in 3D culture and enhanced the enteroid formation ability of the intestinal crypts in matrigel. The results of gene expression analysis of some ISC markers including Lgr5, Bmi1, Ascl2 and PTEN have confirmed that our optimum medium could maintain the stem cell state in this culture system. In a conclusion, these results may help in the enhancement of ISC expansion and understanding the major signaling pathways which maintain the ISC self-renewal and differentiation. In addition, this serum-free medium will be a good tool in the clinical applications.

W-1023
FUNCTIONAL ANALYSIS OF SNAI1 IN THE MOUSE INTESTINAL EPITHELIUM

Abud, Helen E.[1], Horvay, Katja[1], Jardé, Thierry[1], Casagranda, Franca[2], Perreau, Victoria[2], Haigh, Katharina[3], Gridley, Thomas[4], Berx, Geert[5], Haigh, Jody[3], Polo, Jose Maria[1], Hime, Gary R.[2]
[1]Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia, [2]Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Australia, [3]Australian Centre for Blood Diseases, Monash University, Clayton, Australia, [4]Center for Molecular Medicine Maine Medical Center Research Institute, Monash University, Scarborough, ME, USA, [5]Molecular and Cellular Oncology, Inflammation Research Center, VIB, Ghent, Belgium

The Snail family of transcriptional regulators have an important role in mediating epithelial to mesenchymal transitions and cell motility during both embryonic development and cancer metastasis. Although they are generally regarded as markers of mesenchymal cells, Snail proteins have also recently been implicated in regulating stem cell populations in several organs. The objective of our study is to investigate the role of Snai1 in the mouse intestinal epithelium that is continuously renewed via a population of multipotent stem cells that reside in the base of crypts. Our studies have revealed that Snai1 is normally expressed in the intestinal epithelium with strong nuclear expression in crypt base columnar stem cells and proliferating transit amplifying cells but is expressed mostly in the cytoplasm of differentiated enterocytes. We have also found that both the expression and cellular localisation of Snai1 is dependent on canonical Wnt signalling, a key regulatory pathway of intestinal stem cells. Snai1 is up-regulated in polyps from Apcmin/+ mice indicating that Snai1 may also play a part in the early stages of tumorigenesis in addition to promoting metastasis of intestinal tumours. We have investigated Snai1 function in the intestinal epithelium using an inducible conditional knockout approach and found that Snai1 is required for survival of the crypt base columnar (CBC) stem cell population in vivo. Analysis of the effects on the crypt base columnar stem cell population using a combination of Fluorescent Activated Cell Sorting (FACS), lineage tracing and in vitro organoid culture demonstrates that loss of Snai1 results in a decrease in cell proliferation and apoptotic loss of stem cells. Elevation of Snai1 levels using a conditional transgenic approach increases epithelial proliferation and markers of CBC stem cells. Analysis of tissue from Snai1 knockout and transgenic tissue has also revealed a role for Snai1 in differentiation of absorptive cell types. In conclusion, these results suggest that Snai1 has a key role in stem cell maintenance and control of cellular differentiation.

W-1025
GENERATION OF FUNCTIONAL HUMAN INTESTINAL ORGANOIDS FROM LYMPHOBLASTOID B-CELL LINES: DEVELOPING A "DISEASE IN A DISH" MODEL OF INFLAMMATORY BOWEL DISEASE

Barrett, Robert[1], Yeager, Nicole[1], Rushton, David[1], Ornelas, Loren A.[1], Sareen, Dhruv[1], Svendsen, Clive[2], Targan, Stephan[1]
[1]Cedars Sinai Medical Center, Los Angeles, CA, USA, [2]Cedars-Sinai Regenerative Medicine Institute, Los Angeles, CA, USA

Genome wide association studies have identified 163 loci that are associated with Inflammatory Bowel Disease (IBD), some of which may contain causative single nucleotide polymorphisms (SNPs) in or close to genes that are associated with intestinal epithelial cell function.

However, establishing the functional consequences of these SNPs in the intestinal epithelium is difficult; no animal models exist which contain such SNPs, nor are there any suitable in vitro models. As there are a number of repositories which contain genotyped lymphoblastoid B-cell lines (LCLs) derived from IBD patients, the aim of this study was to establish a methodology in which to utilize these repositories and generate intestinal epithelium which may contain these SNPs of interest. To achieve this, a multistep protocol was established whereby LCLs were firstly reprogrammed to form induced pluripotent stem cells (IPSCs) and then subsequently directed to form three-dimensional human intestinal organoids. Initially, a control LCL (Coriell, GM22649) was directed to form IPSCs using an episomal plasmid-based reprogramming method. These LCL-derived IPSCs were alkaline phosphatase positive, expressed the typical pluripotency-associated transcription factors, Sox2 and Oct 4, and the surface markers SSEA4, Tra 1-60 and Tra 1-81. They gave rise to mesodermal, ectodermal and endodermal lineages via embryoid body formation, were karyotypically normal and retained a similar V(D)J rearrangement in the heavy chain immunoglobulin locus as compared to the parental LCL. Three dimensional intestinal organoids were generated from LCL-derived IPSCs by culturing them with Activin A to induce definitive endoderm formation, Wnt3A and FGF4 to induce hindgut formation and then ultimately EGF, noggin and R-Spondin-1 to induce and maintain organoid formation. After 60 days, it was found that the human intestinal organoids expressed the intestinal transcription factor, CDX2, and contained FABP2+ enterocytes, chromogranin A+ enteroendocrine cells, Muc2+ goblet cells and lysozyme+ Paneth cells. The intestinal cells within the human intestinal organoids expressed the tight junctional marker ZO-1, which was polarized towards the lumen, and the adherens junction marker E-Cadherin. Interestingly, transepithelial resistance (TER) could be measured in the human intestinal organoid system and the functionality of these organoids was demonstrated by showing there was a significant decrease in the TER after administration of EGTA. Given the large number of IBD-LCLs in existence, this methodology would allow for the development of a "disease in a dish model" of IBD, whereby human intestinal organoids containing any causative SNP of interest could be generated. This approach would be especially useful for rare variants and may ultimately allow for an assessment of the intrinsic consequences of IBD associated SNPs in the intestinal epithelium.

W-1026
DEVELOPMENT OF A SERUM-FREE AND DEFINED MEDIUM FOR THE ESTABLISHMENT AND MAINTENANCE OF MOUSE INTESTINAL ORGANOID CULTURES

Conder, Ryan, Riedel, Michael J., Thomas, Terry E., Eaves, Allen C., Louis, Sharon A.
STEMCELL Technologies Inc., Vancouver, BC, Canada,

The recent demonstration that intestinal stem cells can be maintained *in vitro* within 3D 'organoid' structures will facilitate our understanding of normal and tumorigenic intestinal stem cells and advance disease modeling and regenerative medicine efforts (Sato *et al.*, *Nature*, 2009). Organoids can be derived from intestinal crypts isolated from primary tissues, or from single Lgr5+ cells purified from crypts. These organoids can be serially passaged and maintained long term in specialized culture medium and 3D matrix that mimic the stem cell niche. We have developed a defined medium (IntestiCult™ Organoid Growth Medium) and protocols to support consistent and efficient establishment and long term maintenance of mouse intestinal organoids. Crypts were mechanically dissociated from dissected upper intestines of C57BL6/J mice and incubated for 20 min with Gentle Cell Dissociation Reagent (GCDR) at room temperature (RT). Following several PBS washes, crypts were counted and re-suspended in a 50:50 mixture of Matrigel® and IntestiCult™ Organoid Growth Medium at 6000 crypts/mL. A 50 µL droplet of the suspension was gently placed into the center of pre-warmed 12-well culture plate wells, creating a hemisphere containing ~300 crypts/well. The hemispheres were solidified at 37°C for 5 min and wells then flooded with 750 µL of IntestiCult™ Organoid Growth Medium. Crypts were cultured at 37°C for 4 - 7 days with 3 times weekly medium changes. Organoid formation was determined using light microscopy. After 7 days, organoids were passaged by treating cultures with GCDR for 15 min at RT followed by mechanical disruption into smaller aggregates. The resultant suspension was mixed with IntestiCult™ Organoid Growth Medium at a 1:5 ratio and then re-plated as above to establish secondary cultures. This protocol was repeated to generate long term cultures. Organoids were characterized at different time points by immunocytochemistry for expression of Lgr5, a putative marker for intestinal stem cells, and markers for polarized enterocytes (Villin), goblet cells (MUC2), enteroendocrine cells (Chg A) and Paneth cells (Lysozyme). At 12-16 hours after plating, growing organoids could be identified as spherical structures, and were counted. New crypt-like structures typically budded from the spheres within 3 days after plating, creating prototypical organoids. The efficiency of organoid formation from primary intestinal tissue-derived crypts was assessed on day 4 at 59 ± 6% (mean ± SD; n = 10), yielding approximately 180 organoids per well. By day 7 organoids generally had multiple buds, each containing Lgr5+ cells surrounded by Paneth cells at the base of each bud, and were ready for passaging. The inter-bud regions were primarily comprised of Villin+ enterocytes interspersed with MUC2+ goblet and Chg A+ enteroendocrine cells. Organoid formation efficiency increased to 89 ± 2 % (mean ± SD; n = 3 mice) after the first passage and remained consistently high over at least 10 passages (91 ± 2%, mean ± SD; n = 3 mice), and organoids maintained expression of Lgr5 and markers of the major differentiated cell types of the mature intestine at later passages. In summary, we have developed a defined Organoid Medium and protocols that efficiently promote the formation and maintenance of 3D organoids from primary mouse intestine. This new culture system provides researchers with a valuable tool for studying stem cell biology and developing diagnostic procedures and therapeutics for intestinal disorders.

LUNG CELLS

W-1029
POTENTIAL OF RESVERATROL AND STEM CELL THERAPY FOR AGEING MOUSE LUNG

Navarro, Sonia[1], Reddy, Raghava[1], Lee, Jooeun[1], Sedrakyan, Sargis[1], Perin, Laura[2], Warburton, David[2], Driscoll, Barbara[3]
[1]Department of Surgery, University of Southern California, Children's Hopsital Los Angeles, Los Angeles, CA, USA, [2]Children's Hospital Los Angeles, Los Angeles, CA, USA, [3]University of Southern California, Los Angeles, CA, USA

Rationale:Ageing is marked by a decline in organ function, stem cell population, homeostasis, and telomere shortening. In mice, we have shown that ageing causes degeneration in lung tissue, characterized by reduced numbers of alveolar epithelial type 2 cells (AEC2), a reduction in structural elasticity resulting in enlargement of air spaces, and diminished capability to resolve a lung injury.1,2,3 We have used the telomerase null (terc-/-) mouse as a model to examine the impact of accelerated ageing on progenitor AEC2. Our preliminary data using this model show decreased whole lung function and

mitochondrial DNA damage in AEC2.The interrelationship between AEC2, whole lung function and mitochondrial homeostasis is not well understood. Resveratrol is an agonist of silent information regulator two proteins deacetylase (SIRT1). Activated SIRT1 deacetylates and activates PGC1a, a master regulator of mitochondrial biogenesis genes4, 5 and deacetylates p53 for protein degradation to decrease apoptosis.6 Resveratrol may offer a novel prophylactic therapy that stimulates SIRT1 to restore or modify mitochondrial dysfunction in AEC2. Mesenchymal-type stem cells have also shown some efficacy in treatment of acute and chronic lung injury.7 We wished to determine if the accelerated ageing phenotype of terc-/- lung, including whole lung and AEC2 mitochondrial dysfunction could be modulated by administration of Resveratrol, stem cells, or a combination of both. Hypothesis: Prophylactic treatment with Resveratrol, Amniotic Fluid Stem Cells (AFSC), or a combination of AFSC and Resveratrol will improve lung function and restore AEC2 integrity in prematurely aged lung. Methods: Cohorts of 2 month old terc-/- mice received 3 doses of 1x106 AFSC, 1mg/kg Resveratrol, 1x106 AFSC plus 1mg/kg Resveratrolor vehicle (N=6-10) by intratracheal instillation at 1 month intervals. One month after the last treatment, lung function was assessed, lung morphologyand AEC2 were isolated for western blotting of ageing and mitochondrial biogenesis marker expression. Results: Pulmonary mechanics showed significant improvement, with decreased dynamic compliance and increased elastance in the AFSC, Resveratrol, and AFSC plus Resveratrol combination treated terc-/-animals as compared to vehicle and non-stem cell controls. The combination of AFSC plus Resveratrol was more effective than the use of AFSC or Resveratrol alone. In AEC2 isolated from treated lung, both AFSC and Resveratrol treatments were shown to stimulate increased expression of anti-ageing protein SIRT1, transcription factors ATF-2 and NRF-2, and oxidative stress protectant Hsp25, and reduced expression of stabilized p53. Conclusions: Prophylactic treatments improved lung function in rapidly ageing mice. Data suggest Resveratrol and AFSC treatment may activate pathways for mitochondrial biogenesis, cell function and cell survival of the normally senescent and injury vulnerable phenotype of terc-/- AEC2. Stem cell and pharmacological or combination interventions show potential to slow lung ageing. We speculate that these reagents may also be useful tools for pinpointing pathways in epithelial cells and structures within the lung that deteriorate with age than can be manipulated to improve aged lung function. These studies should also provide a platform for better understanding the lung aging process.

W-1030
INVESTIGATIGATING CHROMATIN REGULATION OF LUNG TUMOR PROPAGATING CELLS AND LUNG STEM CELLS VIA SMALL MOLECULE INHIBITORS

Rowbotham, Samuel, Beede, Alexander, Sinkevicius, Kerstin, Kim, Carla
Boston Children's Hospital, Boston, MA, USA

Proper epigenetic control of transcription is known to be important for the homeostasis of embryonic and adult stem cells, and is frequently disrupted in cancer. We hypothesize that lung stem cells are maintained by distinct mechanisms of epigenetic regulation, and cancer stem cell-like lung tumor propagating cells (TPCs) may utilize these mechanisms to confer their stem cell and enhanced tumorigenic phenotypes. We have demonstrated that a combination of cell surface markers enrich for murine lung tumor cells with enhanced tumor propagation and metastatic abilities, as well as normal multipotent lung stem cells (BASCs). High-throughput screening of lung TPC cell lines has revealed small molecules that both positively and negatively regulate levels of the stem cell marker Sca-1; inhibition of readers of transcriptionally active histone acetylation, BRD2,3,4 reduces Sca-1 levels, and inhibition of repressive H3K9me1/2 methyltransferases G9a/Glp increases Sca-1. Up and down regulation of Sca-1 by G9a/Glp and BRD2,3,4 inhibition correlated with the ability of cell lines and primary tumor cells to form colonies in 3D growth assays. Pre-treatment of tumor cells with G9a/Glp inhibitors also increases the in vivo burden of metastases in mice following intravenous injection ($p=0.017$, $n=18$). G9a/Glp inhibition of BASCs in 3D culture also restricts the formation of alveolar differentiated colonies ($p=0.007$, $n=6$) and increases the proportion of undifferentiated cells ($p=0.042$, $n=6$). These findings suggest that common mechanisms of chromatin regulation exist between lung stem cells and lung tumor propagating cells. Determining the precise mechanisms of this regulation and the in vivo consequences remain important questions to be answered.

W-1031
P63/KRT5+ DISTAL AIRWAY STEM CELLS ARE ESSENTIAL FOR LUNG REGENERATION

Zuo, Wei[1], Wu, Daniel Z.[1], Guan, Shou Ping[1], Zhang, Ting[1], Liew, Audrey-Ann[1], Lessard, Mark[2], Kumar, Pooja Ashok[3], Nakajima, Noriko[4], Yamamoto, Yusuke[3], Crum, Christopher P.[5], Xian, Wa[3], McKeon, Frank[3]
[1]Genome Institute of Singapore, Singapore, Singapore, [2]The Jackson Laboratory, Bar Harbor, ME, USA, [3]The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA, [4]National Institute of Infectious Diseases, Tokyo, Japan, [5]Department of Pathology, Harvard Medical School, Boston, MA, USA,

The potential for lung regeneration was long discounted due to the irreversible character of chronic lung diseases. However, patients who sustain massive loss of lung tissue during acute infections often recover full pulmonary function. Correspondingly, we demonstrated lung regeneration in mice following H1N1 influenza infections and implicated distal airway stem cells (DASCs) in this process. We show here that rare, preexisting DASCs undergo a proliferative expansion in response to H1N1 influenza infection and can be lineage-traced to nascent alveoli assembled at sites of liquifactive necrosis. We also show that the conditional, in vivo ablation of DASCs prevents the regeneration of lung tissue following H1N1 influenza infection. Finally, we transplant DASC derived from single cells to lungs of H1N1 influenza-infected mice and show that these exogenous stem cells readily contribute to the regenerating alveoli. These data demonstrate that DASCs are required for lung regeneration and may have significant potential for mitigating acute and chronic lung diseases.

EPITHELIAL CELLS (NOT SKIN)

W-1032
COMBINED KIT/FGFR2B SIGNALING REGULATES THE EXPANSION OF EPITHELIAL PROGENITORS IN BRANCHING ORGANS

Lombaert, Isabelle, Abrams, Shaun, Holmberg, Kyle, Patel, Vaishali, Hoffman, Matthew
Laboratory of Cell and Developmental Biology, NIH, Bethesda, MD, USA

Various somatic organs such as prostate, lung and salivary glands can be formed and/or regenerated by organ-specific epithelial KIT+ progenitors. However, the mechanism by which KIT+ progenitors create and regenerate these branching tissues is not well understood. Using fetal salivary gland 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 to form ductal structures. Reiterative rounds of this multicellular communication create the branching architecture of the developing organ. This molecular mechanism is maintained during adult tissue homeostasis, and we predict it will be important for adult gland regeneration. We have already successfully stimulated the KIT/FGFR2b pathway in vitro to expand both mouse and human adult salivary gland KIT+ progenitors. These KIT+ progenitors can be used for transplantation to regenerate the damaged organ. Our novel findings also provide a template for future applications to (re-)generate branching organs.

W-1033
CELL OF ORIGIN AFFECTS SUSCEPTIBILITY TO TRANSFORMATION AND THE RESULTANT PROPERTIES OF DE NOVO GENERATED PRIMARY HUMAN BREAST TUMORS

Nguyen, Long V.[1], Pellacani, Davide[1], Kannan, Nagarajan[1], Osako, Tomo[2], Cox, Claire L.[1], Carles, Annaick[3], Makarem, Maisam[1], Beer, Philip[1], Moksa, Michelle[3], Kennedy, William[1], Hirst, Martin[3], Aparicio, Samuel[2], Eaves, Connie[1]

[1]Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, BC, Canada, [2]Molecular Oncology, British Columbia Cancer Agency, Vancouver, BC, Canada, [3]Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada

Human breast cancers are clonal outgrowths that have diversified genetically and biologically by the time they are clinically evident. Nevertheless, histologic and transcriptomic analyses indicate variably retained similarities to normal mammary populations with a preponderance of basal or luminal cell features. These findings have stimulated speculation as to the potential role of the cell type that first acquires malignant potential. To address this question directly, we have taken a reverse genetic approach in which basal cells (BCs) and luminal progenitor cells (LPs) isolated from normal human mammoplasty samples were transduced with *PIK3CA*^{H1047}, *TP53*^{R273C} and *KRAS*^{G12D} alone or in combination plus a barcoding vector and tumorigenesis then tracked over time in immunodeficient (NOD-SCID-IL2Rγc$^{-/-}$) female mice transplanted under the kidney capsule with the cells embedded in a collagen gel. *PIK3CA*^{H1047}and *TP53*^{R273C} were chosen as these genes have the highest prevalence of mutation in human breast cancers. *KRAS*^{G12D} activates the RAS/ERK pathway which is also commonly perturbed. Mice transplanted with 3x10^4-1.4x10^6 FACS-purified BCs (EpCAM^{lo}CD49f$^+$) or LPs (EpCAM^{hi}CD49f$^+$) that had been exposed simultaneously to 3 lentiviral vectors, each encoding a single mutant gene and a different fluorescent reporter, produced tumors in 6-8 weeks from 8 (42%) and 13 (68%) of the 19 different paired samples tested, respectively. Most of the tumors resembled invasive ductal carcinomas, were highly mitotic (and Ki67$^+$), and had invasive margins. Interestingly, barcode analysis of 1 and 8 of these revealed that the number of clones in the BC-derived tumors (normalized for input dose) was 2-fold higher than in the LP-derived tumors (on average 1/500 as compared to 1/10^3 LPs). However, the clones comprising the LP-derived tumors were larger (up to 9x10^6 cells/clone) than their BC-tumor counterparts (10^2-3x10^4 cells/clone). Tumors were also generated in 6-8 weeks from BCs and LPs expressing *KRAS*^{G12D} alone (2/5 and 6/6, respectively), or in combination with either *PIK3CA*^{H1047R} (3/6 and 4/6) or *TP53*^{R273C} (3/6 and 6/6). From these, a total of 3 and 13 tumors of BC and LP-origin again revealed the number of clones was 2-fold lower but 4 to 8-fold larger in those of LP-origin. Immunohistochemical analysis revealed that 3/5 (60%) of tumors derived from BC transduced with all 3 genes were ER$^+$, whereas 6/6 (100%) LP-derived tumors were ER$^-$, suggesting that the phenotype of cells in breast cancers may be determined by the cell of origin but not necessarily reflect its normal properties (as normal BCs do not express ER). To determine whether this change in phenotype occurs early in transformation, we examined xenografts at 4 weeks (before tumors are readily detectable), and found *KRAS*^{G12D} alone significantly expands the number of cells generated from both transduced BC and LP cells (p=0.043 and 0.048, respectively), compared to matched GFP-transduced control cells, but preferentially promotes luminal differentiation only in the transduced BCs (p=0.004). In contrast, *KRAS*^{G12D}+*TP53*^{R273C} preferentially generated cells with a basal phenotype from transduced LP cells (p=0.024). Taken together, these findings provide the first evidence that normal human mammary basal and luminal epithelial cells may have different susceptibilities to transformation, as well as unanticipated influences on the phenotypes of the transformed cells they generate and their subsequent growth characteristics.

W-1034
CONSERVED TRANSCRIPTIONAL AND EPIGENETIC REGULATION OF CANCER STEM CELLS

Rane, Jayant K.[1], Droop, Alastair P.[2], Pellacani, Davide[3], Ylipää, Antti[4], Scaravilli, Mauro[5], Polson, Euan S.[2], Mann, Vincent[6], Collins, Anne T.[1], Simms, Matthew S.[7], Caves, Leo SD[1], Visakorpi, Tapio[5], Maitland, Norman J.[1]

[1]University of York, York, United Kingdom, [2]Leeds Institute of Cancer and Pathology, Leeds, United Kingdom, [3]Terry Fox Laboratory, Vancouver, BC, Canada, [4]Tampere University of Technology, Tampere, Finland, [5]University of Tampere, Tampere, Finland, [6]Hull York Medical School, Hull, United Kingdom, [7]Castle Hill Hospital, Cottingham, Hull, United Kingdom

Identification of shared transcriptional and epigenetic regulatory mechanisms in normal and cancer stem cells (CSCs) should result in the development of novel CSC-specific treatments. Here we show that a conserved two-step regulation of transcriptional and epigenetic machinery by nuclear receptors defines the prostate epithelial hierarchy. Furthermore, comparative large-scale bioinformatic analyses imply that a similar two-step regulation is common in other human epithelial tissues. The analysis was based on mRNA and miRNA expression profiles from patient-derived prostate epithelial sub-populations. Genes differentially expressed in stem and committed basal (CB) cells fell into 4 distinct co-expression groups, not only in prostate, but also in more than 150 human cell types. Cell fate studies revealed that one gene set was regulated by retinoic acid (RA), which could drive prostate epithelial stem cell differentiation to basal cells, but failed to induce terminal luminal differentiation. The same gene set, in CB cells, then preferentially responded to androgens (AR), which did induce terminal luminal differentiation i.e. the steroidal nuclear receptors collaborate in a cell sub-type dependent manner on the same set of genes to control successive differentiation events. Epigenetic regulation of prostate differentiation displayed a similar, higher order two-step control. The distinct miRNA expression signatures of each cell implied that a differential miRNA expression pattern was crucial for cell sub-type-specific responses. To evaluate this, we integrated our mRNA-miRNA expression microarray datasets, which identified miR-99a/100 as two of the top stem cell fate regulatory miRNAs. CSCs exhibit lower expression of miR-99a/100 (about 8 fold), and can repair DNA more efficiently than CB cells. So, we hypothesised that lower expression of these 2 miRNAs is essential for efficient DNA repair in CSCs and hence for their radio-resistance. Lower miR-99a/100 expression indeed directly correlated with radio-resistance of prostate cancer cell lines. Inhibition of miR-99a/100 expression in CB cells allowed them to efficiently repair damaged DNA, without inducing de-differentiation

or EMT. The effect was principally mediated through SMARCA5 and SMARCD1, which participate in relaxation of damaged chromatin to rapidly recruit DNA repair complexes, such as BRCA1 and RAD51. Recruitment of SMARCA5 was dependent on PARP1 expression, while SMARCD1 recruitment was PARP1-independent. Concurrent transfection of SMARCA5/D1 esiRNAs abrogated efficient DNA repair triggered by miR-99a/100 inhibition. Further mechanistic loss and gain of function studies revealed that glucocorticoid receptor (GR)-miR-99a-SMARCD1 and AR-miR-99a-SMARCD1 regulation form a positive feedback loop in androgen-independent and androgen-dependent cancer cells respectively, to maintain treatment-resistant cancer cells. These results show that the overarching transcriptional and epigenetic regulation of human prostate epithelial differentiation is under fine control by RA, GR and AR in a cell sub-type dependent manner. The clinical implications are: (i) RA-focussed differentiation therapy would fail to differentiate CSCs into treatment sensitive luminal cells, and (ii) miR-99a/100-SMARCA5/D1 signalling should be a good target for drug development in the management of treatment-resistant prostate cancer.

W-1035
STEMNESS OF HUMAN ORAL MUCOSAL EPITHELIAL CELLS

Shahdadfar, Aboulghassem
Ophthalmology, Oslo University Hospital, Oslo, Norway

Human oral mucosal epithelial cells (HOMECs) represent an appealing source of epithelial cells for ocular surface reconstruction and are easily obtained and expanded in vitro. Culture of HOMECs generates two different populations. In this study, it has been investigated the differentiation potential of HOMECs populations to ocular surface cells. Human oral mucosal epithelial biopsies (HOMEBs) and blood as a source for preparation of autologous serum (As) were obtained from donors. HOMEBs were in parallel cultured in media containing AS (DMEM/F12, 100 U/ml antibiotic, and 10% autologous human serum) or FBS (DMEM/F12, 100 U/ml antibiotic, 5% FBS, 2 ng/ml EGF, ITS (5 µg/ml insulin, 5 µg/ml transferrin and 5 ng/ml sodium selenite), 30 ng/ml cholera toxin A, 0.5% dimethylsulfoxid, 15 µM hydrocortisone) at 37^0 C with 5% CO2 in a humidified atmosphere. HOMECs populations were harvested in different steps during 4 weeks and analyzed by qRT-PCR, immunohistochemistry, transmission electron microscopy (TEM). Only one subpopulation of HOMECs contains highly proliferative capacity with stem cell characteristic in both media. Expansion of cells in AS containing medium depends essentially on AS quality and nutritional condition, the important factors that affect cell proliferation, differentiation capacity and gene expression. qRT-PCR analysis of *MKI67, ABCG2, CK3, CK13, CK19, GJA1* (Connexin 43), *OCLN*, *P53*, OCT4, and *SOX9* genes, colony forming, Immunohistochemistry of related proteins, and transmission electron micrographs (TEM) confirm these observations. A sub-cell population of expanded Human Oral Mucosal Epithelial biopsies shows stem cell characteristics with differentiation potential to ocular surface cells. Tissue engineering of these cells could be useful for regeneration of human ocular surface tissues.

W-1036
TROPHOBLAST STEM CELLS: IMMUNOPHENOTYPE, MULTIPOTENCY AND ANTICANCER PROPERTIES

Svitina, Hanna[1], Shablii, Volodymyr[1], Kyryk, Vitalii[2], Kuchma, Maria[1], Skrypkina, Inessa[3], Halina, Kuznietsova[4], Dzhus, Olena[4], Denis, Evgenii[4], Shablii, Yulia[1], Rybalchenko, Volodymyr[4], Garmanchuk, Liudmila[4], Lobintseva, Galina[1]
[1]Institute of Cell Therapy, Kiev, Ukraine, [2]State Institute of Genetics and Regenerative Medicine Academy of Medicine of Ukraine, Kiev, Ukraine, [3]Institute of Molecular Biology and Genetics of National Academy of Science of Ukraine, Kiev, Ukraine, [4]Institute of Biology, Taras Shevchenko National University of Kyiv, Kiev, Ukraine

The main purpose was to determine the effect of intravenous allogeneic and xenogeneic transplantation of trophoblast stem cells (TSC) at dimethylhydrazine (DMH)-induced colorectal mid/late-stage cancer in rats. Methods: TSC were obtained from human and rat placenta by tissue explant culturing. TSC were immunophenotyped by flow cytometry and immunocytochemistry, gene expression was analyzed by RT-PCR. Experimental colon carcinogenesis was induced in male albino Wistar rats by injecting DMH (20 mg*(kg body mass)-1) once weekly for 20 consecutive weeks, and administering of the rat TSC (rTSC) and human TSC (hTSC) at 22nd week after first DMH-injection. We studied the histopathological alterations (number, size and characteristics of the tumors in the colon). ANOVA was used to compare multiple groups and individual comparisons were made using a Manna-Whitney test. Regression analysis was used to examine the doze-dependent effect of TSC on number and size of lessioned tissue. p values less than 0.05 were considered statistically significant. Results: The obtained rTSC expressed specific genes of the trophoectoderm (CDX-2) and of the stemness (ID2), do not express the inner cell mass gene (NANOG) and different trophoblast type cells appropriate genes, such as PRL3B (prolactin 3b) of trophoblast giant cells and TPBPA (trophoblast specific protein alpha) of labyrintotrophoblast and spongiotrophoblast. Lack of mRNA of differentiating cell type proves that the cells relate to the trophoblast stem cells. hTSC similar to rTSC expresses trophoblast related genes such as CDX-2, eomesodermin and had capacity to differentiating into three mesodermal lineages (osteogenic, chondrogenic and adipogenic). Flow cytometry shown that both rTSC and hTSC were positive for mesenchymal stromal markers (CD90, CD44, CD29, CD105, CD73). 5 weeks after intravenous rTSC allogeneic transplantation was observed the exponential dose-response relationship for both number and size of tissue lesions (R2=0.646 for number of lesions [p=0.002]; R2=0.9 [p≤0.001] for size of tissue lessions). The transplantation of more than 1,2x106 cells/kg b.m. resulted the stopping of following tumor progression as compared with control group, and transplantation of more than 2x106 cells/kg resulted decreasing the area of hyperplasic or dysplastic lesions. The exponential dose-response relationship demonstrates the association between the number of transplanted cells and the resulting normalization of colon mucosa. Xenogeneic transplantation of hTSC did not have any effect on tumor growth. It may suggest about huge role of immune system in stem cells modulation of cancer cells. Conclusions. In this study, we demonstrated the similarity of placenta-derived cell culture to both mesenchymal and trophoblast stem cells. In-depth understanding of TSC properties may provide information regarding the biological anti-tumor action. We showed that allogeneic intravenous transplantation of TSC to DMH-induced colorectal cancer rats had doze-related effect on adenocarcinomas further growth and tumorigenic processes in "initiated" sites at the same time no effect were observed at xenogeneic administration of TSC.

W-1037
HUMAN AMNION EPITHELIAL CELLS INFLUENCE MACROPHAGE AND REGULATORY T CELL RESPONSE TO REDUCE LUNG FIBROSIS

Tan, Jean, Chan, Siow, Wallace, Euan M., Lim, Rebecca
Ritchie Centre, Monash Institute of Medical Research/Monash University, Melbourne, Australia

Human amnion epithelial cells (hAECs) have protective and reparative capabilities when administered immediately after and following established lung injury. hAECs can modulate macrophage response during repair by altering their polarity and function. However, the importance of other immune cell types in hAEC mediated lung repair is yet unknown. hAECs exert their reparative effects by inducing the maturation of naïve T cells into regulatory T cells (Tregs) and this step precedes the polarization of macrophages from proinflammatory M1 to proreparative M2. We first determined the role of Tregs by treating bleomycin challenged Foxp3-GFP knock in mice with hAECs and measured the local Treg population by flow cytometry. The reliance of hAEC mediated lung repair on Tregs was further assessed by challenging Rag1-/- mice with bleomycin, followed by adoptive transfer of either Tregs or CD45+/FoxP3- cells. The extent of lung fibrosis and inflammation, and macrophage polarity and function were measured 7 and 14 days later. Administration of hAECs to bleomycin challenged Foxp3-GFP knock in mice induced Treg expansion in the lungs. Further, lung repair in bleomycin challenged Rag1-/- mice was most significant in the cohort of animals administered hAECs following adoptive transfer of Tregs. In vitro, hAECs directly induced FoxP3 transcription in naive CD4+ cells, primarily though TGF-β signaling. Additionally, hAEC mediated polarization of macrophages in vivo towards an M2 anti-inflammatory phenotype was only observed in animals that received hAECs as well as adoptively transferred Tregs. Interaction between hAECs and Tregs contribute to their protective and reparative properties. And polarization of macrophages occurs as a consequence of this cellular interaction.

W-1038
A REGULATORY NETWORK CONTROLS NEPHROCAN EXPRESSION AND MIDGUT PATTERNING

Wei, Wei[1], Hou, Juan[1], Saund, Ranajeet S.[2], Xiang, Ping[1], Cunningham, Thomas J.[3], Lu, Daphne Y.D.[1], Savory, Joanne G. A.[4], Krentz, Nicole A. J.[1], Montpetit, Rachel[1], Cullum, Rebecca[1], Lohnes, David[4], Humphries, R. Keith[5], Duester, Gregg[3], Saijoh, Yukio[2], Hoodless, Pamela A.[1]
[1]BC Cancer Agency, Vancouver, BC, Canada, [2]University of Utah, Salt Lake, UT, USA, [3]Sanford-Burnham Medical Research Institute, La Jolla, CA, USA, [4]University of Ottawa, Ottawa, ON, Canada, [5]University of British Columbia, Vancouver, BC, Canada

While many regulatory networks involved in defining definitive endoderm have been identified, the mechanisms through which these networks interact to pattern the endoderm are less well understood. To explore the mechanisms involved in midgut patterning, we dissected the transcriptional regulatory elements of Nephrocan (Nepn), the earliest known midgut specific gene in mice. We observed that Nepn expression is dramatically reduced in Sox17-/- and Raldh2-/- embryos compared to wild-type embryos. We further show that Nepn is directly regulated by Sox17 and the retinoic acid receptor via two enhancer elements located upstream of the gene. Moreover, Nepn expression is modulated by Activin signaling with high levels inhibiting and low levels enhancing RA-dependent expression. In FoxH1-/- embryos in which Nodal signaling is reduced, the Nepn expression domain is expanded into the anterior gut region suggesting that Nodal signaling can modulate expression in vivo. Together, Sox17 is required for Nepn expression in the definitive endoderm, while retinoic acid signaling restricts expression to the midgut region. A balance of Nodal/Activin signaling regulates the anterior boundary of the midgut expression domain.

W-1039
ONTOGENY OF THE BARRETT'S PRECANCEROUS LESION DEDUCED FROM CLONED STEM CELL PEDIGREES

Yamamoto, Yusuke[1], Wang, Xia[1], Bertrand, Denis[2], Kern, Florian[2], Zhang, Ting[2], Duleba, Marcin[1], Srivastava, Supriya[3], Hu, Yuanyu[2], Teh, Ming[3], Khor, Chiea Chuen[2], Crum, Christopher P.[4], Nagarajan, Niranjan[2], Ho, Khek Yu[5], McKeon, Frank[1], Xian, Wa[1]
[1]The Jackson Laboratory for Genomic Medicine, Farmington, CT, USA, [2]Genome Institute of Singapore, Singapore, [3]Department of Pathology, National University Heath System, Singapore, Singapore, [4]Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA, [5]Department of Medicine, National University Heath System, Singapore

Esophageal adenocarcinoma is a highly lethal cancer whose incidence has quadrupled in the past four decades. Efforts at chemotherapy and surgical resection have not appreciably altered survival rates, and therefore much hope is placed on detecting and treating less aggressive precursors of this disease. Barrett's esophagus ("Barrett's") is widely believed to be the non-cancerous precursor of esophageal adenocarcinoma as its presence confers a 30- to 100-fold increased risk of this cancer. Strategies for selective eradication of Barrett's have been stymied by our inability to identify the Barrett's stem cell. Here we employ novel technologies to clone patient-matched stem cells of Barrett's, gastric, and esophageal epithelium. Whole genome expression microarray data Barrett's and gastric stem cell pedigrees from 10 patients showed similar patterns of differential gene expression within this patient cohort. SNP-array based copy number variation and exome capture sequencing data of Barrett's stem cells reveal a patient-specific mutational spectrum ranging from low somatic variation similar to patient-matched gastric epithelial stem cells to ones marked by extensive heterozygous alteration of genes implicated in tumor suppression, epithelial planarity, and epigenetic regulation. Transplantation of transformed Barrett's stem cells yields tumors with hallmarks of esophageal adenocarcinoma, whereas transformed esophageal stem cells yield squamous cell carcinomas. Thus Barrett's develops from cells distinct from local eponymous epithelia, emerges 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.

EPIDERMAL CELLS

W-1040
EFFICIENT MULTIPLE LINEAGE DIFFERENTIATION OF CD105 POSITIVE DERMAL CELLS IN DERMIS

Lee, Seung Bum[1], Kim, Min-Jung[1], Shim, Sehwan[1], Shin, Hye-Yun[1], Seo, Seong-Won[1], Jang, Won-Suk[1], Lee, Sun-Joo[2], Jin, Young-Woo[1], Park, Sunhoo[1], Lee, Seung-Sook[1]
[1]Laboratory of Radiation Exposure and Therapeutics, Korea Institute of Radiological and Medical Science, Seoul, Republic of Korea, [2]Division of Clinical and Translational Research, Korea Institute of Radiological and Medical Science, Seoul, Republic of Korea

Dermis in the skin include a variety of multipotent stromal cells including skin-derived mesenchymal stem cells (MSC) and dermal multipotent fibroblasts (DFs). Although several studies have shown that DFs share mesenchymal phenotypes with stem cells, the

characterization of these dermal cells is not fully understood. In this study, we isolated the dermal cells from skin of newborn of C57/B6 mice and investigated the cell characteristic of multipotency. Isolated dermal cells exhibited the expression of a similar antigen profiles of MSC except CD73 (one of major markers of MSC) and the positive expression for fibronectin and alpha SMA (fibroblast markers). Interestingly, the expression of CD105 among surface markers of MSC was only reduced upon continue passage and correlated with decreased differentiation potential into adipogenic and ostogenic phenotypes. The comparison analysis demonstrated that CD105 positive cells significantly enhanced proliferation rate and *in vitro* multilineage differentiation (adipogenesis and osteogenesis), suggesting that the expression of CD105 may be important to modulate the capacity of multipotency of fibroblast. In addition, CD105 positive cells highly expressed the stemness related gene including nestin when compared with CD105 negative cells, even mouse bonemarrow-derived MSC. These results suggested that CD105 expression may be important to maintain the capacity of multipotency of fibroblast by upregulating stemness related genes. This finding thus highlights a potential avenue to contribute multipotent fibroblast expressing CD105 antigen to the regeneration of tissue damaged by various harmful stimuli.

W-1041
IN VIVO TRANSCRIPTIONAL GOVERNANCE OF MURINE HAIR FOLLICLE STEM CELLS BY CANONICAL WNT REGULATORS

Lien, Wen-Hui[1,2], Polak, Lisa[1], Lin, Mingyan[3], Lay, Kenneth Wei Jian[1], Zheng, Deyou[3], Fuchs, Elaine[1]
[1]*Laboratory of Mammalian Cell Biology and Development, Rockefeller University, New York, NY, USA, de Duve Institute and Universite Catholique de Louvain,* [3]*Departments of Genetics, Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA*

In mammals, Wnt signaling is known to play a role in many adult stem cells, but exactly how it functions and for what purpose has been a matter of much debate. The downstream effector of canonical Wnt signaling is β-catenin, which can act as a bipartite transcription factor for the lymphoid enhancer-factor 1 (LEF1) and/or T-cell factor (TCF) DNA binding proteins. Much of what is known about Wnt signaling and stem cells comes from studies on the intestine, where Wnt/β-catenin signaling is essential for maintenance of their intestinal crypts and allows intestinal stem cells to maintain long-term organoid cultures in vitro. Wnt signaling can also stimulate stem cell proliferation as reported for cultures of hematopoeitic stem cells and embryonic stem cells (ESCs). However, more recent evidence suggests that β-catenin is dispensable for ESC proliferation under some culture conditions, and ablation of Tcf3 in these cells can even promote pluripotency. Despite intensive investigation, it remains unclear for most if not all stem cell types as to whether β-catenin and its LEF1/TCF DNA binding partners act to maintain stem cells, promote their proliferation, and/or determine their lineage fate in their native tissue context. Our study begin to address these issues, focusing on the adult hair follicle stem cells (HFSCs) that fuel cyclical bouts of active HF regeneration and hair growth (anagen), followed by destruction (catagen) and then rest (telogen). HFSCs reside in a niche known as the bulge located at the base of the telogen phase HF. Using mice harboring Wnt reporter, we show that at the telogen to anagen transition, some HFSCs within the bulge begin to respond to Wnt signaling. As β-catenin plays a critical role for canonical Wnt activity, we next dissect the physiological roles of β-catenin in HFSC maintenance, proliferation and/or fate determination. By transcriptionally profiling β-catenin-null HFSCs, we then identify β-catenin-dependent genes whose expression changes in response to Wnt activity upon normal HFSC activation. As TCF3 and TCF4 are the two LEF1/TCFs expressed by HFSCs, we turn to delineating the direct DNA binding-targets for these β-catenin transcriptional partners and address how Wnt/β-catenin target gene expression changes when TCFs are either elevated or ablated. Finally, by performing immunoprecipitations, we show that in quiescent HFSCs where Wnt/β-catenin activity is absent, TCF3/4 bind to chromatin in a complex with TLE/Groucho repressor proteins and that physiologically these proteins repress Wnt target genes. Notably, in addition to those Wnt/β-catenin-dependent genes, many TCF3/4-bound targets are HFSC signature genes and Wnt/TCF-refractory within the niche. Our most recent data indicates that NFATc1, an established BMP-regulated gene, selectively binds and co-regulates this cohort. This suggests that as long as BMP/NFATc1-signaling remains sufficiently high in the niche, most HFSCs will still transcribe their signature genes and maintain stemness even in the Wnt/β-catenin activated niche. Altogether, our results resolve a number of existing paradoxes and provide several unexpected new insights into how Wnt signaling functions in orchestrating SC behavior.

W-1042
INDUCTION OF HAIR FOLLICLE NEOGENESIS BY DEFINED EXTRACELLULAR FACTORS

Lin, Sung-Jan, Fan, Sabrina M., Yen, Chien-Mei, Wang, Wei-Hong
National Taiwan University, Taipei, Taiwan

When wounded, our body tends to repair with fibrosis. In skin, this often leads to scar formation with loss of associated appendages including hair follicles. Since hair follicles are composed of various distinct epithelial and mesenchymal cells in an organized three-dimensional structure, direct conversion of cells into a single cell type is not able to regenerate a functional hair follicle. To regenerate functional skin, we asked whether hair follicle regeneration can be facilitated by defined extracellular factors. We found that tissue extraction from a specific developmental stage was able to induce hair follicle neogenesis from non-follicular cells. Adult fibroblasts with short-term exposure to the extraction in vitro were able to induce hair follicle neogenesis from keratinocytes when they were transplanted back in vivo. Hence, such pro-regeneration effect worked through regulating adult fibroblasts, but not epithelial cells, to initiate their cross-talk with keratinocytes for hair follicle neogenesis. In further analysis, we discovered defined extracellular factors that together were sufficient to initiate hair follicle neogenesis. Therefore, organ neogenesis can be facilitated by creating a pro-regeneration environment with defined extracellular factors. Identification of such factors can be combined with other schemes for functional regeneration of tissues and organs.

W-1043
MECHANISMS OF CELL FATE DETERMINATION IN EPIDERMAL PROGENITORS DURING SWEAT GLAND DEVELOPMENT

Lu, Catherine P., Polak, Lisa, Fuchs, Elaine
Rockefeller University, New York, NY, USA

Sweat gland is the most abundant gland in human body, and its proper maintenance and functioning is crucial for optimal thermoregulation and water balance throughout our lifetime. Despite their importance for human survival and quality of lives, little is known about sweat gland development at the molecular level. Like other skin appendages, hair follicles or mammary glands, they originate from epidermal progenitors. Using lineage tracing, we identify multipotent progenitors in the sweat duct that transition to unipotency after developing the sweat gland. Exploiting differences in the mesenchymal signals that affect epidermal progenitor cell fate, our findings provide novel

insight into mechanisms of cell fate determination within multipotent epidermal progenitors.

W-1044
LIVE IMAGING OF REGULATED EPITHELIAL CELL DEATH DURING TISSUE REMODELING

Mesa, Kai, Greco, Valentina
Genetics, Yale University, New Haven, CT, USA

Cell death is an essential component of tissue development and maintenance. Physiological cell death programs promote the rapid removal of excess or dysfunctional cells without eliciting harmful effects on surrounding healthy tissue. Studying this process under physiological conditions in vivo has been difficult due to the rapid rate by which dead cells are normally removed as well as the counterbalancing effects of proliferation with the same tissue. This has limited our understanding of the signals and cells involved in the regulation of physiological cell death and tissue remodeling in vivo. Recent technological advances have been made in our lab to visualize dynamic cellular behaviors non-invasively in vivo in the skin of live mice over time by two-photon live microscopy (Rompolas et al., 2012). The mammalian skin hair follicle cycles through stereotypic rounds of growth, programmed cell death, and quiescence which are morphologically well-defined, making it an ideal model to study tissue dynamics. Combining these unique features with tools to genetically manipulate, label and track cells in vivo, we have a temporal approach to uncover the mechanisms that regulate physiological cell death and tissue remodeling as they contribute to tissue homeostasis, which has broad implications for the therapeutic treatment of neoplastic and degenerative diseases.

W-1045
TRANSPLANTATION OF AUTOLOGOUS NON CULTURED OUTER ROOT SHEATH DERIVED MELANOCYTES IN VITILIGO

Mohamadi, Parvaneh, Sshafiiyan, Saeid, Orouji, Zahra, Vaezi, Fatemeh, Khalajasadi, Zahra, Fallah, Nasrin
Royan Institute for Stem Cell Biology and Technology, Tehran, Iran

Vitiligo, one of the most prevalent skin pigmentation disorders is characterized by the selective destruction of melanocytes, leading to the development of depigmented macules that may appear anywhere on the body. It is a visible cosmetic defect that leads to serious emotional stress. The patients are characterized by low self-esteem, suffer more frequently from depression, and have difficulties in finding a job or starting a family. Different treatment strategies such as topical corticosteroids or other local therapies, systemic steroid or immunosuppressive and cytotoxic drugs, UV therapy and Laser have yielded unsatisfactory results in significant number of the patients. Therefore most current treatment trends focused on surgical options such as mini-grafting, Epidermal grafting and epithelial sheet grafting. Cell transplantation options by autologous melanocytes-keratinocytes in order to selectively replenish melanocytes have been implicated as a promising treatment of vitiliginous macules. Since overall proportion of melanocytes is low in the epidermis, we tried follicular outer root sheath to harvest hair follicles as a source of melanocytes. Therefore in this study we assessed the safety and efficacy of non Cultured Melanocyte-Keratinocyte from hair in treatment of vitiliginous macules. Methods: Autologous epithelial cells from shaved biopsy skin sample and occipital hair follicles scalp by using enzymatic and mechanical method were separated. Cell suspension is then injected in affected area in 13 stable vitiligo patients. None of the patients received adjuvant therapy. Patients were followed by examination and questionnaire in a prospective setting at least for 6 months. Percentage of repigmentation was evaluated with digital imaging analysis system and graded as marked with >75% pigmentation, moderated with 51-75% pigmentation, mild with 26-50% pigmentation and poor with less than 25% pigmentation of the treated area. Less than 25% repigmentation seen at the end of 6 months was labeled as treatment failure. Result: Seven patients received injection in symmetrical depigmentation areas in order to evaluate method safety. No side effects were observed in any patients with injected non-cultured melanocyte-keratinocyte cells from skin and hair follicle. Six patients were evaluated for efficacy of the method. In six cases that received epithelial hair cells, repigmentation started during 2 months after transplantation. Six months after transplantation, a marked repigmentation in one, moderate repigmentation in one and mild repigmentation in three patients were observed. In only one patient signs of repigmentation began not until 6 months after treatment. Disscution: Autologous Non Cultured Outer Root Sheath Derived Melanocytes is an effective, simple, non-invasive and allows easy immediate method for vitiligo treatment. When patients suffer from large vitiliginous macules instead of using skin melanocytes its better to use these sources of cells.

W-1046
COUPLING OF THE RADIOSENSITIVITY OF MELANOCYTE STEM CELLS TO THEIR DORMANCY DURING A HAIR CYCLE

Nishimura, Emi, Ueno, Makiko
Tokyo Medical and Dental University, Tokyo, Japan

Current stem cell studies have revealed that stem cells are more radiosensitive than mature cells. As somatic stem cells are mostly kept in a quiescent state, this conflicts with Bergonié and Tribondeau's law that actively mitotic cells are the most radiosensitive. In this study, we focused on hair graying to understand the stress-resistance of melanocyte stem cells (McSCs). We used Dct-H2B-GFP transgenic mice which enable the stable visualization of McSCs and an anti-Kit monoclonal antibody which selectively eradicate amplifying McSCs and found that quiescent McSCs are rather radiosensitive but the coexistence of quiescent and non-quiescent McSCs provide the stem cell pool with radioresistance. The irradiated quiescent McSCs prematurely differentiate in the niche upon their activation without sufficiently renewing themselves nor providing mature melanocytes to the hair bulb for hair pigmentation. These data indicate that tissue radiosensitivity is largely dependent on the state of somatic stem cells under their local microenvironment. Furthermore, the coexistence of non-quiescent McSCs in the niche ensures the resistance of the McSC pool to different kinds of stresses to prevent hair graying.

KIDNEY CELLS

W-1047
NATURAL SCAFFOLDS FOR IN VITRO STUDIES OF KIDNEY DEVELOPMENT, DISEASE, AND TISSUE ENGINEERING

Batchelder, Cynthia A.[1], Lee, C. Chang I.[1,2], Meyers, Frederick J.[3], Tarantal, Alice F.[1,4]
[1]California National Primate Research Center, University of California, Davis, CA, USA, [2] Department of Cell Biology and Human Anatomy, University of California, Davis, CA, USA, [3]Department of Internal Medicine, University of California, Davis Health System, Sacramento, CA, USA, [4] Departments of Pediatrics and Cell Biology and Human Anatomy, University of California, Davis, CA, USA

In vitro studies of kidney development and disease are challenging due to the complex three-dimensional (3D) architecture, which must be overcome in tissue engineering strategies for this vital organ. Our studies have previously demonstrated that decellularized rhesus monkey kidneys of all age groups provide a natural extracellular matrix (ECM) with sufficient structural properties to support migration of cells from kidney explants in an age-dependent manner, and the ability of these scaffolds to provide spatial and organizational influences on human embryonic stem cell (hESC) migration and differentiation. To further explore the use of decellularized renal scaffolds for *ex vivo* studies of development, disease, and as engineered tissue replacements, strategies to improve recellularization were assessed. The ability of a decellularized natural kidney scaffold to influence cell migration and phenotype was studied with undifferentiated hESC seeded in sections of kidney versus whole kidneys. For studies on the recellularization of whole kidneys, the delivery of the cells was by the renal artery versus ureter perfusion. Scaffold and cellular interactions were also explored using human renal cell carcinoma (hRCC) specimens. A repository of decellularized whole-kidney and sections of kidney scaffolds was established from rhesus monkey tissues (age range: fetal to aged; N=36), which were collected according to established methods and decellularized with 1% sodium dodecyl sulfate. After multiple wash steps, decellularized scaffolds were stored at 4°C until use. Recellularization studies were carried out in 6-well plates (sections) or custom perfusion bioreactors (whole-kidney) for up to 3 weeks. Morphology, recellularization, and gene expression were assessed and serial sections of the entire paraffin-embedded constructs evaluated. Undifferentiated hESC seeded onto decellularized kidney sections infiltrated the scaffold and formed tubules with markers of renal development (Pax2), proximal tubule (Aquaporin 1), and ascending loop of Henle/distal tubule (Calbindin). Some tubules with large lumens contained cells that were Vimentin+ (mesenchymal marker). In comparison, hRCC seeded onto decellularized scaffolds did not migrate within the scaffold but were found to line large lumens and expressed Vimentin and Aquaporin 1. When hESC were administered by slow perfusion through the renal artery or ureter within intact whole kidney ECM, cells were found within the vascular or tubular space of seeding. hESC administered via the ureter were shown to express loop of Henle/distal tubule markers while cells administered via the vasculature more frequently expressed markers of proximal tubules or glomeruli (Wilm's tumor 1 or Synaptopodin). In comparison, when hRCC were seeded onto a novel 3D polysaccharide scaffold (PSS), cells aggregated and expressed proteins of multiple cell lineages resembling typical *in vivo* cancer cell phenotypes. These results suggest that: (1) differentiation is directed, at least in part, by the ECM to which cells are exposed; and (2) PSS can be used to study complex interactions of hRCC in a 3D setting that is more relevant to the *in vivo* environment when compared to routine two-dimensional culture methods.

ENDOTHELIAL CELLS / HEMANGIOBLASTS

W-1049
EFFICIENT AND LESS LABOR-INTENSIVE METHODS FOR INDUCING VASCULAR ENDOTHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Ohta, Ryo, Niwa, Akira, Nakahata, Tatsutoshi, Saito, Megumu
CiRA, Kyoto University, Kyoto, Japan

Background and objective - Understanding mechanisms in normal and impaired human vascular development, and developing the cell therapy for vascular diseases are of great interest in the medical scientific fields. For those purposes, it is important to generate vascular endothelial cells from human pluripotent stem cells (PSCs) such as embryonic stem cells and induced pluripotent stem cells. Although some methods for in vitro endothelial differentiation have been reported, there remain significant hurdles before translation of hPSC-based research into clinical situation, including the way to get cells in sufficient yield and purity. To overcome the problems, we established a novel and less labor-intensive culture system for functional endothelial cell induction requiring no cell sorting. Methods and Results - We previously established a 2-dimensional serum-free culture for mesodermal differentiation ,which can generate blood cells and vascular endothelial cells (Niwa et al., 2011). Modifying that culture, ES/ iPS cells on matrigel (BD)-coated plates were stimulated with BMP4 for 3 days. After that, about 80% cells expressed a lateral plate mesodermal marker KDR. We next dispersed whole culture into single-cell level and replated them onto new matrigel-coated plates at low density. After additional 4-days culture with VEGFA stimulation, endothelial cells positive for VE-cadherin, CD34 and KDR emerged with more than 70% efficiency. The potential of them as endothelial cells were confirmed by function assay such as tube formation and LDL-uptaking test. Conclusions - We succeeded in generating human endothelial cells from PSCs at higher efficiency with less-intensive methods. This can contribute to the development of PSC-based vascular regenerative medicine, and also can be applied for dissecting the mechanism of vascular endothelial diseases.

W-1050
ENDOTHELIAL CELL ACTIVATION MEDIATES LEUKEMIA CELL GROWTH, PROLIFERATION, AND SUSCEPTIBILITY TO CHEMOTHERAPY CONTRIBUTING TO LEUKEMIA RELAPSE

Pezeshkian, Bahareh[1], Donnelly, Christopher[1], Tamburo, Kelley[1], Geddes, Timothy[2], Madlambayan, Gerard[3]
[1]Biological Sciences, Oakland University, Rochester, MI, USA, [2]Radiation Oncology, William Beaumont Health System, Royal Oak, MI, USA, [3]Oakland University, Rochester, MI, USA

Acute myeloid leukemia (AML) is a debilitating blood malignancy with high rates of relapse among patients. Overall, > 80% of patients relapse following initial chemotherapy indicating a lack of effective treatment methods for AML. Studies have demonstrated a supportive role of endothelial cells (ECs) in normal hematopoiesis. Recently, we demonstrated that ECs also play a role in leukemogenesis. We found that AML cells themselves initiate this intercellular interaction by inducing EC activation. Through this inflammatory response, activated ECs produce increased levels of E-selectin, a key cell

adhesion molecule, inducing a subset of leukemia cells to adhere on EC layers. These adherent AML cells then become quiescent and survive chemotherapy. We postulate that this mechanism may contribute to the formation of minimal residual disease. The observation that adherent AML cells can later detach and become proliferative further suggests a role of this process in relapse. We are now studying whether or not leukemia initiating stem and progenitor cells participate in this process. Preliminary data suggests that these cells are affected by the aforementioned EC activation-based mechanisms. Interestingly, we recently we found that EC activation concomitantly enhances the growth and proliferation of the non-adherent subset of AML cells through a paracrine mechanism. Therefore, EC activation acts as a 'double-edged sword' generating microenvironments that promote chemoresistance and survival as well as AML cell growth leading to the progression of the disease. We are now using this knowledge to develop novel therapies that overcome this EC activation effects to enhance patient treatment outcomes.

W-1051
TGF-BETA2 AND SNAIL OVEREXPRESSION IS REQUIRED FOR ENDOTHELIAL-MESENCHYMAL TRANSITION (ENDMT) IN DIFFERENT CELL LINEAGES

Pinto, Mariana Tomazini[1], Melo, Fernanda Ursoli Ferreira[2], Palma, Patrícia Viana Bonini[2], Rodrigues, Evandra Strazza[1], Malta, Tathiane Maisto[2], Covas, Dimas Tadeu[3], Kashima, Simone[1]
[1]Regional Blood Center of Ribeirão Preto, University of São Paulo (USP); Faculty of Pharmaceutical Sciences of Ribeirão Preto, USP, Brazil, Ribeirão Preto, Brazil, [2]Regional Blood Center of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, Brazil, [3]Regional Blood Center of Ribeirão Preto, University of São Paulo (USP); Faculty of Medicine of Ribeirão Preto, USP, Brazil., Ribeirão Preto, Brazil

Endothelial-mesenchymal transition (EndMT) is a biological process in which polarized endothelial cells undergo multiple biochemical changes that enable them to assume a mesenchymal stem cell phenotype. These changes include loss of the endothelial markers and intercellular junctions, increased expression of mesenchymal markers, and enhanced invasiveness, and migratory capacity. However, the molecular mechanism involved in this process is not totally elucidated. The aim of this work was to evaluate the role of TGF-β2 and the transcription factor Snail for the generation of mesenchymal phenotype in endothelial cells (EC) from distinct anatomical sources. For this propose, primary pulmonary artery endothelial cell (HPAEC) and primary pooled umbilical vein endothelial cell (PHUVEC) lineages were induced under three distinct conditions: I) 10 ng/mL of TGF-β2; II) ectopic expression of Snail; III) ectopic expression of Snail associated with 10 ng/mL of TGF-β2. The endothelial cells were induced for 48 hours. The mammary epithelial cell (MCF-10A) lineage was used as a control, and was induced under same three conditions. MCF-10A containing 10 ng/mL of TGF-β2 (condition I) and ectopic expression of Snail in MCF-10A (condition II) showed higher gene expression levels of mesenchymal markers and their morphology resembled mesenchymal cells. However, the major difference observed was in ectopic expression of Snail in MCF-10A containing 10 ng/mL de TGF-β2 (condition III). Overexpression of Snail associated with TGF-β2 induced MCF-10A cells to undergo epithelial-mesenchymal transition (EMT), evidenced by the fibroblastoid morphology, downregulation of epithelial markers (E-cadherin), and upregulation of the mesenchymal markers (fibronectin, vimentin, α-SMA, Slug, Zeb1/2). The same results were observed in EndMT using PHUVEC and HPAEC cells induced with ectopic expression of Snail associated with 10 ng/mL of TGF-β2 (condition III). The EC assumed a fibroblast shape and a significant increase ($p<0.05$) of mesenchymal markers (CD90, fibronectin, vimentin, FSP1, α-SMA, Col1A1, and Slug). However, the VE-cadherin was not decreased. Moreover, HPAEC and PHUVEC had the same behavior when induced with ectopic expression of Snail in MCF-10A containing 10 ng/mL de TGF-β2. These results indicate that expression of both Snail and TGF-β2 is required to induce EndMT. Furthermore, our results suggest that the anatomic origin of EC did not alter the process of EndMT using ectopic expression of Snail associated with TGF-β2. Therefore, the overexpression of Snail associated with TGF- β2 is a potent inductor for EndMT and for mesenchymal cell generation.

W-1052
PARACRINE FACTORS SECRETED BY ENDOTHELIAL PROGENITOR CELLS PROMOTE ANGIOGENESIS, MIGRATION AND SURVIVAL OF ENDOTHELIAL CELLS IN VITRO, BUT ARE INSUFFICIENT TO PREVENT PULMONARY ARTERIAL HYPERTENSION

Suen, Colin, Deng, Yupu, Stewart, Duncan
Ottawa Hospital Research Institute, Ottawa, ON, Canada

Background: Endothelial progenitor cells (EPCs) have been shown to be effective in the prevention of pulmonary arterial hypertension (PAH) in preclinical models. Previously, our group has also demonstrated that xenotransplantation of human early EPCs in the rat monocrotaline (MCT) model prevented pulmonary arterial hypertension, despite the near complete loss of human cells from the lung after even 24 hours. Therefore, we hypothesize that the therapeutic effects of EPCs in PAH are mediated by a paracrine mechanism, which in turn, may enhance endothelial repair and integrity. Methods: EPCs were derived from human peripheral blood mononuclear cells obtained via leukapheresis by adherence to fibronectin and growth in endothelial growth medium (EGM-2MV). 24 hour conditioned medium from Day 6 EPCs (EPC-CM) was collected and concentrated by ultrafiltration prior to in vitro and in vivo studies. PAH was induced by a single intraperitoneal injection of (MCT) in nude athymic rats (150-200 g). EPC (5x10^6/kg) or EPC-CM from an equivalent number of cells were administered via jugular vein at day 3 after MCT and hemodynamics and echocardiography were assessed at day 24. Results: EPCs were characterized as such based on expression of CD14, CD31, CD45 by flow cytometry as well as the ability to ingest acetylated LDL and bind UEA-1 lectin. Incubation of human umbilical vein endothelial cells (ECs) with EPC-CM enhanced Matrigel capillary-like network formation by approximately 2-fold compared with serum-free media ($p<0.05$). EPC-CM increased migration in the scratch wound-healing assay compared to control media (4-fold, $p<0.001$). EPC-CM also significantly improved survival of HUVECs after serum deprivation ($p<0.05$) in a dose-dependent manner. Injection of EPCs resulted in decreased right ventricular systolic pressure ($p<0.05$) and improvement in pulmonary artery acceleration time (+28%, $p<0.05$). However, infusion of an equivalent amount of EPC-CM as well as a 10-fold increased dose of EPC-CM failed to prevent PAH. Conclusions: Therefore, EPC-CM enhanced in vitro EC angiogenesis, migration, and survival, consistent with potential of a paracrine mechanism. However, in an in vivo preclinical model, EPC conditioned media did not prevent PAH, suggesting that intact cells act through mechanisms that may involve cell-cell signaling.

W-1053

EMBRYONIC STEM CELLS AND THEIR DERIVATIVE NEURAL PROGENITOR CELLS ENHANCE BLOOD-BRAIN BARRIER PROPERTIES OF MONKEY BRAIN CAPILLARY ENDOTHELIAL CELLS

Tatsumi, Rie[1], Nakagawa, Shinsuke[1], Suzuki, Yutaka[2], Aruga, Jun[1]
[1]Department of Pharmacology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan, [2]Mitsubishi Tanabe Pharma Corporation, Yokohama, Japan

Brain capillary endothelial cells (BECs) have a highly specialized system for regulating the substance trafficking between bloodstream and brain, providing the structural and functional basis of the blood-brain barrier (BBB). The integrity of BBB does not only depend on BECs but also on other neurovascular cell components such as astrocytes, pericytes, perivascular microglia, oligodendroglia and neurons. The cross-talk among these neurovascular cells contributes to induce the highly organized cell-cell adhesion comprising tight junctions (TJs) and adherens junctions (AJs), which act for brain homeostasis by controlling paracellular permeability between BECs. To date, the co-culturing with astrocytes and pericytes has been widely known for the induction of the BBB properties in the primary cultured BECs. In addition, a recent study has reported that neural progenitor cells (NPCs) play important roles in BBB development, maturation and maintenance. Although the underlying mechanism remains unclear, NPCs can differentiate to various brain cells including neurons, astrocytes and oligodendrocytes. Stem cell-derived NPCs are thus possible cell sources for *in vitro* BBB modeling. Here we present embryonic stem cells (ESCs) or their derivative NPCs have a potential to enhance BBB barrier integrity of the cultured BECs, which is one of critical properties of BBB. Briefly, in our experiments, monkey BECs were cultured with the undifferentiated monkey ESCs themselves or the ESC-derived NPCs using the conventional cell culture insert system. After the co-culture, both ESCs and ESC-derived NPCs improved the barrier integrity and TJ organization in the monolayer formed by BECs, as measured by increases in the trans-endothelial electrical resistance (TEER) and decreases in passive permeability (TEER values, 700-900 $\Omega \times cm^2$ for BECs with ESCs or ESC-derived NPCs). Interestingly, these TEER values were relatively higher than those observed in the co-culture with primary astrocytes isolated from rat brains (400-500 $\Omega \times cm^2$), indicating that more effective induction of BBB properties had occurred by the co-culturing BECs with ESCs or ESC-derived NPCs than the approaches reported previously. During the co-culture with BECs, the ESC-derived NPCs (Pax6+, Sox1+) were differentiating into early stage of neurons (beta III tubulin+), and ESC population was also changing their differentiated state from undifferentiated (Nanog+, Oct4+, Sox2+) to neuroectoderm (Sox2+, Pax6+). Taken together, these results raise a possibility that some secreted factors from early neural differentiation processes of ESCs or NPCs are involved in the induction of BBB properties. Thus, ESCs or NPCs would provide us a new approach to obtain improved BBB properties of the *in vitro* BBB model. Further analysis is in progress to clarify the molecular basis of NPC/ESC-mediated BBB functional enhancement.

W-1054

EPIGENETIC PROGRAMMING DURING ENDOTHELIAL DIFFERENTIATION: NOVEL ROLES OF INNATE IMMUNITY

Tian, Xiaoyu[1], Wong, Wing Tak[1], Cooke, John P.[2]
[1]Cardiovascular Sciences, Houston Methodist Research Institute, Houston, TX, USA, [2]Center for Cardiovascular Regeneration, Houston Methodist Research Institute, Houston, TX, USA

We aim to discover novel epigenetic modifiers critical for endothelial cell differentiation from pluripotent stem cells and its regulation by NF-kB signaling, in order to generate functional endothelial cells with high efficiency and fidelity. These cells could then be used for therapeutic angiogenesis for ischemic injuries such as coronary heart disease and peripheral arterial disease. Our current understanding of the regulation of vascular development and differentiation by transcription factors and epigenetic modifiers is still limited. In the present study, we use a heterokaryon model (interspecies cell-to-cell fusion) as a discovery tool in which endothelial cells instruct pluripotent stem cells to activate an endothelial gene profile, which recapitulates the endothelial differentiation. We performed RNA-seq on the stem cell transcriptome in the heterokaryon model and found several previously unrecognized regulators, which might be important for endothelial differentiation. In this work, we have first identified critical innate immune signaling responses induced in the heterokaryon and examined whether this activation of innate immunity is responsible for the rapid and robust induction of endothelial genes from stem cells. Second, we applied this knowledge to better understand the influence of innate immune signaling on the commitment of stem cells to endothelial differentiation by loss- and gain-of-function studies, in the hope of identifying novel endothelial transcription factors regulated by NF-kB. Finally, based on the candidate genes (epigenetic modifiers) we identified from heterokaryon RNA-seq, we examined their potential function on the regulation of endothelial phenotype through histone demethylation to remove the repressive histone mark on endothelial gene promoters to facilitate the transcriptional activation by endothelial transcription factors. Our data provides a systematic and mechanistic approach by employing multiple methods to identify key regulators for endothelial differentiation, which will provide insights into formulating methods for directed endothelial differentiation from pluripotent stem cells for therapeutic angiogenesis.

HEMATOPOIETIC CELLS

W-1055

MOLECULAR CLASSIFICATION OF BONE MARROW FAILURE SYNDROME: ANALYSIS OF PROTEIN EXPRESSION PATTERNS OF HEMATOPOIETIC STEM CELLS DERIVED FROM PLASTIC ANEMIA, HYPOPLASTIC MYELODYSPLASTIC SYNDROME AND PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS

Alaiya, Ayodele, Shinwari, Zakia, Owaidah, Tarek, Al Mohareb, Fahad, Al Sharif, Fahad, Al Zahrani, Hazzaa, Al Nounou, Randa, Syed, Osman Ahmed, Chaudhri, Naeem, Al Jurf, Mahmoud
King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

Background/Purpose: Bone marrow failure syndrome is an example of disease entity where accurate diagnosis of Aplastic Anemia (AA), Paroxysmal Nocturnal Hemoglobinuria (PNH) and Hypoplastic Myelodysplastic Syndrome (MDS) is difficult. The aim of this study was to analyze global protein expression profiles of hematopoietic stem cells derived rom bone marrow samples from

patients diagnosed with subtypes of bone marrow failure syndromes. Methodology: We have analyzed global protein expression profiles of bone marrow and serum, samples from 20 patients with newly diagnosed AA/MDS/PNH and normal BM samples using the classical expression proteomics platforms (2-DE and label free quantitative liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The goal was to identify potential disease-specific /disease-associated proteins biomarkers for accurate differential diagnosis of diseases of bone marrow failure and better prediction of disease prognosis. Results: A higher similarity in protein expression profiles between pairs of AA vs. MDS was observed compared with pairs of AA/PNH and MDS/PNH. Approximately 50% of all resolved protein spots are common to all the three disease entities. However, the fraction of spots that are uniquely expressed in single disease entity is very small. We identified a panel of 13 differentially expressed proteins (> 2- ∞- fold change, $p < 0.001$) between AA and MDS (Table 1). Six (6) of the 13 identified proteins were filtered and mapped as potential biomarkers using Ingenuity Pathway Analysis. Our data indicates that multivariate analysis of quantitative proteome data can potentially be useful as a means of discovery of disease related or disease specific biomarkers for bone marrow syndromes. Conclusions: We conclude that label-free quantitation proteomics can, objectively be used for discovery of disease related or disease specific for bone marrow failure syndromes patients, thus opening the possibility that validated studies can lead to the identification of clinically useful biomarkers.

W-1056
ENHANCEMENT OF FUNCTIONAL ERYTHROID DIFFERENTIATION AND ENUCLEATION OF HUMAN HEMATOPOIETIC STEM CELLS ON POSITIVELY CHARGED POLY-L-LYSINE SUBSTRATE

Park, Kwang-Sook[1], Ahn, Jongchan[1], Kim, Ji Yeon[2], Kim, Hyun Ok[2], Kim, Jin-Su[1], Arai, Yoshie[1], Moon, Bo Kyung[1], Park, Hansoo[3], Lee, Soo-Hong[1]

[1]CHA University, Seongnam-si, Republic of Korea, [2]Yonsei University, Seoul, Republic of Korea, [3]Chung-Ang University, Seoul, Republic of Korea

Hematopoietic stem cells (HSCs) are continuously stimulated by physical interactions with bone marrow or umbilical cord niches, and chemical factors found within these niches. The niche can be mimicked by modification of the cytokine composition, elasticity, topography, and/or charge. This work employed positively charged cell culture plates coated with several concentrations of poly-l-lysine (PLL). The PLL coated plate significantly increased the total number of differentiating erythroid cells during erythroid differentiation of CD34+ cells compared to non-coated plate. Furthermore, the PLL stimulated enucleation of erythroid cells resulting in an increase in the number of erythrocytes. Interestingly, PLL substrate enhanced oxygen-carry ability of erythroid cells compared to even peripheral blood. Thus, PLL would be a useful material to enhance the production of functional erythroid cells from human HSCs in vitro.

W-1057
REDUCED ACTIVITY OF GROWTH-FACTOR ACTIVATED HUMAN SHORT-TERM HEMATOPOIETIC REPOPULATING CELLS TRANSPLANTED INTO IMMUNODEFICIENT MICE

Miller, Paul Harry, Rabu, Gabrielle, Knapp, David JHF, Beer, Philip A., Wei, Lisa, Humphries, R. Keith, Eaves, Connie

Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Transplantation of hematopoietic cells is now a critical component of treatments used to cure a range of malignancies and congenital disorders. Cells with rapid but short-term repopulating ability (STRCs) are the main source of early neutrophils and platelets post-transplant, and their relative under-representation in human cord blood (CB) explains the clinical inadequacy of single CB collections for adult patients. Extensive ex vivo expansion of hematopoietic progenitors can be readily achieved with currently available growth factors and culture conditions, but these expanded populations do not significantly accelerate neutrophil and platelet recoveries when transplanted. To investigate the mechanism underlying this apparent deficiency, we have used NOD/SCID-IL-2Rγc-null mice constitutively producing human IL-3, GM-CSF and SF (NSG-3GS) as an in vivo recipient model for assessing STRCs derived from cultured human CD34+ CB cells. Initial experiments showed that 7-day serum-free cultures supplemented with human insulin, FLT3-L, SF, IL-6, IL-3 and G-CSF (6 GFs) contained 10-fold more in vitro colony forming cells (CFCs) than the input cells but the rapid (week 3) granulopoietic repopulating activity of the cells in these cultures was reduced 10-fold. Similar (5-fold) reductions in this early in vivo repopulating activity were observed when the culture period was shortened to 24 hours, but not if all the GFs except insulin were omitted from the medium or in the presence or absence of insulin in medium supplemented with 2% FCS. We also found that the inhibitory effect of the 5 GFs could be replicated by SF+FLT3L, or G-CSF+IL6, or IL3 only - strongly suggesting that all of these GFs activate a common pathway to mediate this effect. In a next series of experiments, we showed that this effect on rapid neutrophil recovery was mirrored by the numbers of fresh and cultured (24 hours in 6 GFs) CB CFCs that could seed the bone marrow (BM) of NSG mice 16-24 hours after being injected IV and this latter effect was also mimicked by similar GF treatment of several human leukemia-derived cell lines (AML5, HL60, and KG1 with a 3.5 to 10-fold GF-induced reduction in BM seeding efficiency). In fact, even when the GF concentrations were decreased 1000-fold, the BM seeding efficiency of AML5 cells remained at 50% of that obtained when the same cells were cultured in the absence of added GFs. Preliminary phosphoflow analyses to compare the 30 minute signalling responses of CB CD34+CD45RA- and AML5 cells to insulin vs IL-3 have identified pSTAT5 as a candidate differentially responsive target. In summary, exposure to several growth factors (but not insulin) rapidly induces an engraftment defect in human STRCs as well as several human leukemia cell lines. Comparison of the molecular changes invoked by the 5 GFs versus insulin should lead to an improved understanding of the defective engraftment of cultured cells, and thereby enable expansion strategies that can generate large numbers of functional STRCs for clinical use.

W-1058
HUMAN MESENCHYMAL STEM CELLS DOWNREGULATE LPS-INDUCED MYELOID DENDRITIC CELL MATURATION AND CYTOKINE REPONSES

Mun, Chin Hee, Shin, Yong Dae, Kyu-Hyung, Park, Lee, Sang-Won, Lee, Soo-Kon, Park, Yong-Beom

Department of Internal Medicine, Division of Rheumatology, Yonsei University College of Medicine, Seoul, Republic of Korea

Background: Mesenchymal stem cells (MSCs) have profound immunomodulatory properties. Using their properties, MSCs-based therapies have been applied in several inflammatory diseases. The immune modulation of MSCs is related to inhibition of immune cell proliferation and activation. Dendritic cells (DCs) play pivotal roles in initiating immune response as DCs are important antigen-presenting cells. DCs are able to mature into inflammatory DCs, sustaining a continuous activation of adaptive immune system. As the effects of MSCs on DCs have been not fully understood. We investigated

the immunomodulatory property of MSCs on DCs. Methods: Bone marrow-derived CD11c+ mononuclear cells of DBA/1 mice were differentiated cultured with GM-CSF and IL-4, to immature DCs (iDCs) for 7 days. The iDCs were maturated using lipopolysaccharides (LPS). We co-cultured human bone marrow-derived MSCs (BM-MSCs) and mouse DCs directly with 1:1 and 1:10 ratio. The effect of BM-MSCs on DC maturation was assessed by maturation markers by flow cytometry, and supernatants for induced production of cytokines. Results: The expressions of CD86 and MHCII were highly induced under LPS (100 ng/ml) treatment. Human BM-MSCs significantly inhibited the DCs maturation by decreasing CD86 and MHCII expressions in both 1:1 and 1:10 co-culture ratio. And, human BM-MSCs decreased the levels of IL-12p70 and TNF-α in both 1:1 and 1:10 co-culture ratio, and decreased the level of IL-6 effectively in 1:1 ratio. Conclusion: Our data showed that human BM-MSCs inhibit the maturation of DCs in in vitro. This study was supported by a grant of the Korean Health Technology R and D Project, Ministry of Health and Welfare, Republic of Korea. (HI13C1270)

W-1059
THE ROLE OF HISTONE METHYLTRANSFERASE EHMT2/ G9A IN ACUTE MYELOID LEUKEMIA PROGRESSION

Nguyen, Phuong Ha[1], Lehnertz, Bernhard J.[1], Yi, Lin[1], Jin, Jian[2], Humphries, Keith[1], Rossi, Fabio M.V.[1]
[1]The University of British Columbia, Vancouver, BC, Canada, [2]UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC, USA

Posttranslational modifications of chromatin are central to the regulation of many chromosomal functions and are intimately tied to transcriptional regulation. The histone methyltransferase (HMTase) G9a, encoded in human by the EHMT2 gene, is responsible for methylation of lysine 9 of histone H3 (H3K9me). This modification is commonly associated with gene repression but G9a can also activate transcription at least in part by acting as a cofactor for the Mediator complex. Despite the importance of this histone methyltransferase to gene expression and development, the cohort of genes regulated by G9a has not been reported and its functions in lineage specification of adult cells is still not fully understood. Recently, we have identified a significant role of G9a in the development of acute myeloid leukemia (AML). In mouse model, loss of histone methyltransferase G9a selectively delays disease progression and significantly reduces the leukemic stem cell (LSC) frequency. In fact, the leukemogenic transcription factors HoxA9 was shown to strongly interact with G9a and primary human AML cells are also sensitive to G9a inhibition. These results suggest a therapeutic approach based on inhibiting G9a activity as means to suppress the high proliferation rate and self-renewal of AML cells. Moreover, identifying G9a's target genes is critical to understand the mechanisms underlying G9a's role in AML. Some remaining barriers include the toxicity, instability as well as the high level of degradation of the available G9a inhibitors when exposed to biological fluids. Using a newly-developed lipid nanoparticle delivery system, we hope to overcome these difficulties and achieve effective inhibition of G9a in leukemic cells.

W-1060
ANTI CANCER THERAPY USING TUMOR TARGERTED NATURAL KILLER CELLS DERIVED FROM HUMAN PLURIPOTENT STME CELLS

Ni, Zhenya[1], Hermanson, David[1], Knorr, David[2], Bendzick, Laura[1], Mao, Chao[1], Kaufman, Dan S.[1]
[1]University of Minnesota, Minneapolis, MN, USA, [2]University of Minnesota, Roseville, MN, USA

Natural killer (NK) cells are known to be key components of the innate immune system with the ability to recognize diverse types of tumors and virally-infected targets. NK cells represent an attractive option for adoptive immunotherapy due to their ability to kill target cells in a human leukocyte antigen (HLA) non-restricted manner and without prior sensitization. Generation of NK cells from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) is becoming an unlimited source of cell-based gene/immunotherapy against a variety of tumors and virally-infected diseases. Chimeric antigen receptors (CARs) targeting various tumor/viral-associated antigens have been developed and tested with promising clinical results. Recently, the studies of specific tumor-targeted T cells derived from T-iPSCs showed exciting tumor regression in a xenograft model, independent of HLA restriction. However, the endogenous TCR could still be a problem, potentially causing graft versus host disease or other off-target effects. Here, we hypothesized that the combination of CAR strategy and hESCs/iPSCs would be able to produce "off-the-shelf" NK cells with tumor-targeted properties. Using the Sleeping Beauty transposon system, both hESCs and iPSCs have been genetically engineered to express 3rd generation CARs (including a single chain antibody fragment specific for CD19 antigen, a CD8α hinge region, the transmembrane protein CD28, a co-stimulatory protein 4-1BB, and the activating domain CD3ζ). NK cells can be routinely derived from hESCs/iPSCs with an anti-CD19CAR. These CAR-expressing hESC/iPSC-derived NK cells are phenotypically similar to NK cells derived from unmodified hESCs/iPSCs (hESC-NKs, iPSC-NKs) and NK cells isolated from peripheral blood (PB-NKs). These NK cells are CD56+, CD94+/CD117-, NKp44+, NKp46+, NKG2A+, NKG2D+, and KIR+. In 51Cr release cytotoxicity assays against a variety of CD19+ B lymphoma cells, NK cells derived from either CD19CAR expressing hESCs or iPSCs (CD19CAR-hESC-NKs, CD19CAR-iPSC-NKs) show an enhanced anti-tumor activity compared to NK cells derived from hESCs/iPSCs, even at low effector-to-target ratios. To investigate the anti-tumor activity of CD19CAR-hESC/iPSC-NK cells in vivo, NOD-SCID IL2Rγcnull (NSG) mice were inoculated with the human CD19+ B lymphoma Raji cells expressing firefly luciferase protein. Three days later, when the tumors were confirmed by their bioluminescent signal, anti-CD19CAR-iPSC-NK cells or PB-NK cells were injected i.p. along with IL-2 and IL-15. Tumor burden was evaluated by bioluminescent imaging weekly after NK cell treatment. Tumor progression was significantly delayed in mice treated with either anti-CD19CAR-iPSC-NK cells or in vitro expanded PB-NK cells, compared to tumor-carrying mice. Furthermore, when anti-CD19CAR-iPSC-NK cells were used to treat 018Z (a human B-cell precursor acute lymphoblastic leukemia (ALL) line)-engrafted NSG mice, a significant survival advantage was observed than tumor-carrying mice with no treatment. The bioluminescent imaging also indicated delayed tumor progression in CD19CAR-iPSC-NK cells treated mice. Together, these studies demonstrate engineering hESCs and iPSCs with tumor-specific receptors provides a novel strategy to produce targeted NK cells suitable for immune therapies against refractory malignancies.

W-1061

SPHINGOSINE-1-PHOSPHATE RECEPTOR SIGNALING MEDIATES HEMATOPOIETIC STEM CELL MOBILIZATION THROUGH PERTURBATION OF OPPOSING BONE MARROW NICHE AND VASCULAR CHEMOTACTIC GRADIENTS.

Ogle, Molly E.[1], Segar, Claire[1], Botchwey, Edward[2]
[1]Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA, USA, [2]Georgia Institute of Technology, Atlanta, GA, USA

Hematopoietic stem and progenitor cells (HSCs) are a powerful endogenous cellular resource that can be exploited therapeutically for long-term repopulation of bone marrow in hematological malignancies and novel regenerative and tissue-engineering goals. The egress of these primitive progenitor cells from the bone marrow niche to peripheral blood and engraftment into an extramedullary site in response to injury and inflammation is regulated by complex signaling modules. HSCs are localized to the bone marrow niche in part through antiparallel gradients of stromal derived factor-1 (SDF-1) in the bone marrow and sphingosine-1-phosphate (S1P) in the blood. SDF-1 is a key protein governing HSC niche tethering and S1P is a bioactive lipid signaling molecule with pleiotropic signaling functions including cell adhesion, migration, and homing. Inflammatory injury elevates plasma S1P as an endogenous trafficking cue and therefore the S1P signaling axis presents a promising therapeutic target. The current study examines the hypothesis that S1P receptor signaling alters the competing chemotactic gradients of bone marrow SDF-1 and plasma S1P to control the trafficking of progenitor cells. Our studies demonstrate that systemic antagonism of S1P receptor 3 (S1P3) in mice produces a robust and transient mobilization of HSCs into peripheral blood within 1.5 hours. The plasma isolated from mobilized animals was significantly more chemotactic for HSCs by in vitro migration assay. To determine whether the increased chemotactic potential of the plasma was due to changes in gradients, S1P and SDF concentrations were investigated in plasma and bone marrow by HPLC-MS and ELISA, respectively. Interestingly, S1P concentrations were significantly increased and SDF-1 concentrations decreased in the plasma with S1P3 antagonism. SDF-1 bone marrow concentration was also significantly decreased, while S1P was below the level of detection. S1PR expression was characterized by immunofluorescence and mRNA expression profiles to begin to identify cell types responsible for altering chemotactic gradients in response to S1P receptor signaling. In vitro studies with the OP9 mesenchymal stromal cell line and human endothelial cells supported the finding that SDF-1 secretion is reduced by S1P3 antagonism. Further in vitro studies demonstrate that while HSCs migrate dose dependently toward either S1P or SDF-1 in a transwell assay, HSCs do not migrate from physiological competing gradients of bone marrow SDF-1 concentration ranges toward S1P (500nM). Alteration of the gradient slope by increasing the S1P (1uM) relative to the SDF-1 concentration overcomes "SDF-1 tethering" and increases HSC migration toward the S1P compartment. Taken together, the coordinated changes in S1P and SDF-1 concentrations by S1PR signaling alters the slope of the chemotactic gradients between blood and bone marrow which controls the migration response of HSCs. A better understanding of the endogenous signals that govern HSC trafficking, including the role of opposing gradients of S1P and SDF-1 between the bone marrow and blood, will improve strategies for harnessing the therapeutic potential of this rare cell population. These studies will not only provide insight to the underlying biology of HSC trafficking through the S1P signaling axis, but also will inform further therapeutic applications of S1PR targeting compounds in regenerative medicine research and endogenous tissue engineering approaches.

W-1062

CONNECTIVE TISSUE GROWTH FACTOR (CTGF/CCN2) PRODUCED BY NICHE CELLS IS REQUIRED TO MAINTAIN HEMATOPOIETIC STEM CELLS IN CULTURE.

Vilne, Baiba[1], Istvanffy, Rouzanna[1], Bock, Franziska[1], Schreck, Christina[1], Grziwok, Sandra[1], Prazeres da Costa, Olivia[2], Schiemann, Matthias[2], Peschel, Christian[1], Mewes, Hans Werner[2], **Oostendorp, Robert A.J.**[1]
[1]Klinikum rechts der Isar der TU Munchen, Munchen, Germany, [2]TU Munchen, Munchen, Germany

Hematopoietic stem cells (HSC) are regulated by intrinsic and extrinsic signals. We studied the interactions of HSC (lineage-negative Sca-1+ c-Kit+: LSK) in co-cultures with niche stromal cells. Microarray analyses from cells prior to co-culture and cells sorted separately from the cultures revealed that most changes in gene expression occur within the first 24 hours of co-culture. Interestingly, connective tissue growth factor (Ctgf/Ccn2), was strongly upregulated in both stromal and LSK cells, both on mRNA and protein level. In co-cultures of LSK cells with siCTGF knockdown stromal cells, short-term HSC activity was unchanged, but siCtgf-stromal cells were unable to sustain long-term repopulating ability. This finding indicates that Ctgf in stromal cells is required for maintenance of HSC in culture. To study underlying mechanisms, we studied co-cultures of LSK cells with control and Ctgf-knockdown stroma. In the absence of extrinsic Ctgf, Pten was increased in with a concomittant decrease in phosphorylation of both pS308 and pS473 Akt while Erk phosphorylation was unchanged. Also, canonical Wnt (LRP6, Gsk3b, b-catenin) was inhibited, and Tgfb (Smad2/3) signaling was significantly increased. This resulted in a downregulation of G1 transition, as was exemplified by downregulation of Cyclin D1, upregulation of p27Kip1 and upregulated phosphoryalation of both Rb and p53. Equally interestingly, we found that extrinsic Ctgf deficiency also downregulates induction of Ctgf in LSK cells, suggesting the existence of intrinsic-extrinsic feedback regulation of Ctgf induction. In summary, co-culture of LSK cells with stromal cells results in cellular activation of both stromal cells and LSK cells, involving Akt-, Smad2/3- and b-catenin-mediated signaling pathways. Furthermore, reduced expression of extrinsic Ctgf functionally results in an increased production of myeloid progenitors and decreased long-term repopulating ability of HSC. Our studies give insights how the niche regulates early regenerative responses in hematopoiesis.

W-1063

SCL DETERMINES HEMATOPOIETIC OVER CARDIAC FATE VIA PRIMED ENHANCERS DURING HEMOGENIC ENDOTHELIUM SPECIFICATION

Org, Tonis[1], Duan, Dan[1], Ferrari, Roberto[1], Montel-Hagen, Amelie C.M.[1], Van Handel, Ben[1], Kerenyi, Marc[2], Sasidharan, Rajkumar[1], Fujiwara, Yuko[2], Orkin, Stuart H.[2], Kurdistani, Siavash[3], Mikkola, Hanna K A[1]
[1]MCDB, University of California, Los Angeles, Los Angeles, CA, USA, [2]Boston Children's Hospital, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA, [3]Department of Biological Chemistry, UCLA, Los Angeles, CA, USA

Uncovering the mechanisms directing mesoderm specification to hematopoietic and cardiovascular stem/progenitor cells is important to advance the development of cell-based therapies for blood and heart diseases. The bHLH transcription factor Scl is known as the master regulator of the hematopoietic fate. We discovered that, in addition to its critical function in promoting the establishment of hemogenic endothelium during hematopoietic stem/progenitor cell (HS/PC) development, Scl is also required to

repress cardiomyogenesis. In the absence of Scl, the endothelium in hematopoietic tissues and endocardium in the heart differentiated into beating cardiomyocytes. However, the mechanisms how Scl directs hematopoietic vs. cardiac fate choice have remained unknown. Combining ChIP-sequencing and microarray analysis of Flk1+ mesoderm differentiated from mouse ES cells, we show that Scl binds to both its activated genes, including a broad network of regulators required for hemogenic endothelium and HS/PC development (e.g. Runx1, cMyb, Lyl1, Mef2C, Sox7 etc.), and its repressed genes, including transcriptional regulators of cardiogenesis (e.g. Gata4, Gata6, Myocd, etc.). Repression of cardiac program occurs during a short developmental window through Scl binding to distant enhancers that are primed with enhancer mark histone H3K4me1. In contrast to hematopoietic enhancers that retain Scl binding and H3K4me1 throughout HS/PC development, cardiac enhancers lose H3K4me1 as well as Scl binding. Although there was no evidence of Polycomb involvement in the silencing of the cardiac enhancers in hematopoietic cells, the final silencing of the Scl dependent cardiac genes was accompanied by Ezh2 binding and increase in H3K27me3 mark at the TSS. However, analysis of Scl deficient mesoderm revealed that the recruitment of the Lysine-Specific Histone Demethylase Lsd1 to the H4K3me1 primed enhancers and polycomb to the TSS of cardiac genes in mesoderm can occur independently of Scl, raising the hypothesis that rather than directly recruiting co-repressors, Scl hampers cardiogenesis "passively" by blocking the cardiac enhancers from competing cardiac master regulators. Indeed, comparison of Scl and cardiac transcription factor Gata4 binding sites in mesoderm showed that at least one enhancer in cardiac and hematopoietic genes can be bound by both factors, nominating these enhancers as "master enhancers" that act as the battlefield where the initial fate choice between the competing cell fates is made. Compared to all Scl binding sites, these co-shared "master enhancers" were evolutionarily more conserved and showed higher levels of Scl and Lsd1 binding in mesoderm. These results uncover a dual function for Scl in dictating hematopoietic vs. cardiac fate choice and suggest that lineage-specific bHLH/Gata factors compete for H3K4me1 primed "master enhancers" to establish mutually exclusive cell fates.

W-1064
MICRORNA-29A MAINTAINS HEMATOPOIETIC STEM CELL SELF-RENEWAL

Hu, Wenhuo[1], Dooley, James[2], Chung, Stephen Shiu-Wah[3], Shin, Yusup[1], de Strooper, Bart[4], Liston, Adrian[2], **Park, Christopher Y.**[3]
[1]Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA, [2]VIB and Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium, [3]Pathology, Laboratory Medicine, and Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA, [4]VIB and Center for Human Genetics and Leuven Institute for Neuroscience and Disease, Memorial Sloan Kettering Cancer Center, Leuven, Belgium

MicroRNAs (miRNAs) are important regulators of both embryonic and somatic stem cell self-renewal. We previously showed that ectopic expression of miR-29a, a miRNA highly expressed in HSCs as well as in human acute myeloid leukemia (AML) stem cells, in immature mouse hematopoietic cells is sufficient to induce a myeloproliferative disorder that progresses to AML. During the early phase of this disease, overexpression of miR-29a is sufficient to confer aberrant self-renewal to committed myeloid progenitors, strongly suggesting a role for miR-29a in regulating HSC self-renewal. In order to determine the role of miR-29a in HSC function, we evaluated HSCs from miR-29a/b1 null mice. Homozygous deletion of miR-29a/b1 resulted in reduced bone marrow cellularity and reduced colony forming capacity of hematopoietic stem and progenitor cells (HSPCs). The phenotype was mediated specifically by miR-29a,since miR-29b expression was not significantly altered in HSCs, and reconstitution of miR-29a/b null HSPCs with miR-29a, but not miR-29b, rescued in vitro colony formation defects. miR-29a deficient HSCs exhibited defects in self-renewal in both competitive and non-competitive transplantation assays, and these deficits were associated with increased HSC cell cycling and apoptosis. Gene expression studies of miR-29a deficient HSCs demonstrated widespread gene dysregulation including a number of predicted miR-29a target genes including DNA methylation enzymes (Dnmt3a, -3b) and cell cycle regulators (e.g. Cdk6, Tcl1, Hbp1, Pten). Dnmt3a is a key mediator of miR-29a's function in HSCs, as knockdown of Dnmt3a in miR-29a deficient HSCs resulted in restoration of colony formation, and functional defects in miR-29a deficient HSCs were rescued in the setting of Dnmt3a heterozygosity. Together, these studies demonstrate that miR-29a is an important regulator of HSC function by positively regulating HSC self-renewal through its inhibitory effect on Dnmt3a activity.

W-1065
ELUCIDATION OF NOVEL GENETIC REGULATORS OF HEMATOPOIETIC STEM CELL DEVELOPMENT USING A ZEBRAFISH FORWARD GENETIC SCREEN

Perlin, Julie[1], Carr, Logan A.[1], Tamplin, Owen[1], Zhou, Yi[1], Pan, Weijun[2], Zon, Leonard I.[1]
[1]Stem Cell Program and Division of Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA, [2]Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Shanghai Jiao Tong University School of Medicine, Shanghai, China

Marrow transplantation of hematopoietic stem cells (HSCs) is an important therapy for patients with a variety of anemias, immunodeficiencies, and leukemias. The transplant relies on the homing and engraftment of donor HSCs to the recipient marrow, yet the genetics of such cell mobility are not understood. In development, HSCs arise from the aorta, enter circulation, and then undergo dynamic interactions with associated endothelial cells to form the blood stem cell niche. We have undertaken the first large-scale genetic screen to uncover pathways essential for HSC engraftment into the stem cell niche in the zebrafish. 35 mutants were found that were morphologically normal, but that had specific alterations in the expression of cmyb, a critical hematopoietic transcription factor, in the fetal liver equivalent of the zebrafish, or caudal hematopoietic tissue (CHT). cmyb is a conserved transcription factor expressed in hematopoietic progenitors, which is required for hematopoietic progenitor specification, maintenance, and differentiation. Six mutant lines have been cloned and the mutated genes responsible for the cmyb phenotype participate in cell cycle regulation, cell proliferation, and cell-cell interactions. Six additional mutant lines have been identified with a reduction of hematopoietic stem cells (HSCs) in the CHT. The majority of these lines also have a reduction of T cells in the thymus, a mature hematopoietic organ, as observed by rag1 staining. In order to examine the cellular behavior of the hematopoietic stem cells and to understand the reduction of niche occupation we examined HSCs using a transgenic line that specifically labels blood stem cells. For the first time, we are able to visualize the HSC-endothelial cell dynamics required for niche formation in a panel of genetic mutants affecting HSC development. Transgenic lines labeling HSCs revealed specific engraftment defects. These include fewer HSCs exiting circulation to engraft in the vascular niche, decreased HSC proliferation within the niche, and HSCs that appear unable to leave the niche and reenter circulation to progress to the mature hematopoietic organs. These studies will provide insight into HSC development and hematopoietic

niche formation, and the genes and pathways uncovered may be able to be manipulated to augment therapeutic marrow transplantation.

W-1066

NERVE GROWTH FACTOR PRODUCED BY PERIVASCULAR MESENCHYMAL STROMAL CELLS IS REQUIRED FOR BONE MARROW INNERVATION

Peyer, James G., Murphy, Malea M., Morrison, Sean J.
Children's Research Institute at UT Southwestern, Dallas, TX, USA

Innervation of the bone marrow by sympathetic nerve fibers has been shown to regulate hematopoietic stem cell (HSC) function and hematopoiesis. However, nothing is known about the mechanisms that regulate bone marrow innervation, and the evidence for neural regulation of hematopoiesis came from studies that ablated large portions of the sympathetic nervous system. Sympathetic nerve fibers enter the bone marrow through the nutrient foramen and extend along arteries in the central marrow. These nerve fibers synapse upon mesenchymal stromal cells surrounding arterioles in the central marrow, but not arterioles near the endosteum. HSCs are distributed throughout the bone marrow such that some HSCs are found near nerve fibers but quiescent HSCs overall exhibit little skewing in their distribution toward nerve fibers or innervated arterioles. We identified a subpopulation of Leptin receptor-expressing peri-arteriolar cells in the central marrow that produce Nerve Growth Factor (NGF). Conditional deletion of NGF from these cells using Prx1-Cre eliminated bone marrow innervation. This did not lead to HSC depletion, demonstrating that bone marrow innervation is not required for HSC maintenance. Nerve innervation is thus regulated by NGF expression by peri-arteriolar mesenchymal stromal cells in the central marrow that are not necessarily associated with HSCs or their niches. This suggests that bone marrow innervation regulates HSC function and hematopoiesis through indirect mechanisms that do not generally involve innervation of niche cells themselves.

W-1067

CENTRAL CHOLINERGIC SIGNALS REGULATE HEMATOPOIETIC STEM CELL MOBILIZATION

Pierce, Halley[1], Magnon, Claire[1], Lucas, Daniel[1], Huggins, Matthew[1], Schwartz, Gary[2], Frenette, Paul S.[1]
[1]Cell Biology, Gottesman Stem Cell Institue, Albert Einstein College of Medicine, Bronx, NY, USA, [2]Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY, USA

Hematopoietic stem cells (HSCs) are multipotent self-renewing cells that give rise to the cellular constituents of the blood throughout the lifetime. Transplantation of healthy HSCs is the only curative therapy for many hematologic disorders. Collection of these cells is usually achieved through the administration of the cytokine granulocyte colony-stimulating factor (G-CSF), which mobilizes HSCs from specialized niches within the bone marrow to peripheral blood. However, a subset of patients (e.g. those that have previously received myeloablative therapies) fails to mobilize sufficient numbers of HSCs to peripheral blood for transplantation. A better understanding the mechanisms through which HSCs are mobilized might improve bone marrow transplantation efficiency and may also shed light on how other stem cells migrate. Results from our laboratory have revealed that adrenergic signals from the sympathetic nervous system (SNS) target the HSC niche to elicit mobilization of HSCs after G-CSF, identifying a distinct role for neural stimuli in HSC behavior. Often in mammalian biology the SNS is antagonized by the parasympathetic nervous system, which is characterized primarily by cholinergic nerve fibers that release acetylcholine to effected tissues. Additional studies in our laboratory have shown that the muscarinic receptor type 1 (Chrm1) promotes the invasion and migration of prostate cancer-initiating cells (Science. 2013, Jul 12). This led us to explore the role of cholinergic signaling in HSC migration. We have found that Chrm1-/- mice exhibited a 3.28 fold reduction (p=0.0083) in the number of phenotypic HSCs mobilized into peripheral blood after G-CSF stimulation. Additionaly, when G-CSF mobilized blood was transplanted into irradiated recipients, Chrm1-/- donors displayed a decreased ability to reconstitute irradiated hosts, suggesting lower numbers of mobilized functional HSCs. Notably, Chrm1 was not expressed in HSCs or in various stromal cell populations contributing to HSC niche, including Nestin+ mesenchymal stem cells, osteoblasts, osteocytes, endothelial cells, and macrophages, suggesting that the target of cholinergic activity was located outside of the bone marrow. To test the hypothesis that Chrm1 expression in the brain might regulate HSC migration from the bone marrow, we tested HSC mobilization in mice injected either peripherally (i.p.) and intracerebrally with pirenzepine, a Chrm1 specific antagonist. The chemical characteristics of this drug prevent it from crossing the blood brain barrier, allowing us to distinguish between a peripheral and central effect of the receptor. We found that only mice treated intracerebrally with the Chrm1 antagonist exhibited a reduction in HSC mobilization similar to Chrm1-/- mice. This indicates that cholinergic signals from the central nervous system are required for robust mobilization. Ongoing experiments are investigating the mechanisms through which central Chrm1 signals are transduced to the bone marrow. Identifying the factor that links the CNS to the bone marrow could provide an alternate therapeutic target for enhancing HSC yield by mobilization in both healthy and patients predicted to be poor mobilizers.

W-1068

CHARACTERIZATION OF AML TUMOR GENOMES AND TRANSCRIPTOMES FOR DEVELOPMENT OF EFFECTIVE, TARGETED CANCER TREATMENTS

Pilsworth, Jessica[1], Chiu, Readman[2], Nip, Ka Ming[2], Birol, Inanc[2]
[1]Medical Genetics, The University of British Columbia, Vancouver, BC, Canada, [2]Genome Sciences Centre, Vancouver, BC, Canada

Background:More than 80% of myeloid leukemias are associated with at least one chromosomal rearrangement. These translocations frequently involve genes encoding transcription factors that have roles in hematopoietic lineage development. The resulting chimeric transcripts often play causal roles in tumorigenesis and therefore represent ideal diagnostic and therapeutic targets. We use high-throughput sequencing (HTS) platforms on tumor samples from large patient cohorts and assemble short sequence reads using a scalable de novo assembly tool, ABySS for genomic data and Trans-ABySS for RNA-seq data. Our analysis of resulting contig sequences represents an unbiased approach, which is a crucial feature for identifying and characterizing complex genetic changes and rearrangements in the genome. Results: Here, we report on the development of a clinical pipeline that identifies mutations and variations rapidly and consistently. We have performed de novo genome and transcriptome assemblies of tumor samples (and matched normal, when available) from cancer patients diagnosed with acute myeloid leukemia (AML). For leukemogenesis to occur, two types of mutations are required: 1) a mutation that improves hematopoietic cells' ability to proliferate (class I) and 2) a mutation that prevents the cells from differentiating (class II). Our analysis pipeline consistently identifies both class I and class II mutations, including fusion events between PML and RARA, fusion events between MYH11 and CBFB, ITD events in FLT3, PTD events in MLL, and sequence insertion in NPM1. These events are all clinically relevant markers for AML treatment. Conclusions: This

project will enable clinicians to evaluate the best treatment options for their patients based on genomic profiling. We are proposing that if we have a reliable way of detecting variations and associating them with a particular disease, it will assist in the development of better treatment approaches. Conventionally, clinicians use standard treatment regimes based on a general understanding of a disease, and move to an alternative treatment if patients do not respond. Clinical genomics holds great promises to change that paradigm, where each cancer patient will be evaluated at the genomic level first, results of which will inform clinicians to develop treatment plans on a per patient basis. The reported work offers a bioinformatics pipeline that contributes to this new vision of cancer care.

W-1069
MEGAKARYOCYTES REGULATE HEMATOPOIETIC STEM CELL QUIESCENCE VIA CXCL4 SECRETION

Pinho, Sandra[1], Bruns, Ingmar[1], Lucas, Daniel[1], Ahmed, Jalal[1], Lambert, Michele P.[2], Kunisaki, Yuya[1], Scheiermann, Christoph[1], Ahamed, Jasimuddin[3], Poncz, Mortimer[2], Bergmann, Aviv[4], Frenette, Paul S.[1]

[1]Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, Bronx, NY, USA, [2]Children's Hospital of Philadelphia, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA, [3]Rockefeller University - Laboratory of Blood and Vascular Biology, New York, NY, USA, [4]Department of Systems and Computational Biology, Albert Einstein College of Medicine, Bronx, NY, USA

Hematopoietic stem cells (HSCs) adapt to the varying needs for the replenishment of terminally differentiated blood cells by switching between quiescence, self-renewal and differentiation. In the bone marrow (BM), HSCs reside in specialized niches that regulate their behavior. All putative HSC niche cells have a non-hematopoietic origin. Using three-dimensional whole-mount BM imaging we have uncovered an association between HSCs and megakaryocytes (Mk), in that up to 21% of Lineage-CD41-CD48-CD150+ HSC were in direct contact with Mk compared to ~7% in simulation of random HSC localization. To assess the functional role of Mk in HSC regulation in vivo, we bred Cxcl4-cre mice (in which Cre recombinase is under the control of the Mk-specific CXCL4 promoter, also called platelet factor 4) with iDTR mice (where cre recombination induces expression of the diphtheria toxin receptor). Strikingly, Mk depletion after DT treatment led to a specific loss of HSC quiescence and a 5.5-fold increase ($P<0.001$) in proliferation. This led to a marked (~11-fold, $P<0.001$) expansion of phenotypic Lineage−Sca-1+c-kit+CD105+CD150+ HSC, confirmed by competitive reconstitution assays. Importantly, Mk depletion did not result in aberrant hematopoiesis as reflected by WBC and RBC counts in the peripheral blood and progenitor cell numbers in the BM that were indistinguishable from control mice. To investigate the mechanisms through which Mk regulate HSC, we screened for factors that have been shown to maintain cell quiescence. Of those, CXCL4 itself, which is mainly expressed by Mk, had the greatest reduction in the BM extracellular fluid upon Mk depletion as determined by ELISA. We therefore hypothesized that CXCL4 contributed to Mk-mediated maintenance of HSC. In vitro culture experiments revealed that recombinant CXCL4 restrained HSC proliferation (20% reduction) and this effect was abrogated when CXCL4 was neutralized by heparin. Similarly, in vitro co-cultures confirmed that MK induce HSC quiescence, whereas Cxcl4−/− MK have a reduced capacity to induce HSC quiescence. In vivo CXCL4 injection reduced HSC numbers (by 50%; $P<0.001$) via increased quiescence. By contrast, Cxcl4−/− mice exhibited increased HSC numbers (1.5-fold; $P<0.01$) and proliferation. These results demonstrate a feedback loop where an HSC progeny, the MK, can directly regulate HSC quiescence via CXCL4. Recently we described the non-random association of quiescent HSC and BM arteriolar niches. Thus, we have analyzed the distribution of 260 HSCs relative to MK and arterioles, using computational modeling of observed and random HSC distribution. These analyses revealed that a meaningful fraction of HSC are found in a niche comprised of both arterioles and MK, with 15% of HSCs closer than 25μm to both structures, while only 6% of HSC fell into this configuration in our random simulation. Interestingly, compared to the 7% of randomly placed HSC, 20% of the actual HSC were in close proximity to MK (<25μm) but distant (>100μm) from arterioles, thus suggesting the existence of a distinct Mk niche. In summary, our results show that MK directly regulate HSC quiescence via CXCL4. In addition, our data implicate a role for MK in the arteriolar HSC quiescent niche as well as in a niche distant from arterioles.

W-1070
MIRNOME SCREENING IN HUMAN ERYTHROPOIESIS

Rayabaram, Janakiram[1], Mayuranathan, Thiyagaraj[1], Srivastava, Alok[1], Palakodeti, Dasaradhi[2], **Velayudhan, Shaji R.**[1]

[1]Centre for Stem Cell Research, Christian Medical College, Vellore, India, [2]Institute for Stem Cell Biology and Regenerative Medicine, Bangalore, India

Erythropoiesis, the process of differentiation of hematopoietic stem cells to mature erythrocytes, is highly regulated at both transcriptional and epigenetic levels. Several transcription factors and cofactors have been identified to be involved in the differentiation of haematopoietic stem cells to erythroid cells. Recent studies revealed the potential role of miRNAs during hematopoietic stem cell differentiation and a few miRNAs have been identified to be critical for erythropoiesis and globin gene regulation. Comprehensive analysis of miRNAs involved in human erythropoiesis has been lacking and it is important for the better understanding of the role of these transcriptional regulators in maintenance of haematopoietic stem cells and erythroid lineage determination and erythroid disease pathogenesis. We performed ex-vivo erythroid differentiation of CD34+ haematopoietic stem cells and progenitors (HSPCs) and the RNA obtained from the cultured erythroid cells at five different stages of erythropoiesis were used for small RNA sequencing. Small RNA library was prepared and analyzed in Illumina Hi Seq platform and the data was analyzed by using in-house perl script. Transcription factor binding near the miRNAs was determined using publicly available ChIP-sequencing data in human erythroid cells. We identified 25 miRNAs that are upregulated greater than 10 fold in the cultured erythroid cells compared to undifferentiated HSPCs. Among these, 6 miRNAs have been reported earlier to be involved in mouse and human erythropoiesis. We found 10 upregulated miRNAs whose role in erythropoiesis has not been known so far. These include mir-4732, miR-584, miR-5001, miR-5695, miR-7155, miR-3200, miR-3688, miR-3117 and miR-561 and miR-375. We found strong occupancy of GATA1 or TAL1 in the promoter region (~5kb region upstream) of most of these miRNAs suggesting the erythroid cell specific role of these miRNAs. We found 23 miRNAs that were downregulated greater than 20 fold during ex-vivo erythropoiesis. Out of these, miR-155, miR-221, miR- 222 and miR-145, have been shown earlier to have critical roles in erythropoiesis and the molecular basis through which they regulate erythropoiesis have been well studied. The roles of other downregulated miRNAs in erythropoiesis are not yet known. Target prediction analysis of these miRNAs on miRDB indicated the potential miRNA targets associated with erythroid compartment, especially the membrane proteins. Other targets include epigenetic factors including TET1 and haematopoietic regulatory proteins like EPO, GATA1 and LIN28. Interestingly, we found several miRNA

clusters in which all the miRNAs are co-regulated in erythropoiesis. It has been previously shown that miR-144 and miR-451 present in a cluster and they are upregulated during erythropoiesis and we identified miRNA-4732 present in the vicinity of miR-144 and miR-451 and it also showed >600 fold increase in erythroid cells suggesting that all these three miRNAs form an erythroid specific cluster. Our results suggest that ex-vivo erythropoiesis is a valid tool to study the transcriptional regulation of human erythropoiesis. We identified several miRNAs which are differentially expressed in haematopoietic stem cells and erythroid cells and there are several miRNA clusters that are transcriptionally co-regulated in these cells. This study helped us to identify the miRNAs that are required for haematopoietic stem cell maintenance and erythropoiesis.

W-1071
PHARMACOLOGICAL MOBILISATION OF MURINE MESENCHYMAL STEM CELLS: IDENTIFYING POTENTIAL MECHANISMS

Redpath, Andia Nicole, Fellous, Tariq G., Rankin, Sara M.
Leukocyte Biology, Imperial College London, London, United Kingdom

Mesenchymal stem cells (MSCs) expanded *ex vivo* display hypoimmunogenicity, immunomodulatory and regenerative properties in addition to the ability to home to areas of tissue damage and inflammation. Due to these beneficial properties MSCs are prominently used in cell-based therapies. Our group focuses on understanding endogenous MSC properties, as such characterisation is currently in its infancy; with the aim to manipulate MSCs *in vivo.* We have thus initiated an investigation into pharmacological mobilisation of endogenous MSCs as a novel therapeutic strategy. We have previously demonstrated that treatment of BALB/c mice with Vascular Endothelial Growth Factor (VEGF) in combination with the CXCR-4 antagonist, AMD3100, induced mobilisation of MSCs from the bone marrow (BM) to the peripheral blood. The aim of this study was to identify potential mechanisms involved in this mobilisation regime. We hypothesised that VEGF may be acting on MSCs directly, priming them for migration upon exposure to the SDF-1/CXCR-4 axis induced by AMD3100. At first, we investigated the direct effect of VEGF on MSCs *in vitro* as an attempt to elucidate which VEGF receptor (VEGFR) signalling would induce downstream mediators involved in MSC migration - however VEGFR-1 and -2 surface expression was downregulated with culture. Further limitations discontinued the examination of these downstream mediators which led us back to investigate our model of mobilisation *in vivo* by applying principles surrounding haematopoietic stem/progenitor cell (HSPC) mobilisation. We therefore explored the widely researched HSPC mobilising agent Granulocyte-Colony Stimulating Factor, G-CSF, in parallel to our VEGF regime. Unlike VEGF, G-CSF does not induce MSC mobilisation when quantified by Fibroblast Colony Forming Unit (CFU-F) assays. Flow cytometry analysis of Ki67 expression revealed that whilst VEGF had no effect on the proliferation of MSCs *in vivo*, G-CSF decreased proliferation of BM MSCs from 43% to 16%. Further, we investigated whether VEGF has an indirect role on MSCs *in vivo* by affecting BM resident macrophages (MΦs) - a cell type which was reported to interact with MSCs and coordinate MSC-derived SDF-1. SDF-1 is known to contribute to the BM stem cell retention system. Clodronate liposomes were administered in mice (10µl/g i.v.) at day 0; a 98.6% decrease in the BM MΦ population was confirmed at 24hrs post administration and remained depleted by day 5. Consistent with previous reports, we confirmed that MΦ depletion induces HSPC mobilisation and significantly enhances AMD3100-induced HSPC mobilisation. In contrast, we showed that MΦ depletion attenuated mobilisation of MSCs by the VEGF/AMD3100 regime. There was an 11-fold decrease in the number of CFU-Fs/ml of blood in these mice, suggesting that distinct mechanisms regulate HSPC and MSC mobilisation. We conclude that the presence of BM MΦs is important for MSC mobilisation following pharmacological treatment. These findings provide insight into potential mechanisms involved, with the aim to ultimately optimise the MSC mobilising regime as an alternative or accessory to exogenous MSC therapy. We are currently assessing the effect of treatments to the niche microenvironment, its resident cells and the SDF-1/CXCR-4 axis.

W-1072
MUSASHI2 REGULATES PRIMITIVE HUMAN HEMATOPOIETIC CELL SELF-RENEWAL

Rentas, Stefan, Belew, Muluken, Holzapfel, Nicholas, Hope, Kristin
Stem Cell and Cancer Research Institute, McMaster University, Hamilton, ON, Canada

The RNA binding protein Musashi2 (Msi2) has recently been shown to regulate murine hematopoietic stem cell (HSC) activity; however, it remains unknown if this is conserved in humans. At the transcript level we show that MSI2 is highly expressed in human HSCs and decreases as cells lose self-renewal potential. Knockdown of MSI2 had minimal effects on colony forming cell capability but instead resulted in an almost complete deficiency in the capacity of human HSCs to provide long-term reconstitution to immunodeficient mice. Importantly, lentiviral overexpression of MSI2 led to a pro-self renewal phenotype resulting in a significant increase in the replating capacity of multipotential colony forming cells. Furthermore, ectopic expression provided a significant expansion of total colony forming cells in short-term in vitro cultures and increased the number of short-term reconstituting cells as measured at early post-transplant time points. Altogether, this work demonstrates for the first time that MSI2 is an essential positive regulator of primitive human hematopoietic cell activity, impacting directly on their self-renewal capacity.

W-1073
INFORMING DERIVATION OF HEMATOPOIETIC STEM CELLS FROM EMBRYONIC STEM CELLS VIA COMPARATIVE PROFILING OF THE WNT PATHWAY IN HEMATOPOIETIC DEVELOPMENT

Richter, Jenna
Cellular and Molecular Medicine, University of California, San Diego, CA, USA

Hematopoietic stem cells (HSCs) are the adult stem cells that give rise to all terminally differentiated blood cells. The restorative properties of these cells thus underlie the efficacy of hematopoietic cell transplantation (HCT), which can cure many hematopoietic diseases, including leukemia, lymphoma, and multiple myeloma. Human pluripotent stem cells (hPSCs) are a potential source to derive patient-matched HSCs, however, to date, definitive HSCs suitable for HCT have not been generated. To gain a better understanding of HSC development, we are using zebrafish where HSC specification, emergence, expansion and maintenance can be observed in real time and perturbed using various methods. Consistent with several published studies, we have found that activation or inhibition of the Wnt signaling pathway in zebrafish embryos leads to increases or decreases in HSC numbers, respectively. To further characterize the role of Wnt signaling in hematopoietic development we have profiled the expression of Wnt ligands, receptors (encoded by the Fzd gene family), and coreceptors (encoded by select LRP genes) in the developing zebrafish embryo at critical stages of HSC specification, emergence, expansion, and maintenance. From this analysis, we have selected Wnts, Fzds, and LRP coreceptors that are

potentially involved in HSC development. We are currently performing overexpression and knockdown experiments to establish a functional link between these Wnt signaling components and HSC development. In parallel, we are analyzing expression of Wnt pathway components as hPSCs differentiate into cells with hematopoietic characteristics. To facilitate these experiments, we are using hPSC lines harboring reporter elements (SOX17-GFP and RUNX1c-GFP) that become activated in HSC precursor cell populations. Comparative analysis of Wnt signaling in the developing zebrafish and differentiating hPSCs will yield new insights into the molecular mechanisms underlying HSC development and will inform future strategies to derive HSCs from hPSCs.

W-1074
CYTOKINE-REGULATED GADD45G INDUCES DIFFERENTIATION AND LINEAGE SELECTION IN HEMATOPOIETIC STEM CELLS

Thalheimer, Frederic B.[1], Wingert, Susanne[1], De Giacomo, Pangrazio[2], Haetscher, Nadine[1], Brill, Boris[2], Theis, Fabian[3], Hennighausen, Lothar[4], Schroeder, Timm T.[5], **Rieger, Michael A.**[1]

[1]LOEWE Center for Cell and Gene Therapy, Goethe University Frankfurt, Frankfurt (Main), Germany, [2]Georg-Speyer-Haus, Frankfurt (Main), Germany, [3]Institute of Bioinformatics, Helmholtz Center Munich, Munich, Germany, [4]Laboratory of Genetics and Physiology, NIDDK, National Institutes of Health, Bethesda, MD, USA, [5]Swiss Federal Institute of Technology (ETH) Zürich, Basel, Switzerland

The balance of self-renewal and differentiation in long-term repopulating hematopoietic stem cells (LT-HSC) must be strictly controlled to maintain blood homeostasis and to prevent leukemogenesis. Hematopoietic cytokines can induce differentiation in LT-HSCs, however, the molecular mechanism orchestrating this delicate balance requires further elucidation. We identified the tumor suppressor Growth Arrest and DNA-Damage-inducible 45 gamma (GADD45G) as an instructor of murine LT-HSC differentiation under the control of differentiation-promoting cytokine receptor signalling. GADD45G immediately induces and accelerates differentiation in LT-HSCs, and overrides the self-renewal program by specifically activating MAP3K4-mediated MAPK p38. Conversely, the absence of GADD45G enhances the self-renewal potential of LT-HSCs in Gadd45g knock-out mice. Long-term time-lapse microscopy-based cell tracking of single LT-HSCs and their progeny revealed that, once GADD45G is expressed, the development of LT-HSCs into lineage-committed progeny occurred within 36h, and uncovered a selective lineage choice with a severe reduction in megakaryocytic-erythroid cells. Here we report an unrecognized role of GADD45G as a central molecular linker of extrinsic cytokine differentiation and lineage choice control in hematopoiesis.

W-1075
HYPERCHOLESTEROLEMIA INCREASES GENOTOXICITY OF HEMATOPOIETIC STEM CELLS IN APOLIPOPROTEIN E-DEFICIENT MICE

Rodrigues, Bianca P.[1], Porto, Marcella L.[1], Bezerra, Geisiane S.[1], Campagnaro, Bianca Prandi[2], Meyrelles, Silvana S.[1], Vasquez, Elisardo C.[3]

[1]Graduate Program of Physiological Sciences, Federal University of Espirito Santo, Vitória, Brazil, [2]Pharmaceutical Sciences Graduate Program, University of Vila Velha, Vila Velha, Brazil, [3]Emescam Health Sciences, Vitória, Brazil

Background: A decline in tissue stem cell functionality may be a key component in cardiovascular disease. It is well known that hypercholesterolemia contributes to the development of atherosclerosis and can enhance the DNA damage leading to apoptosis. Therefore, the aim of this study was to evaluate how hypercholesterolemia affect bone marrow hematopoietic stem cell (HSC) function in mice. Methods: Two month-old male mice (n= 26) were divided into C57 and apoE$^{-/-}$ groups. Mice were euthanized and bone marrow was flushed out of tibias and femurs, mononuclear cells were placed on culture. Hematopoietic stem cells (HSC) were obtained by immunomagnetic depletion of non-adherent cells. Briefly, HSC were incubated with Lineage antibody cocktail (CD3e, CD11b, CD45R/B220, LY-6G and LY-6C, TER-119/Erythroid) conjugated with magnetic beads and subjected to the BD IMag magnetic column. HSC^{Lin-} were stained with antibody against Sca-1 (stem cell antigen), CD 90.2, CD 133, CD 117 or matched isotype control. Reactive oxygen species production was evaluated by flow cytometry with the fluorescent dye dihydroethidium (DHE). The genotoxicity analysis was performed using the alkaline comet assay to obtain from individual cells, an array with a fluorescent halo in the format of a comet corresponding to the amount of the fragmented DNA. The DNA helices ruptures extensions were evaluated by the image intensification method, where 50 cells were randomly selected (25 in each slide) and analyzed using the software CASP. The measurements of the comets were obtained by the parameters of the percentage tail DNA and tail moment. For apoptosis detection, HSC were resuspended in Binding Buffer and incubated with Annexin V-FITC and propidium iodide (PI). Data by flow cytometry were acquired and analyzed using BD FACSDiva and FCS Express 4 softwares. Plasma cholesterol was also measured. Data are mean±SEM. Statistical analysis was performed using Student´s *t* test. Results: Flow cytometric analysis showed an increase in reactive oxygen species production, but statistically difference was not found. Thus, HSC from apoE$^{-/-}$ mice showed a 3-fold augmented DNA fragmentation compared with C57 in both parameters, percentage tail DNA (C57: 20 ± 5; apoE$^{-/-}$: 51 ± 0.2, $p<0.05$) and tail moment (C57: 26 ± 9; apoE$^{-/-}$: 73 ± 3, $p<0.05$). Apoptosis was also increased with hypercholesterolemia (C57: 5 ± 0.8; apoE$^{-/-}$: 9 ± 2.1, $p<0.05$). Conclusion: Our data shows that hypercholesterolemia increases DNA fragmentation and apoptosis can impair HSC function. Such findings contribute to new insights into the mechanisms by which high levels of total cholesterol can alter cells involved in maintaining of the immune system. Financial Support: Bioclin, CNPq, CAPES, FACITEC, FAPES-PRONEX.

W-1076
EXPERIMENTAL MODEL TO EVALUATE THE EFFECT OF MESENCHYMAL STEM CELLS ON HEMATOPOIETIC STEM CELLS SELF-RENEWAL

Rodriguez-Pardo, Viviana Marcela[1], Jaimes-Leal, Diana[1], Barreto-Prieto, Alfonso[1], Jiménez-Borrego, Camilo[1], Vernot-Hernandez, Jean Paul[2]

[1]Pontificia Universidad Javeriana, Bogota D.C., Colombia, [2]Universidad Nacional de Colombia, Bogota D.C., Colombia

The self-renewal in stem cells is associated with symmetrical or asymmetrical division models that occur in response to regeneration processes or maintenance of tissue homeostasis. It has been shown that the microenvironment can influence division pattern of stem cells and contact with other cell populations and promote asymmetric proliferation associated with differentiation or instead a symmetrical division that aims for conservation multipotent features. In the model of hematopoiesis, bone marrow mesenchymal stem cells (BM-MSC) are involved in the regulation of different physiological processes of the hematopoietic stem cells (HSC); however, is unclear of the role of the BM-MSC in the modulation of proliferation associated with self-renewal HSC. In addition, there is not in vitro model to

evaluate this phenomenon directly. In this work, we have isolated BM-MSC from human bone marrow (hBM) and have studied their ability to induce symmetric or asymmetric proliferation in human umbilical cord blood-derived HSC (hUCB-HSC). hBM-MSC were isolated and characterized by morphologic and immunophenotypic criteria (CD34-/CD45-/CD73+/CD105+) and by their ability to differentiate into osteoblasts, chondrocytes and adipocytes in suitable conditions. hBM-MSC after passage 3 were used as a feeding-layer to expand hUCB-HSC (CD34+) cells in the presence or absence of thrombopoietin (TPO), Flt3-L and stem cell factor (SCF) (50ng/ml) on 7 days. After that, both experimental conditions were evaluated by confocal microscopy (Olympus FV1000) using monoclonal antibodies to CD34 antigen, α-tubulin and nucleus stained DAPI with Olympus Fluoview® software. Contact pictures between metaphase hUCB-HSC and hBM-MSC (n = 120, 3D images with X, Y, and Z coordinates per image) were obtained and a mathematical model of vector algebra is used to evaluate the angle of the vector between the centrosomes of metaphase hUCB-HSC regarding contact hBM-MSC. We established that contact between hUCB-HSC and hBM-MSC (no exogenous addition of cytokines) induces angles (50o to 70o) lower than with the addition of cytokines (between 87o and 93o) ($p<0.05$). This indicates that the contact between these cellular populations induces a parallel location of the hematopoietic cells relative to the hBM-MSC which can be associated with a symmetric proliferation, while in the co-culture with hBM-MSC and cytokines, the angle of inclination of the hUCB-HSC was more perpendicular what might be related to a model of asymmetric proliferation. This work proposes a new strategy for evaluating phenomena of UCB-HSC self-renewal in physical contact with cells of the marrow microenvironment. These results demonstrate that only contact between UCB-HSC and hBM-MSC (cell microenvironment population) promotes symmetrical self-renewal of these hematopoietic cells may be useful in models of tissue regeneration.

W-1077
HEMATOPOIETIC STEM CELL ACTIVATION UPON STRESS CONDITIONS CRITICALLY DEPENDS ON CDK6

Scheicher, Ruth Maria, Sexl, Veronika
Institute of Pharmacology and Toxicology, University of Veterinary Medicine, Vienna, Austria

The kinases CDK6 and CDK4 exert several redundant functions in regulating cell-cycle progression. Cdk6-/- mice are viable and display only minor defects in hematopoiesis under homeostatic conditions, where the absence of CDK6 is presumably compensated for by its homologue CDK4. Recently, we demonstrated a key role for CDK6 in lymphoid tumor formation where CDK6 transcriptionally regulates Vegf-A and p16INK4a. We here describe a novel role for CDK6 in hematopoietic stem cell (HSC) activation upon stress that goes beyond its function as a cell-cycle regulator. HSC homeostasis requires the precise regulation of cell proliferation as the maintenance of long-term repopulation capacity is crucial for the retained ability to produce blood cells. Dormant hematopoietic stem cells represent the reservoir for hematopoiesis, ready to be rapidly activated when needed. Under homeostatic conditions HSCs rarely divide and remain quiescent in the G0-phase of the cell-cycle. Under stress conditions dormant HSCs enter the cell-cycle, leading to a rapid increase in multipotent progenitor (MPP) numbers that subsequently differentiate into distinct mature hematopoietic cells. The transition of d-HSCs from quiescence to cycling is tightly controlled by a network of transcription factors. The details of maintaining this delicate balance of HSC quiescence and proliferation are still only partially understood. We here show that Cdk6-/- HSCs cannot efficiently repopulate recipient mice in competitive and serial transplant assays and that Cdk6-/- mice are significantly more susceptible to the chemotherapeutic drug 5-fluorouracil. When dormant HSCs proliferate in response to interferon signalling significantly fewer Cdk6-/- HSCs exit the quiescent state. These results define CDK6 as a crucial player in HSC activation required to allow for hematopoietic recovery after stress. Molecular alterations triggered by the novel transcriptional activity of CDK6 required for stem cell quiescence and homeostasis will be presented.

W-1078
HEMATOPOIETIC STEM CELLS REQUIRE HIGHLY REGULATED PROTEOSTASIS MECHANISMS

Signer, Robert A.J.[1], Magee, Jeffrey[2], Morrison, Sean J.[1]
[1]Children's Research Institute at UT Southwestern, Dallas, TX, USA, [2]Washington University, St. Louis, MO, USA

Proteostasis is crucial for maintaining cellular homeostasis and healthy aging. The network of biological pathways that coordinate proteostasis include protein synthesis, protein folding and protein degradation. However, little is known about how these processes are regulated in somatic stem cells and whether proteostasis mechanisms affect stem cell function. We quantified protein synthesis and proteasome activity in hematopoietic stem, progenitor and differentiated cells. We found that the amount of protein synthesized per hour in hematopoietic stem cells (HSCs) in vivo was lower than in most other hematopoietic cells, even if we controlled for differences in cell cycle status or forced HSCs to undergo self-renewing divisions. We also found that proteasome activity was lower in HSCs than in whole bone marrow cells and myeloid progenitors, but was higher than in some lymphoid progenitors. These data suggest that proteostasis mechanisms are highly regulated and differ among hematopoietic cells, raising the possibility that precise regulation of proteostasis mechanisms is essential for maintaining stem cell function. To test this, we examined the consequences of either decreasing or increasing protein synthesis in HSCs using genetic mouse models. Reduced ribosome function in *Rpl24*$^{Bst/+}$ mice reduced protein synthesis in HSCs and impaired HSC function. *Pten* deletion increased protein synthesis in HSCs but also reduced HSC function. *Rpl24*$^{Bst/+}$ cell-autonomously rescued the effects of *Pten* deletion in HSCs, blocking the increase in protein synthesis, restoring HSC function, and delaying leukemogenesis. *Pten* deficiency thus depletes HSCs and promotes leukemia partly by increasing protein synthesis. These data demonstrate that the rate of protein synthesis is tightly regulated in HSCs, and both decreases and increases in translation can impair stem cell function. Since proteostasis regulates aging and lifespan, our data raise the possibility that long-lived stem cells may depend on highly regulated proteostasis mechanisms.

W-1079
MODULATION OF HUMAN HEMATOPOIETIC STEM CELLS IN SEPSIS

Skirecki, Tomasz[1], Zielińska-Borkowska2, Urszula[2], Kawiak, Jerzy[1], Hoser, Grażyna[1]
[1]Laboratory of Flow Cytometry, Centre of Postgraduate Medical Education, Warsaw, Poland, [2]Department of Anesthesiology and Intensive Care, Centre of Postgraduate Medical Education, Warsaw, Poland

Background: Sepsis remains one of the most common cause of deaths in the intensive care units. One of the main disturbances in sepsis is development of immune reprogramming frequently associated with the loss of immune cells. Hematopoietic stem cells (HSCs) are responsible for an efficient replenishment of the immune cells thanks to their ability to self-renew and give rise to differentiating progenitors.

As HSCs express Toll-like receptors and receptors for inflammatory cytokines they may be affected during systemic inflammation present in sepsis. We aimed to investigate the effect of sepsis upon circulating HSCs in patients and in vitro effects of bacterial lipopolysaccharide (LPS) on these cells. Also, a humanized mouse model was applied to analyze human HSCs in the bone marrow(BM) in the models of endotoxemia. Methods: Peripheral blood samples from 20 septic patients were collected. Blood cells were stained with antibodies against CD34, CD38, Ki-67, CD133, Lin and CD45. BM derived CD34+ cells were cultured in hypoxia (1%O2) and the effects of stimulation with LPS on their proliferation (CellTrace Violet), differentiation (appearance of CD38), mitochondrial potential (JC-1), reactive oxygen species (ROS) production (DCFDA) and activation of NF-κB pathway (immunofluorescence detection of p-IκB) were evaluated. Furthermore, a model of endotoxemia (40μg LPS i.v.) on the humanized NSG mice was performed. The BM cells were analyzed 24h after LPS injection by CFU assay with medium supporting growth of human cells. Results: Analysis of blood samples from septic patients demonstrated a 5-fold reduction in the CD34+CD38- HSC count ($p<0.05$) but an increased number of CD34+CD45-Lin- VSELs (2150/ml vs. 180/ml in healthy). The number of CD34+ progenitor cells expressing Ki-67 was increased 4-fold in septic patients ($p<0.05$). Notably, the number of circulating VSELs was significantly higher in non-survivors. The in vitro experiments showed that stimulation with LPS in 30 minutes up-regulated the staining of p-IκB in CD34+ cells. LPS also led to an 1.3-fold increase in the mitochondrial potential of CD34+CD38- cells after 90 minutes of culture. At the same time, the production of ROS in CD34+ cells increased 4-fold, but in the CD34+CD38- subset ROS level remained unchanged. After 9 days of culture LPS enhanced the proliferation of CD34+ cells by 2.3-fold. LPS also reduced the frequency of CD34+CD38- cells by 50%, but the proliferation rate of these cells was unchanged by LPS. Analysis of BM cells from humanized mice that received LPS revealed their significantly decreased potential to form hematopoietic colonies. Conclusions: Sepsis reduces number of circulating HSCs, but increases the number of progenitors that entered cell cycle what can reflect disturbed mechanisms of HSCs self-renewal and homing. Dissimilar kinetics of the VSELs and HSPCs circulation imply their differential trafficking mechanisms. Although the in vitro stimulation of HSCs with LPS seems to promote their survival and proliferation mediated by NF-κB and metabolic alterations, analysis of HSC from BM of endotoxemic humanized mice reveled that their functional capacities may be diminished. Our findings shed light on the impact of sepsis on the HSCs and suggest they play an important role in the host responses to those conditions. Supported by 'Innovative methods of stem cells applications in medicine', EU Innovative Economy Operational Programme, POIG 01.02-00-109/09.

W-1080
IN VIVO PHARMACOLOGIC MODULATION OF BONE MARROW MICROENVIRONMENTAL SIGNALS PROMOTES LONG-TERM HEMATOPOIETIC STEM CELL FUNCTION

Smith, Julianne Noel Petterson[1], Goodman, Alexandra N.[2], Hoffman, Corey M.[3], Calvi, Laura M.[2]

[1]Department of Pathology and Laboratory Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA, [2]Department of Medicine - Endocrine Metabolism, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA, [3]Department of Pharmacology and Physiology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

Osteoblasts (OBs) and the bone marrow (BM) vasculature constitute hematopoietic stem cell (HSC) niches. We previously demonstrated that OB-activation by Parathyroid Hormone (PTH) expands HSCs microenvironmentally. To test if the BM vasculature is also modulated during PTH-induced HSC expansion, we examined effects on BM endothelial structures. Ten days of PTH treatment increased BM microvessels (167±18 vs 348±39 per hindlimb section, n=5 mice per group) and endothelial cells (ECs) (0.09±0.02% vs 0.2±0.02%, n=4-5 mice per group). Furthermore, intravital analysis of the vascular network within calvarial BM revealed greater branching (14±2 vs 27±4 branchpoints, n=10-12 three-dimensional areas) after only three days of PTH treatment, suggesting rapid angiogenesis. OB stimulation is sufficient to drive these angiogenic changes as comparable effects were seen in mice with OB-specific PTH receptor activation. To determine whether vascular remodeling was due to locally increased proangiogenic signaling downstream of PTH, effects on VEGF-A and FGF2 were assayed. PTH rapidly increased VEGF in various cultured OB cell types as well as in endosteal cells in vivo. FGF2 expression was similarly increased in the same OBs that showed PTH-dependent VEGF induction. Because the BM perivascular milieu is heterogeneous and capable of supporting either HSC quiescence or maturation depending on the specific vascular cell type and stimuli, we went on to test whether tuning BM angiogenic responses to PTH could manipulate HSCs more specifically. Treatment with the anti-VEGF-A monoclonal antibody bevacizumab (αVEGF) precluded PTH-induced vascular branching in calvarial BM (18±3 vs 19±2 branchpoints, n=9-12 three-dimensional areas), while PTH-induced bone anabolism, EC and perivascular stromal cell expansion were sustained following ten days of PTH + αVEGF. αVEGF therapy, alone or in combination with PTH, did not alter immature mesenchymal cell pools reported to regulate HSCs. Remarkably however, inhibiting PTH-induced VEGF augmented HSC expansion with no impairment to hematopoietic progenitors or precursors. The enhancement of functional HSCs was sustained throughout 22 weeks in lethally-irradiated, BM transplant recipients (13.4± 3.17% vs 20.6± 4.19% donor-derived cells in the peripheral blood, from PTH alone vs PTH + αVEGF treated BM donors, n=21 mice per group). Moreover, this activity was transplantable into secondary lethally-irradiated recipients, in contrast to the activity of HSCs modulated by PTH alone that was largely unable to reconstitute secondary transplant recipients. Because FGF2 is also increased by PTH and has been reported to expand HSCs directly and microenvironmentally, we tested its role in PTH + αVEGF-induced HSC expansion. Inhibiting FGF receptor 1 diminished hematopoietic stem and progenitor cell expansion by PTH + αVEGF (0.20± 0.041% vs 0.13± 0.013%, n=9-10 mice per group), suggesting the two angiogenic signals have opposing effects on HSCs during microenvironmental activation by PTH. These data demonstrate that niche heterogeneity can be targeted to achieve specific stem cell outcomes and identify a potentially useful strategy in the treatment of BM injury or hematopoietic malignancy.

W-1081
HEMATOPOIETIC STEM CELL NICHES MATRIX TENASCIN C PROMOTES DEFINITIVE HEMATOPOIESIS FROM HUMAN PLURIPOTENT STEM CELLS IN A COMPLETELY DEFINED SERUM- AND FEEDER-FREE SYSTEM

Uenishi, Gene Ichiro[1], Vodyanik, Maxim A.[2], Stewert, Ron[3], Kumar, Akhilesh[1], Lee, Jeong-Hee[1], Theisen, Derek J.[1], Raymond, Matthew[1], Thomson, James A.[3], Slukvin, Igor I.[4]

[1]Pathology and Laboratory Medicine, University of Wisconsin, Madison, WI, USA, [2]National Primate Research Center, Madison, WI, USA, [3]Morgridge Institute for Research, Madison, WI, USA, [4]University of Wisconsin Madison, Madison, WI, USA

The advent of human pluripotent stem cell technologies has provided the opportunity to produce endothelial and hematopoietic cells in

vitro for functional studies and therapies. Previously, we established a coculture system using the mouse bone marrow stromal cell line, OP9, for the efficient and scalable differentiation of human pluripotent stem cells (hPSCs) into endothelial and blood lineages. However, this system relied on mouse feeder cells and serum, which limits its utility for studying hPSC response to specific growth factors and manufacturing clinical grade therapeutic blood cells. To overcome these limitations, we identified the optimal combination of morphogens, small molecules, and hematopoietic cytokines required to reproduce hPSC differentiation pattern on OP9 in serum-free defined conditions. We found that stage-specific administration of BMP4, Activin A, FGF2, VEGF, and LiCl were sufficient and necessary for hematoendothelial specification, while the addition of hematopoietic cytokines, SCF, TPO, IL-6, and IL-3 greatly increased hematopoietic expansion. In addition, based on molecular profiling of OP9 and stromal cell lines with different hematopoiesis-inducing activity, we found that Tenascin C, a protein associated with HSC niches, strongly promotes hematopoietic development from hPSCs. Phenotypic and functional analyses of cells generated in our Tenascin C-based feeder-free protocol showed that they undergo similar stages of development as hPSCs cocultured on OP9 cells. These stages include, ApelinR+PDGFRα+ primitive posterior mesoderm with mesenchymo- and hemangio-blast forming potentials, KDRhiApelinR+PDGFRα-/lo hematovascular mesodermal precursors, VE-Cadherin+CD73-CD235a- hemogenic endothelial progenitors, VE-Cadherin+CD73+CD235a/43- non-hemogenic endothelial progenitors, VE-Cadherin+CD73-CD235a+ angiogenic hematopoietic progenitors, and multipotent lin-CD34+CD43+CD45-/+ hematopoietic progenitors. hPSCs differentiated in completely defined conditions formed the entire spectrum of hematopoietic colonies including GEMM and possessed T lymphoid potential, indicating the induction of the definitive hematopoietic program. Collectively, this novel differentiation system opens the opportunity to produce clinical grade blood cells de novo for therapies of blood diseases.

W-1082
MIR-146A REGULATES HEMATOPOIETIC STEM CELL MAINTENANCE AND CELL CYCLE ENTRY

Wegrzyn Woltosz, Joanna[1], Knapp, David JHF[2], Copley, Michael[3], Ibrahim, Rawa[1], Umlandt, Patricia[1], Fuller, Megan[1], Baltimore, David[4], Boldin, Mark[5], Karsan, Aly[1]

[1]Genome Science Centre, BC Cancer Agency, Vancouver, BC, Canada, [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada, [3]BC Cancer Agency, Vancouver, BC, Canada, [4]California Institute of Technology, Pasadena, CA, USA, [5]Department of Molecular and Cellular Biology, Beckman Research Institute of City of Hope, Duarte, CA, USA

Maintenance of blood homeostasis depends on the balance between self-renewal of hematopoietic stem cells (HSCs) and their differentiation into blood cell progenitors. A variety of different intrinsic or extrinsic regulators, including multiple microRNA (miRNA) species, have been described to play a role in the regulation of these processes. Disruption of any of these regulators could lead to stem cell exhaustion or increased risk of leukemogenesis. Given recent reports of the role of miR-146a in malignant hematopoiesis, we evaluated its role in hematopoietic stem progenitor cell (HSPC) function. We show that miR-146a is highly expressed in HSCs and its expression decreases in committed progenitors. miR-146a- deficient HSCs had dramatically reduced self-renewal capacity as measured by serial competitive bone marrow transplantation assays. The lower self-renewal capacity was accompanied by decreased quiescence in miR-146a-deficient cells, as revealed by decreased proportion of miR-146a-/- HSPCs (Lin- Sca-1+ c-kit-, LSK) in G0 of the cell cycle (Ki-67- negative), and their increased proliferation, measured by BrdU incorporation. We further showed that increased proliferation of HSPCs is cell intrinsic. By sorting EPCR+ CD48- CD150+ (ESLAM) HSCs and examining cell division kinetics at the single cell level, we found that miR-146a-/- HSC undergo cell division earlier and differentiate more rapidly than wild-type HSCs, thereby producing larger colonies containing more differentiated (Lin+) cells. Our data provide evidence that miR-146a loss attenuates HSC quiescence and impairs their self-renewal ability, leading to hyperproliferation of progenitor cells. The phenotype seen is cell autonomous and the findings suggest that miR-146a plays a critical role in maintaining long term HSC function.

W-1083
DISPARATE RAPID EFFECTS OF FLT3-LIGAND AND GP130-ACTIVATING CYTOKINES ON PRIMITIVE SUBSETS OF CD34+ HUMAN HEMATOPOIETIC CELLS

Wei, Lisa[1], Eaves, Connie[1], Rose-John, Stefan[2], Knapp, David JHF[1]

[1]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada, [2]Institute of Biochemistry, Christian Albrechts Univerity Kiel, Kiel, Germany

Background: Expansion of human hematopoietic stem cells (HSCs) remains an important clinical imperative for therapies with the goal of improving both blood cell recovery and replacement. Current approaches exploit empirically accrued identification of cytokines that expand human cells with longterm hematopoietic output potential in vitro and in transplanted irradiated immunodeficient mice. However the specific mechanisms by which many of these may act on HSCs and closely related cell types remain poorly understood. Previous studies have suggested that FLT3-L and activation of Gp130 (the common signaling β chain of the IL6 receptor family) are independently active stimulators of long-term culture initiating cell (LTC-IC) expansion in short-term (10-day) cultures of CD34+CD38- human cord blood (CB) cells. More recent experiments have shown that CB cells with in vivo repopulating activity are obtained at much higher purity (~10%) in the CD45RA-CD90+CD49f+ subset of the CD34+CD38- population (CD49f cells; ~10% HSCs). Therefore it was of interest to compare the effects of this mode of stimulation on the CD49f+ subset with the total CD34+CD38- fraction of CB cells. Methods: Aliquots of 3150 cells FACS-sorted CD34+CD38- or 100 CD49f+ CB cells were cultured for 12 days in 100 ng/mL FLT3-L + 20 ng/mL of either IL11 or hIL6 (a synthetic peptide consisting of IL-6 connected via a linker to its cognate -chain receptor) and then analyzed for their outputs of CD34+CD45RA-CD90+ cells and LTC-ICs. For molecular analyses, CD34+ cells were stimulated with the same factors for 5, 15, 30 min, or 24 hours, fixed, stained in 2 steps for CD34, CD38, CD45RA, CD90, CD49f, CD135, Cyclin-B1 and Ki67, and then analyzed on a LSR Fortessa. Results: As expected, under both culture conditions, some phenotypically primitive (CD34+CD45RA-CD90+) cells (on average a 5-fold decrease from the input no.'s) and LTC-ICs (~3 and 16 for hIL6/IL11 respectively) were present after 12 days in the cultures initiated with the CD34+CD38- cells. Interestingly, no CD34+ cells were detected in the 12-day cultures initiated with CD49f+ cells. From the molecular analyses, these different biological outcomes were mirrored by a failure of these cytokine combinations to activate Ki67/CyclinB1 expression in the CD49f+ cells, in contrast to the rapid response of the total CD34+CD38- and the CD34+CD38-CD45RA-CD90-CD49f- cells. Individually tracked CD34+CD38- cells also showed more rapid initiation of CD45RA-CD90-CD49f+ cell proliferation in hIL6. Interestingly, a marked instability was observed in FLT3 receptor (CD135) levels following stimulation, suggesting that this may not be a useful marker in the context of manipulated cells. Conclusions: These results show that cytokines active on very primitive human hematopoietic cells can have previously unappreciated differences in

their immediate signaling and subsequent biological effects on closely related subsets. Further careful dissection of these cell-type specific responses are thus likely to provide important new information relevant to the optimization of improved methods to expand primitive human hematopoietic cells *ex vivo*.

W-1084
STEM CELLS EXPRESSING HEMATOPOIETIC MARKERS POPULATE THE INJURED CEREBRAL CORTEX AND CAN PLAY A ROLE IN TISSUE REGENERATION

Wylot, Bartosz, Konarzewska, Katarzyna, Bugajski, Lukasz, Piwocka, Katarzyna, Zawadzka, Malgorzata
Neurobiology Center, Nencki Institute of Experimental Biology, Warsaw, Poland

In response to the central nervous system (CNS) injury cells of hematopoietic origin populate the lesion and contribute to macrophage populations. Recent data demonstrated that certain populations of brain macrophages may support regeneration of the diseased CNS by secreting a plethora of growth factors as well as reducing inflammation via production of immunomodulating cytokines and removal of tissue debris. To date, the source of such pro-regenerative macrophages in the injured brain has not been unambiguously characterized. In addition, the contribution of specific rare hematopoietic stem/progenitor cells to macrophage populations in the injured CNS has not been sufficiently studied. Therefore, the aim of the present study was to examine the potential of hematopoietic stem/progenitor cells to be the source of functional macrophages in the injured CNS. The CD45+lin- hematopoietic stem/progenitors with different pattern of c-kit expression were found in the murine cerebral cortex and their number was increased following traumatic stab wound injury. The hematopoietic populations were sorted with FACS and examined in vitro towards differentiation into microglia/macrophages by co-culture with astrocytes. The potential of these cells to contribute to macrophage populations in vivo was confirmed by their transplantation into the CNS lesion. Results of our study showed that primitive hematopoetic stem/progenitor cells may be the source of macrophages with pro-regenerative potential in the injured central nervous system.

W-1085
OSTEOPROGENITOR HSC NICHE SUPPORTS POSTINJURY RECOVERY FOR BLOOD HOMEOSTASIS

Yoon, Kyung-Ae, Kim, Joo-Hyun, Cho, Je-Yoel
Department of Veterinary Biochemistry and BK21, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

Hematopoietic stem cell (HSC) maintenance requires a specific microenvironment. HSC niches could be compromised by several different cell types, such as Nestin+ MSC, CAR cells, macrophage, and SNS including osteoblasts (OBs) and the vascular marrow sinusoids. In steady state niche condition, these cells could regulate HSC activity and blood homeostasis. However, BM stress by treatment with chemotherapeutic drugs, such as cyclophosphamide and 5-fluorouracil (5FU) induce activated niche condition. These stimuli also generated tissue damaging chemotherapeutic drugs and various cell signaling molecules, such as SDF-1, FGFs. Recent research showed insufficiently the recovery of OBs including other constituents of niches after marrow injury. We therefore investigated that how osteoblastic niche in mice treated with 5FU (150mg/kg) could promote the proliferation and facilitating mobilization hematopoietic stem and progenitor cells (HSPCs) to enhance donor HSC engraft or reconstitution. The OBs isolated from 5FU-treated or control mice were increased niche-related genes, mSDF-1, mJag-1, mScf, mN-cad, mAngpt1 and mVcam-1, and osteoblast marker genes, mOsx, mOpn, mRunx2, mAlp, mColI. Ki67, proliferation marker gene, significantly decreased in 5FU-activated OBs comparing to normal OBs. CFU assay also largely decreased the proliferation rate of OBs isolated from 5FU-treated mice. These results showed BM stress by 5FU treatment induced activated osteoblastic niche. FGF signaling is important for hematopoietic regulation. It was recently reported that FGF ligands in the BM of 5FU-treated mice significantly increase. We confirmed the proliferation rate and the expression of niche marker genes in 5FU-activated OBs from mice was increased during FGF2 treatment. Expression of FGFRs in 5FU-activated OBs were also activated. Because osteoblast lineage cells within osteoblastic niche consist various stage osteoblastic cells, we therefore anticipated that stage-specific interaction between osteoblastic niche cells and HSPCs was important for postinjury recovery after marrow injury. To find regulator gene candidates in OSX-GFP positive cells isolated from 5FU-treated mice using OSX-GFP-CRE mouse, we performed an RNA-sequencing analysis using next generation tool. Taken together, FGF signaling could regulate osteoblastic niche cells to support HSC hemeostasis in response to severe BM damage.

W-1086
PRIMING WITH ERYTHROPOIETIN IMPROVES THE ANGIOGENIC POTENTIAL OF HUMAN PERIPHERAL BLOOD STEM CELLS MOBILIZED BY G-CSF

Yun, Ji-Yeon[1], Hur, Jin[2], Kang, Jeehoon[2], Jin, Yunjung[1], Kang, Jin-A[1], Lee, Hwan[1], Choi, Jae-Il[1], Lee, Hyun-chae[1], Choi, Young-eun[1], Kwon, Yoo-Wook[2], Cho, Hyun-Jai[2], Park, Young-Bae[2], Kim, Hyo-Soo[3]
[1]Seoul National University, Seoul, Republic of Korea, [2]Seoul National University Hospital, Seoul, Republic of Korea, [5]Seoul National Hospital Department of Internal Medicine, Seoul, Republic of Korea

Stem cell therapy in ischemic diseases, such as myocardial infarction (MI) has been regarded as a promising method to repair ischemic tissue and enhance neovascularization. Despite the several results performed by numerous clinical trials of stem cell therapy, there is still limitation in the therapeutic efficacy of current strategy. In our previous clinical trials, we proved that intracoronary infusion of the mobilized peripheral blood stem cells (mobPBSCs) after injection of Granulocyte colony stimulating factor (G-CSF) is significantly effective in improving myocardial contractility and reducing infarct volume in patients with acute myocardial infarction. In addition, we applied a priming strategy to boost the therapeutic effect of mobPBSCs and established our strategy to prime these cells just for several hours' incubation without ex-vivo cell culture. In this study, we examined the effect of erythropoietin (EPO) as a priming agent for mobPBSCs in order to augment the therapeutic efficacy. In general, EPO is known to promote survival, proliferation, and differentiation of erythroid progenitor cells and has long been used for cell based therapy in ischemic diseases. We used mobPBSCs obtained from healthy volunteers after 3 days' subcutaneous injection of G- mcg/kg). About 40% of mobPBSCs were positive for EPO receptor (EPOR) and responded to EPO priming (10 IU/ml) for 6 hours by increasing the expressions of angiogenic cytokines or growth factors (IL8, IL10, bFGF, PDGF, MMP9) as well as adhesion molecules (integrin αV, β1, β2, β8). Various angiogenic factors such as IL8, IL10, and PDGF were contained more in the conditioned media from EPO-primed mobPBSCs compared to the conditioned media from Veh-primed mobPBSCs. The conditioned media from EPO-primed mobPBSCs significantly enhanced migration and tube formation capability of endothelial cells. EPO-primed mobPBSCs also showed increased adhesion on endothelial cells or fibronectin. Augmented angiogenic potential of EPO-primed mobPBSCs was confirmed by Matrigel plug assay and athymic nude mice hindlimb ischemia models, showing direct incorporation of human mobPBSCs

into vasculature and increase in vessels formation and blood stream. Briefly, EPO could be used a novel priming agent to augment the angiogenic potential of human mobPBSCs by induction of angiogenic cytokines and adhesion molecules, which would be helpful to achieve better results after intracoronary infusion in patients with ischemic disease. Our results strongly suggest that ex-vivo EPO priming of mobPBSCs can be a feasible and effective method to improve the efficacy of stem cell therapy.

W-1087
ZFX MODULATES THE GROWTH AND IMATINIB SENSITIVITY OF CD34$^+$ CHRONIC MYELOID LEUKEMIA CELLS

Zhang, Xiuyan[1], Wu, Jie[1], Zhou, Haixia[2], Wu, Depei[2], Zhao, Yun[1]
[1]Cyrus Tang Hematology Center, Soochow University, Suzhou, China, [2]Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University, Suzhou, China

ZFX (Zinc finger protein, X-linked) is a key regulator of both embryonic stem cells (ESCs) and hematopoietic stem cells (HSCs). A recent report has demonstrated that Zfx is required for Notch intracellular domain (NotchIC) induced acute T-cell leukemia and MLL-AF9 induced myeloid leukemia in mice models. Moreover, the implication of ZFX in various human tumors has been documented as well. However, the expression and the role of ZFX in human leukemia (*e.g.* chronic myeloid leukemia, CML) have not been addressed yet. In the present study, we firstly found that *ZFX* had similar expression level in nucleated cells from chronic phase CML patients (n=13) as that from normal bone marrow (NBM, n=7), however *ZFX* was 3-fold up-regulated in CD34$^+$ CML cells compared to that in NBM cells (8 CML vs. 4 NBM, $p<0.01$). In addition, we utilized Hoechst 33342 (Hst) and Pyrosin Y (Py) co-staining to purify the CD34$^+$ quiescent CML and NBM cells (CD34$^+$Hst^{lo}Py^{lo}), and found that ZFX had a 13-fold higher expression level in CML cells than that in NBM cells (3 CML vs. 3 NBM, $p<0.05$). In contrast, the protein expression of ZFX decreased upon the treatment of Imatinib mesylate (IM) in K562 cells. To address the role of ZFX in CML cells, we validated two independent shRNA sequences delivered with lentiviral vector, which resulted in the decreased ZFX protein expression by 67% and 73% in K562 cells, respectively. ZFX silence inhibited the proliferation of K562 cells in liquid culture and impaired their colony-forming cell (CFC) capacities as well. The knockdown of ZFX also decreased CFC production of CD34$^+$ CML cells (ranging from 58% to 85%, n=3, $p<0.01$). Interestingly, ZFX silence sensitized K562 and CD34$^+$ CML cells (both IM sensitive and insensitive samples) to the treatment of IM. To obtain more molecular insights, we generated the global gene expression data comparing ZFX silenced and control K562 cells and we validated that both transcript and protein expression of B4GALT1 (UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 1) was reduced upon ZFX silence as what indicated by the array data. Next, we performed chromatin immunoprecipitate (ChIP) analysis and revealed the direct binding of ZFX to the promoter of B4GALT1 in K562 cells. Lastly, we co-transduced K562 cells with B4GALT1 and shRNA against ZFX, which partially reversed the suppressed CFC production and the enhanced IM sensitivity obtained with ZFX silence alone. Taken together, we have demonstrated the aberrant expression of ZFX modulates the growth and IM sensitivity of CD34$^+$ CML cells possibly via its direct regulation of B4GALT1, which indicates a novel regulatory role of ZFX in human CML and potentially leading to novel therapies to improve the management of the disease.

W-1088
MEGAKARYOCYTES MAINTAIN HOMEOSTATIC QUIESCENCE AND PROMOTE POST-INJURY REGENERATION OF HEMATOPOIETIC STEM CELLS

Zhao, Meng[1], Perry, John[1], Marshall, Heather[1], Venkatraman, Aparna[2], He, XI (CiCi)[1], Li, Linheng[1]
[1]Stowers Institute for Medical Research, Kansas City, MO, USA, [2]Centre for Stem Cell Research, Bagayam, India

Multiple bone marrow (BM) stromal cells have been identified as hematopoietic stem cells (HSCs)-regulating niche cells. Mature hematopoietic cell lineages, including macrophages, can influence HSC stromal niche cells, however, whether HSC progeny can directly serve as an HSC niche has not been shown. Here, we report an unexpected role of megakaryocytes (Mks) in maintaining HSC quiescence during homeostasis and promoting HSC regeneration in response to chemotherapeutic stress. We observed that Mks physically associated with HSCs in the BM (58% within 2 cell distance). Using the Mk-specific Platelet factor 4 (Pf4)-Cre induced diphtheria toxin receptor (iDTR) mouse line, we found that Mk ablation led to activation of quiescent HSCs and increased proliferation (10-fold increase of Lin-Sca-1+c-kit+CD48-CD150+ HSCs, $P<0.001$) under homeostasis. RNA-seq analysis revealed that Tgfβ1 expressed the highest in Mks compared to other HSC niche cells, and Mk ablation led to reduction of biologically active TGFβ1 protein in BM and nuclear-localized pSmad2/3 in HSCs indicating that Mks may maintain HSC quiescence through TGFβ/Smad signaling. Indeed, TGFβ1 injection prevented activation and restored quiescence of HSCs upon Mk ablation, demonstrating that Mks regulate HSC quiescence prominently through TGFβ1. In response to chemotherapeutic stress, Mks expanded in parallel with HSC regeneration near blood vessels in the central marrow, however, Mk ablation resulted in severe HSC regeneration defects ($P<0.01$). Mechanistically, Mks were found to increase FGF1 production to promote HSC expansion in response to chemotherapeutic stress. These observations demonstrate that Mks uniquely serve as an HSC-derived niche, which dynamically regulates HSCs under different conditions maintaining HSC quiescence via TGFβ signaling during homeostasis but supporting HSC expansion via FGF signaling under stress.

CANCER CELLS

W-1092
POMEGRANATE AND THE BREAST CANCER STEM CELLS - A DETAILED STUDY ABOUT THEIR LINK AND A NOVEL NUTRACEUTICAL THERAPY

Edward, Christina
Genomics, Madurai Kamaraj University, Madurai, India

Breast Cancer Stem Cell is a single, genetically abnormal cell. As this one culprit cell divides, it eventually becomes a tumor and develops a blood supply to nourish its continued growth. The target should be the cancer stem cells and not any normal cells. At some point, the cancer stem cells may break off from the primary mass and move through the blood supply or nearby lymph system to other parts of the body and this process is called metastasis. The most common sign of breast cancer is a new lump or mass. Most often when untreated a breast cancer stem cells trigger the cancer growth to spread to underarm lymph nodes even before the original tumor in the breast tissue is large enough to be detected. We are in need of a therapy/treatment to selectively kill cancer stem cells at the original tumor site and in distant metastases with no toxic effects on healthy cells, including normal stem cells. Cancer stem cells are critical to a cancer's ability to recur following

conventional chemotherapies and radiation therapy because they can quickly multiply and establish new tumors that are often therapy resistant. Here comes the role of pomegranate which has a long history of use as a food and medicine in Asia and South America. According to the WHO, this medicinal plant is the best source to obtain variety of drugs. About 80% of individuals from developed countries use them in traditional medicine. The plant part or the compounds derived from the plants are now established recipe of both pharmaceuticals and nutraceuticals. There are many evidences that the pomegranate has good anti-cancer properties against prostate, bowel and liver cancer. But there are no studies so far looking at the use of pomegranate in humans. The objective of this paper is to provide a detailed report about why pomegranate in human breast cancer, scientific research proof for the medicinal quality of pomegranate, Sensitivity of Pomegranate and its hypersensitive reactions, its interactions with drugs and a novel nutraceutical therapy using pomegranate.

W-1093
DIFFERENTIAL EXPRESSION OF SPECIFIC OCT4 TRANSCRIPTS IN BRAIN TUMORS

Furukawa, Gabriela[1], Rodini, Carolina[1], Silva, Patrícia[1], Okamoto, Oswaldo[2]
[1]USP, Sao Paulo, Brazil, [2]University of Sao Paulo, Sao Paulo, Brazil

The *OCT4* gene encodes the transcription factor POU5F1, and its expression is linked to pluripotency and inhibition of cellular differentiation in healthy cells. Ectopic expression of OCT4 in neoplastic cells has also been reported in several types of cancer such as pancreas, bladder, breast, and gliomas. This gene, however, has three alternative transcript variants, Oct4A, Oct4B1 and Oct4B, whose protein products display different functions. OCT4A, the only isoform with a well known function, is involved in the maintenance of pluripotency. Recent studies have suggested involvement of OCT4B1 in apoptosis, stress response and stemness, while OCT4B has been reported as related to genotoxic stress. However, more studies regarding the function of these isoforms are still needed. In addition to these three alternative splicing variants, *OCT4-PG1*, formerly considered an *OCT4* pseudogene, also generates the transcript Oct41B, which shares 95% similarity with Oct4A. A previous study from our laboratory analyzed 37 medulloblastoma samples and revealed that sporadic expression of *OCT4* significantly correlated with poor overall survival, although no information regarding specific *OCT4* transcript expression was known. Here, we have developed a method to discriminate expression of distinct Oct4 transcripts using specific primers developed in exon-exon junctions and exclusive gene sequences of *OCT4-PG1*, in such way that certain combinations of primers would only amplify one of the transcripts by real time PCR. Amplification specificity was confirmed by both the melting curve profile and sequencing of each amplicon. This strategy allowed us to perform further studies regarding expression of *OCT4* alternative transcripts and *OCT4-PG1* in medulloblastoma. The log-rank analysis (P<0,05) of data from 15 medulloblastoma specimens revealed that expression of any of the transcripts on its own does not significantly correlate with poor survival, but a concomitant expression of specific transcripts does. This assay could therefore assist the prescription of post-surgical treatments to medulloblastoma patients. Studies regarding the influence of *OCT4* transcripts in medulloblastoma will contribute to a better understanding of mechanisms underlying tumor aggressiveness.

W-1094
PEDIATRIC AND ADULT LEUKEMIAS EXHIBIT DISPARATE GENOMIC ORGANIZATION AND FUNCTIONAL PROPERTIES

Giambra, Vincenzo[1], Lam, Sonya[1], Belmonte, Miriam[1], Ng, Amy[1], Shevchuk, Olena O.[1], Gusscott, Sam[1], Salehi, Sohrab[1], Chun Chan, Fong[2], Kridel, Robert[2], Lorzadeh, Alireza[3], Jenkins, Christopher[1], Hoofd, Catherine[1], Benz, Claudia[2], Steidl, Christian[2], Eaves, Connie[1], Hirst, Martin J.[4], Andrew, Weng P.[1]
[1]Terry Fox Laboratory, BC Cancer Research Centre, Vancouver, BC, Canada, [2]BC Cancer Research Centre, Vancouver, BC, Canada, [3]Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada, [4]British Colombia Cancer Agency, Vancouver, BC, Canada

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of immature T-cell progenitors which affects both children and adults. While optimization of chemotherapy regimens has led to steady improvements in outcome for pediatric patients over the past 5 decades, new approaches are needed for adults. The basis for this divergence is likely multifactorial, but we sought in this study to investigate whether intrinsic features might underlie the biologic differences between pediatric and adult leukemias. In an effort to model pediatric and adult leukemias, we transduced hematopoietic stem/progenitor cells (HSPCs) derived from mouse fetal liver (FL) and adult bone marrow (ABM) with activated NOTCH1 virus and transplanted these cells into histocompatible recipient animals. We find that whereas fetal and adult HSPCs generate similar primary leukemias in terms of penetrance, latency, disease burden/distribution, and immunophenotype, fetal leukemias exhibit greater cycling activity, but dramatically reduced ability to propagate disease in secondary recipients. In fact, fetal leukemias contain 150- to 500-fold fewer leukemia-initiating cells than adult leukemias, suggesting there may be important differences in their self-renewal activity. Using a combination of gene expression profiling and in vitro culture assays, we further demonstrate that NOTCH1 induces autocrine IGF signaling in FL, but not ABM-derived HSPCs which is responsible for the increased cell cycling phenotype. Importantly, similar results were obtained with human HSPCs derived from CD34+ cord blood and bone marrow. We also show that NOTCH1 dimerization is required for the pronounced IGF expression seen in fetal mouse and neonatal human HSPCs. Interestingly, NOTCH1 induces different histone methylation signatures in chromatin overlying the IGF1 locus in FL and ABM HSPCs, and also mediates a different set of physical interactions with distant loci. We conclude from these studies that NOTCH1 enacts differential, developmental stage-specific transcriptional programs by a combination of local epigenetic patterning and long-range genomic interactions. These findings support the idea that pediatric and adult T-ALL may potentially be regarded as related, but biologically distinct diseases, and that novel, age-specific therapies that exploit these differences may improve clinical outcomes.

W-1095
IDENTIFICATION OF PROGNOSTIC BIOMARKERS FOR HEPATIC CANCER PROGRESSION BASED ON HEPATIC DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELL

Han, Jiyou
College of Life Sciences and Biotechnology, Lab of Stem Cells and Tissue Regeneration, Korea University, Seoul, Republic of Korea

MFG-E8 (Milk fat globule-epidermal growth factor VIII), also called lactadherin or BA46, SED1 is a glycoprotein found in milk and mammary epithelial cells, it is a major protein component associated with milk

fat globule membrane. Previously, our study showed that expression of MFG-E8 is gradually increased with hepatic differentiation of human embryonic stem cells (hESCs). Therefore, we hypothesized that MFG-E8 would be an early cancer stem cell marker, which may predict cancer progression. Our results showed that MFG-E8 was expressed in various human cancer cell lines such as HepG2, Hep3B, and Huh7. Production and secretion of the MFG-E8 were also confirmed in the conditioned media of those three cell lines using enzyme-linked immunosorbent assay. Next, we analyzed the MFG-E8 expression in 11 clinical cases of cholangiocellular carcinoma (CC) and 33 cases of hepatocellular carcinoma (HCC) by immunohistochemistry and examined the potential correlation with β-catenin and AFP, which are known cancer markers. According to hitological criteria, the progression of HCC and CC was evaluated and classified into high, low, metastatic, and well-, moderate-, poor-differentiated, respectively. Our immunohistochemistry data showed that MFG-E8 was expressed both HCC and CC tissue. Interestingly, the MFG-E8 expression was significantly increased with cancer progression ($P<0.05$) in both cases. Additionally, β-catenin expression was increased and its localization was changed from membrane to cytoplasm and nucleus with the degree of HCC. Likely β-catenin, AFP was also increased with the degree of HCC but it was not correlated with severalty of CC. Importantly, both AFP and β-catenin were highly co-localized with MFG-E8 in HCC. These results suggest that MFG-E8 may have important physiological roles and its expression in HCC and CC would be considered as an important prognostic factor. This research was supported by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (No. 2012M3A9C7050139 and No. 2012M3A9B4028636 for JHK) and Korea University Grant (for JH).

W-1096
LEUKEMIC PROPAGATING CELLS MODULATE THE BM NICHE IN RESPONSE TO THERAPY

Hong, Dengli
Shanghai Jiao-Tong University School of Medicine, Shanghai, China

Increasing evidence suggests that the residence of cancer propogating cells (CPCs) in preferential niches may have a major part in evading therapy and in subsequent evolution. However, this topic should be further investigated in terms of the nature of the niches involved and the mechanisms by which CPCs modulate the niche and the niche protects CPCs. We addressed these issues in the context of human ALL in this study. We have investigated the mouse models and patients' BM biopsies. The results reveal the interplay between leukemia propogating cells (LPCs) and their non-malignant supporting cells in the bone marrow (BM) niche and the initial events resulting from primary chemotherapy. Prior to frank leukemic dissemination, residual leukemic cells were located in normal endosteal and vascular niches after initial chemotherapy. However, LPCs resided in a sheath of supporting cells comprising a novel niche once leukemic dissemination substantially devastated the normal BM environment. The novel niche is dynamically transient: beginning with Nestin+ cells, maturating through their transition to α-SMA+ cells, and terminating with fiber residues. The niche then provided the resident LPCs with anti-apoptotic properties. This kind of niche-mediated "reprogramming" in LPCs was achieved by epigenetic cooperation, and the alterations may be memorized by the niche-resident LPCs until they evolve by generating genetically distinct refractory subclones. Therefore, the therapeutic strategies for such disease should prevent the formation of an early protective niche or interfere with its function during the early phase of chemotherapy.

W-1097
IDENTIFICATION OF MIRNA INVOLVED IN MEDULLOBLASTOMA AGGRESSIVENESS

Kaid, Carolini, Silva, Patricia, Rodini, Carolina Oliveira, Semedo-Kuriki, Patrícia, Okamoto, Oswaldo
Genetics Department, Bioscience Institute / São Paulo University, São Paulo, Brazil

Medulloblastoma is a common malignant brain tumor in children and a major cause of morbidity and mortality by childhood cancer. A recent study of our group revealed that ectopic expression of the pluripotent factor OCT4 is associated with poor survival of medulloblastoma patients. Differential expression of miRNAs known to regulate intrinsic stem cell properties has also been recently correlated with poor prognosis in some cancers. In this work, we found increased levels of hsa-miR-367 in human medulloblastoma CHLA-01-Med cells stably overexpressing OCT4A, established in our lab, which prompted us to further investigate a possible direct contribution of this miRNA in medulloblastoma aggressiveness. Expression of primary and mature hsa-miR-367 was detectable by real-time PCR in parental CHLA-01-Med cells, albeit at lower levels when compared with OCT4A overexpressing CHLA-01-Med cells and with human embryonic stem cells. Transfection of parental CHLA-01-Med cells with a synthetic hsa-miR-367 mimic resulted in down-regulation of two bioinformatically predicted transcript targets encoding ITGAV and RAB23, which are involved in cancer. Furthermore, transfection with the miRNA mimic significantly increased viable cell growth after 24h and 48h of cell culture, as well as the amount of medulloblastoma neurospheres formed four days post-transfection ($P<0.05$). These findings suggest a pro-oncogenic activity for this microRNA involved in pluripotency and stem cell self-renewal, which may contribute to tumor aggressiveness as a downstream target of OCT4A in medulloblastoma.

W-1098
BONE MARROW DERIVED MESENCHYMAL STEM CELLS ARE ASSOCIATED WITH THE TUMOR MICROENVIRONMENT OF GASTRIC CARCINOMA

Kasashima, Hiroaki[1], Yashiro, Masakazu[1,2], Masuda, Go[1], Morisaki, Tamami[1], Fukuoka, Tatsunari[1], Hasegawa, Tsuyoshi[1], Hirakawa, Kosei[1]
[1]Department of Surgical Oncology [2]Oncology Institute of Geriatrics and Medical Science, Osaka City University Graduate School of Medicine, Osaka City, Japan

Current studies have reported that stromal cells in the tumor micro-environment contribute the development of gastric carcinoma. However, the origin of the stromal cells remains to be unclear. The aim of this study is to clarify the correlation between bone marrow mesenchymal stem cells (MSCs) and tumor stromal cells in gastric cancer. A gastric cancer cell line, OCUM-2MD3, was used. EGFP-labelled bone marrow was transplanted into BALB/C nude mice treated with irradiation of 4 Gray. Then OCUM-2MD3 cells were respectively transplanted into subcutaneous, stomach, or peritoneal cavity of mice. EGFP-labelled bone marrow-derived cells in each inoculated tumor were determined by fluorescence microscopy. Also each tumor was immunohistochemically stained by α-SMA, and cells with α-SMA -positive were determined as myofibroblasts. Next, we evaluated the effect of MSCs on the tumorigenicity of OCUM-2MD3 cells by inoculating in the presence or absence of MSCs. Taken together, In vitro, we examined the effect of MSCs on the proliferation and migration of gastric cancer cells in vitro. EGFP-labelled bone marrow-derived cells were found in the stroma of each tumor micro-environment. Some of EGFP-labelled cells were α-SMA positive. The tumorigenicity of co-inoculated tumor with cancer cells and MSCs was significantly

(p=0.020) increased, in comparison with that of tumor by cancer cells alone. Moreover, myofibroblasts with α-SMA -positive was frequently found in the co-inoculated tumor, in comparison with tumor by cancer cells alone. Conditioned medium from MSCs significantly increased the proliferation (p<0.001) and invasion (p<0.001) activity of gastric cancer cells. Bone marrow MSCs are associated with the tumor micro-environment, and might affect on the progression of gastric carcinoma.

W-1099
DIFFERENTIAL SENSITIVITY TO CISPLATIN IN A MODEL OF LUNG LINEAGES DERIVED FROM LUNG CANCER IPC CELLS

Kong, Chiou Mee, Xie, Xiaojin, Wang, Xueying
Biochemistry, National University of Singapore, Singapore

Non-small cell lung cancer (NSCLC), accounts for 80% of all lung cancers, is often deadly as majority of patients are detected at late stages. There is, however, paucity of a suitable model for early NSCLC detection that hinders the research progress. Despite resistance to chemotherapeutic drugs, cisplatin (CDDP) is still one of the commonly used drugs used in treating NSCLC cells. A better understanding of cellular events and molecular pathways of the development of resistance to CDDP could improve its therapeutic effect. The advent of induced pluripotency by Takahashi and Yamanaka in cancer research has opened up unchartered territories for oncologists to further understand cancer in order to approach it more effectively. These induced pluripotent cancer (iPC) cells provide unprecedented opportunities in studying the pathogenesis of cancer in vivo and serve as a valuable in vitro tool in studying drug sensitivity. In the present study, a sequential lung lineages differentiation model from NSCLC-derived iPC cells was established which enables us to interrogate CDDP resistance in NSCLC cells. Our results revealed that, in contrary to the parental NSCLC cells, definitive endoderm (DE) and lung epithelial cell lineage (LE), differentiated from NSCLC-derived iPC cells were less sensitive to CDDP. Our results further demonstrated that CDDP phosphorylates ATM and Chk2, which in turn leads to the activation of p53 and subsequently increases p21 expression levels, results in G1/S growth arrest. Taken together, our results showed that the ineffectiveness of CDDP in targeting differentiated NSCLC cells may be contributed from its failure in treating the cancer clones through the p53-p21-cell cycle axis. These findings suggest a more targeted therapy in treating NSCLC clinically. In summary, a sequential lung lineages differentiation model from NSCLC-derived iPC cells not only offers a suitable model for possible early lung cancer detection; but also improves our understanding of cellular events associated with CDDP resistance phenotype in NSCLC.

W-1100
HEDGEHOG-INTERACTING PROTEIN (HHIP) IS A KEY REPRESSOR OF HEDGEHOG SIGNALING THAT DRIVES CELL SURVIVAL, PROLIFERATION, INVASION, AND DRUG-RESISTANCE IN LUNG ADENOCARCINOMA

Kuo, Ting-yu[1], Lin, Erh-Hsuan[2], Yang, Sheng-Ping[3], Wu, Cheng-Wen[1]
[1]Institute of Biochemistry and Molecular Biology, National Yang Ming University, Taipei, Taiwan, [2]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, [3]Institute of Microbiology and Immunology, National Yang Ming University, Taipei, Taiwan

The cross-talk between oncogenic pathways and stemness pathways play important roles during cancer development and contribute to cancer cell stemness. Recent years, the aberrant activations of stemness signaling such as Hedgehog (HH), hypoxia-inducible factor, and Wnt pathways, and the stemness factors like Oct-4 and Sox-2 have been reported in lung cancers. However, most researches to date focused on the impact of positive regulators of stemness pathways in oncogenesis, but less on the importance of negative regulators. Hedgehog interaction protein (HHIP) is a membrane protein that binds to HH ligands with an affinity comparable to Ptch-1 (the native HH ligand receptor), and HHIP overexpression attenuates HH signaling by capturing HH ligands. HHIP has been found to be down-regulated in several types of cancers through promoter hyper-methylation. In lung cancer, however, its role and importance has not been identified. Here, we show that HHIP was significantly repressed in lung cancer cell lines and human lung tumor samples through epigenetic silencing. Overexpression of HHIP in lung cancer cells blocked the auto-loop induction of endogenous HH pathway, and inhibited the invasiveness of cancer cells. We also found that in starvation state, HH pathway was autonomously induced which then mediated the expression of HGF and cMET phosphorylation, while HHIP overexpression blocked such inductions and significantly repressed cell proliferation rate. Furthermore, HHIP reduced the size of spheroids formed by lung cancer cells in serum-free 3D matrix. Finally, we found that HH-induced HGF expression decreased the sensitivity of lung cancer cells to Genfinitib treatment. In summary, our results indicate that HHIP is a key regulator of HH signaling that was silenced in lung cancer and thus potentiates cancer cells to activate stemness pathway during adverse condition. This result also implies that cancer cells can acquire stemness by downregulating negative regulators of stemness pathways for survival, metastasis, and drug resistance.

W-1101
THE EMERGING ROLE OF YAP IN CELL SURVIVAL AND PROLIFERATION AND ITS CROSSTALK WITH EGFR IN LUNG ADENOCARCINOMA

Lee, TingFang, Wu, Cheng-Wen
National Yang-Ming University, Taipei, Taiwan

Lung cancer is one of the leading causes of malignancy-related deaths. The epidermal growth factor receptor (EGFR) pathway, a signaling network that regulates cell growth and survival, is overexpressed or mutated in a majority of lung adenocarcinoma (LAC) cases. Hippo pathway has emerged over the past decade as a major determination of organ growth and contact inhibition. The Hippo signaling effector YAP plays an important role in the maintenance of pluripotency in embryonic stem cells. Here we demonstrate that YAP is crucial in the maintenance of cell survival, cell proliferation and stemness-like properties in LAC cells. Overexpression of YAP promotes oncogenic phenotypes while knocking down YAP attenuates proliferation in LAC cells. We also observed a crosstalk between YAP and EGFR. YAP is regulated by EGFR signaling and overexpressed YAP can further upregulate EGFR, forming a positive feedback loop. EGFR active mutant LAC cell lines showed higher YAP protein levels compared to EGFR wild type cells. Knocking down EGFR contributes to decreased YAP expression in EGFR mutant cells; EGF stimulation rescued YAP expression in EGFR wild type cells. Our findings suggested the emerging role of YAP in proliferation and survival in LAC cells involved with EGFR signaling. YAP can serve as a potential target as a prognosis marker and a drug target.

W-1102
INTERACTION OF GD2 GD3 WITH GROWTH FACTOR RECEPTORS SUSTAIN THE STEM CELLS PHENOTYPE OF BREAST CANCER

Liang, Yuh-Jin
Chang Gung Memorial Hospital, Institute of Stem Cell and Translational Cancer Research, Taoyuan, Taiwan

Glycosphingolipids (GSLs) are ceramide with one or several sugar units attached, primarily localized on the plasma membrane of animal cells to mediate cell adhesion and signal transduction via lipid raft in the membrane. It was also reported that specific GSLs are highly expressed in human cancer cells and enhance tumor phenotypes include cell proliferation, invasion and motility. In this study, we used an EMT model which generate breast cancer stem like cells from immortalized human mammary epithelial cells and assessed the different expression profiles of GSLs between cancer stem cells (CSCs) and non-CSC. We have found that Fuc-(n)Lc4Cer and Gb3Cer were drastically reduced in CSCs, whereas GD2, GD3, GM2, and GD1a were greatly increased in CSCs. Among various glycosyltransferases tested, mRNA levels for ST3GAL5, B4GALNT1, ST8SIA1, and ST3GAL2 were increased in CSCs, which could explain the increased expression of GD3, GD2, GM2, and GD1a in CSCs. We further demonstrated that gangliosides GD2 and GD3 are specifically enriched in CD44hi/CD24lo CSC population. In addition, the stem cell phenotypes, such as mammosphere formation and migration ability, were reversed when GD3 synthase (ST8SIA1) or GD2 synthase (B4GALNT1) were knockdown in breast cancer cell line. Our results demonstrated that GD2 and GD3 may play a function role in maintaining the CSC phenotype in human breast cancer. Since gangliosides combined with their interacting receptors/ adaptors are known to exist in lipid rafts on the surface of plasma membrane and leading to regulate signaling transduction. Thus, how the GD2/ GD3 regulate the breast CSC phenotype by interacting with their interacting receptors/ adaptors in lipid raft and transmit specific signal for cell self-renewal and invasion in breast CSCs were investigated by Enzyme-Mediated Activation of Radical Sources reaction combind with Mass Spectrometry. A number of interacting molecules with GD3 or GD2 on the cell membrane were identified. Representative results of GD3 associating EGFR and GD2 associating c-Met were shown.

W-1103
FUNCTIONAL CHARACTERIZATION OF NOVEL BIOMARKERS FOR SUBTYPE-SPECIFIC MEDULLOBLASTOMA CELL PHENOTYPES

Liang, Lisa[1], Aiken, Christopher[1], Coudière-Morrison, Ludivine[1], McClelland, Robyn[1], Remke, Marc[2], Del Bigio, Marc[1], Taylor, Michael D.[2], Werbowetski-Ogilvie, Tamra[1]
[1]University of Manitoba, Winnipeg, MB, Canada, [2]Hospital for Sick Children, Toronto, ON, Canada

Medulloblastoma (MB) is the most common malignant primary brain tumor in children. Despite improved clinical outcomes, children with MB often suffer from the consequences of treatment such as surgery, chemotherapy and radiation. MB is currently classified into 4 distinct molecular subtypes based on genomic alterations, gene expression profile, response to treatment and cell of origin; Wnt, Sonic Hedgehog (Shh), Group 3, and Group 4. This extensive heterogeneity has revealed a critical need for subtype-specific, functionally validated biomarkers and therapeutic strategies. Here, we utilized a recently developed high-throughput flow cytometry cell surface marker screening platform to identify biomarkers that are differentially expressed in higher self-renewing vs. lower self-renewing Shh MB cells. From 242 cell surface markers, 25 showed differential expression between these two phenotypes. Four of these cell surface markers were also differentially expressed in the Shh variant relative to the other 3 subgroups across 3 independent transcriptome datasets consisting of 548 patient samples: CD271/p75NTR/NGFR, CD106/VCAM1, CD171/NCAM-L1 and EGFR. This differential expression was validated by flow cytometry using multiple cell lines derived from the MB subgroups. As we had previously demonstrated a role for CD271 as a potential marker for tumor propagating cells (TPC) in a Shh variant MB model in vitro, we initially chose this candidate for additional functional analysis. We employed gain and loss of function studies to investigate the effect of CD271 on MB cellular properties including self-renewal, proliferation, differentiation and cell motility/invasion. To date, our results demonstrate that compared to the control, CD271 overexpression cells generate larger tumorspheres, but lose their self-renewal capacity over subsequent passage. Neural lineage analysis of tumorspheres grown in differentiation conditions revealed a decrease in expression of both glial (A2B5 and O4) and neuronal markers (βIII-tubulin). These data suggest that CD271 may amplify progenitor cells. Our study demonstrates the utility of high throughput flow cytometry screening platforms for identifying novel markers associated with specific MB phenotypes. Due to the cellular heterogeneity between MB subgroups, identification of TPC populations for specific MB variants will be crucial for the design of next generation targeted therapies. Exploring the relevance of CD271 in the Shh variant MB will provide insight into a Shh specific TPC signature and the mechanisms responsible for regulating these clinically relevant cells. This will ultimately lessen the broad impact of toxic treatments such as radiation and chemotherapy on the child's developing nervous system and improve the quality of life for those children who survive long-term.

W-1105
THERAPEUTIC TRANSMEMBRANE PEPTIDES NEUROPILINE-1 AND PLEXIN-A1 INHIBIT GLIOBLASTOMA STEM CELL GROWTH

Meyer, Lionel, Jacob, Laurent, Crémel, Gérard, Bagnard, Dominique
INSERM, Strasbourg, France

Glioblastoma multiforme (GBM), the highest grade of glioma according the W.H.O classification is a lethal brain tumour for which no effective treatment is available. It is characterized by high proliferation, invasiveness, angiogenesis and resistance against treatments. The latter confers to GBM a systematic recurrence capacity, partially due to a subpopulation called glioblastoma stem cells (GSC). Here, we propose innovative membrane targeting peptides Plexin-A1 (MTP-PlexA1) and Neuropilin-1 (MTP-NRP1) to interfere on proliferation and differentiation capabilities of the GSCs. GSC were initially subjected to molecular characterization (Immunocytochemistry and RTqPCR) and differentiation assay showing an important oligodendrocyte ratio (O4 marker = 46% of total cells) compared to normal neural stem cells (O4 marker = 19% of total cells $p < 0.0001$). Hence, sphere formation assay revealed the possibility to inhibit GSC proliferation using MTP-PlexA1 and MTP-NRP1 (-32% and -34% respectively, $p = 0.001$). To address whether this inhibitory effect would impede tumor development in vivo we performed xenografts experiment. GCSs were grafted in the flank of nude mice without treatment or when cells were pre-incubated with MTP-PlexA1. This was done using a mCherry fluorescent version of GSC (NCH644-mCherry) allowing live monitoring of tumor apparition. We chose to monitor tumor development over a 5 days period of time as a compromise to reach the size of detectable tumors while conserving significant inhibitory activity of MTP-PlexA1. With control cells we observed the development of 14 tumors out of the 20 grafted mice (70%). However, GCS pre-incubated with the peptides gave rise to 25%

of detectable tumor bulks. Hence, the transmembrane peptide strategy using MTP-PlexA1 and MTP-NRP1 inhibits GSCs growth in different in vitro and in vivo models thereby opening an interesting potential therapeutic option.

W-1106
BAALC PROMOTES LEUKEMOGENESIS BY BALANCING MEK/ERK-DEPENDENT PROLIFERATION WITH KLF4-DERIVED DIFFERENTIATION BLOCK.

Morita, Ken[1], Masamoto, Yosuke[1], Kagoya, Yuki[1], Kataoka, Keisuke[1], Koya, Junji[1], Yashiroda, Hideki[2], Sato, Tomohiko[1], Murata, Shigeo[2], Kurokawa, Mineo[1]

[1]Department of Hematology and Oncology, The University of Tokyo, Tokyo, Japan, [2]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

BAALC (brain and acute leukemia, cytoplasmic) has drawn increasing attention since its clinical significance on worsened outcomes in cases of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), however, the precise mechanisms of BAALC to promote leukemogenesis remain to be addressed. BAALC has been shown to localize mainly in the cytoplasm, which offered us a clue to seek for alterations of signal transduction in cytoplasm by excessive BAALC. Our elaborate profiling of phosphorylated proteins in BAALC-overexpressed HEK293T cell line revealed the enhanced phosphorylation of extracellular signal-regulated kinase (ERK), and its downstream target p90RSK. To find out possible factors that bind to BAALC, yeast two-hybrid screening was conducted, in which BAALC as a bait and human bone marrow-derived cDNA library were simultaneously introduced, and MEKK1 (MAP3K1), a scaffold protein necessary for ERK activation, and KLF4, a differentiation-promoting transcription factor, were identified as partners of BAALC. In multiple cell lines with BAALC-overexpression, the interactions between BAALC and MEKK1 or KLF4 were confirmed by immune-precipitation (IP) assay and the colocalization of these proteins in the cytoplasm was detected by immune-fluorescence assay. The key properties of BAALC overexpression in leukemia cell lines turned out to be its proliferative capacity and differentiation block. The former was dependent on MEK/ERK activation level and the latter was represented by the reduced expression of hematopoietic differentiation-associated surface markers such as CD11b and CD14. Indeed U0126, a potent MEK inhibitor, could sufficiently suppress the augmented proliferation of these cell lines by BAALC, emphasizing the dependence of BAALC on MEK/ERK pathway. Furthermore, BAALC could inhibit the recruitment of MKP3, an ERK-specific cytosolic phosphatase, to ERK, resulting in sustained ERK activation by reduced dephosphorylation of ERK. Overexpression of MKP3 in leukemia cell lines effectively suppressed BAALC-induced proliferative advantage, highlighting the role of BAALC in MEK/ERK signal transduction cascade as a core coupling factor. Additionally, BAALC sequestered KLF4 in the cytoplasm, which might induce differentiation block by reducing nuclear KLF4 expression, and forced expression of KLF4 in leukemia cell lines in turn cancelled the augmented proliferative capacity by BAALC. Interestingly, IP assays further proved that MEKK1 and KLF4 competitively bound to BAALC, suggesting that BAALC might promote leukemogenesis by balancing MEK/ERK-derived proliferation with KLF4-derived differentiation block. Our series of knocking-down of BAALC, ERK or KLF4 in leukemia cell lines ensured consistency with the results by overexpressing these factors. Notably, in primary human AML samples, BAALC mRNA expression showed a positive correlation with the expression of ERK target genes and the sensitivity to U0126, as well as an inverse correlation with KLF4 expression, which could underscore our analyses at molecular level. Taken together, BAALC acts as a key coupling factor that positively regulates MEK/ERK pathway, in addition to as a cytoplasmic KLF4 conveyer, both of which eventually lead to the maintenance and proliferation of immature leukemic cells. These novel functions of BAALC might account for the aggressiveness of leukemia and would be a promising therapeutic target of BAALC-high leukemia.

W-1107
DIFFERENTIATION AND CANCER-SPECIFIC CPG METHYLATION STATES COEXIST IN HIERARCHICALLY RELATED PROSTATE CANCER CELL SUBSETS

Pellacani, Davide[1], Simms, Matthew S.[2], Mann, Vincent M.[2], Collins, Anne T.[3], Maitland, Norman[3]

[1]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada, [2]Castle Hill Hospital, Cottingham, United Kingdom, [3]Department of Biology, University of York, York, United Kingdom

The mature luminal cells of the normal adult human prostate epithelium develop from undifferentiated and basally located stem cells through a continuous series of amplifying divisions. In prostate cancer (CaP), this process is perturbed and an excess of proliferating cancer cells with luminal features (AR+/PSA+/P63-) accumulate. However, also present is a small population of malignant (AR-/PSA-/P63+) basal cells with stem-like proprieties. Altered DNA methylation control profoundly alters normal differentiation in many tissues, and aberrant DNA methylation patterns in cancers are thought to promote their development and progression. However, the status and role of DNA methylation in the hierarchy of cell types in human CaP have not been investigated. To address this issue we isolated phenotypically distinct subsets of prostate epithelial cells from matched normal and cancer biopsies from the same patients and then analyzed their individual DNA methylation profiles. This enabled us to compare the DNA methylation profiles of CaP cells with basal and luminal phenotypes, as well as comparing these to their phenotypically matched normal counterparts. In initial studies, we found a set of genes previously reported to be hypermethylated in CaP to be hypermethylated in luminal (tissue-derived Lin-/CD24+) CaP cells, but unmethylated and actively expressed in basal CaP cells (derived from primary cultures). During normal differentiation of basal to luminal cells, expression of these genes was downregulated by a CpG methylation-independent mechanism. The downregulation and hyper-methylation of these genes, which include the well-studied GSTP1, is therefore not essential for CaP development or expansion. Genome-wide RRBS analysis of freshly isolated cells from patients with organ confined, treatment naïve CaP (first of 4 patients to be analyzed) showed normal and CaP basal (Lin-/EPCAM+/CD24-/CD49f+) cells possessed similar CpG methylation profiles. In contrast, the methylation profiles of the luminal (Lin-/EPCAM+/CD24+/CD49f-) cells from the same samples differed greatly from each other and from those of both sources of basal cells. This implies that CpG methylation is subject to substantial changes during normal prostate epithelial differentiation, and these are then further modified in CaP populations. Nevertheless, almost two-thirds of the CpG islands (CGI) characteristically hyper-methylated in normal luminal cells as compared to basal cells, were also hypermethylated in CaP luminal cells. This suggests that many changes in CGI methylation that occur during normal differentiation are conserved in CaP. Interestingly, however, numerous CGI were hyper-methylated specifically in CaP luminal cells. Moreover, half of the CGI hyper-methylated in basal CaP cells were also hyper-methylated in luminal CaP cells (compared to their normal counterparts), suggesting that many CaP-specific methylation patterns are established in the basal CaP cells and conserved when these cells generate luminal progeny. In summary, by separate analysis of phenotypically distinct subsets of

normal and CaP epithelial cells, we show that a large part of the DNA methylation patterns established during normal prostate epithelial cell differentiation persists in CaP, with many cancer-specific methylation patterns being shared by basal and luminal CaP cells, consistent with a model of luminal CaP cells being derived from an epigenetically defined minor basal CaP cell population.

W-1108
MOLECULAR MECHANISMS ASSOCIATED WITH STEMNESS FEATURES IN CANCER CELLS UNDER HYPOXIC MICROENVIRONMENT

Prasad, Pankaj, Srivastava, Tapasya
Department of Genetics, University of Delhi South Campus, New Delhi, India

The solid tumors are characterized by their genetic heterogeneity which determines their proliferating capacity. Lack of drug penetrance and hypoxia are key features of solid tumor interiors. Prolonged hypoxic micro-environment leads to stemness characteristics in several tumor cell types but how such features get attributed to tumor cells is poorly understood. Also, do these stemness exhibiting cells carry similar molecular profile as of human embryonic stem cells (hESCs) and possess the hallmark properties of cancer stem cells (CSCs) like extensive proliferative potential and maintenance of self-renewal? In this study, we hypothesized that hypoxia up-regulates stem-cells like features in tumor cells via active DNA demethylation and cancer cells exhibit epigenetic reprogramming at the promoter regions of pluripotency-associated genes. Differential methylation analysis, ChIP assay, side population assay, immunolocalization and gene expression profiles of stemness and differentiation associated genes demonstrated that active demethylation of the pluripotency-associated genes help restoration of stem cell transcriptional circuitry in brain tumor cell lines when cultured in hypoxic conditions.

W-1109
MYELOID LEUKEMIA STEM CELL PROGRESSION SIGNATURE BASED ON ISOFORM EXPRESSION PATTERNS

Sadarangani, Anil P.[1], Court, Angela[2], Chun, Hye-Jung[3], Barrett, Christian L.[2], Minden, Mark D.[4], Storb, Rainer F.[5], Frazer, Kelly[2], Jamieson, Catriona HM[1]
[1]Medicine, University of California San Diego Moores Cancer Center, La Jolla, CA, USA, [2]University of California San Diego Moores Cancer Center, La Jolla, CA, USA, [3]Canada's Michael Smith Genome Sciences Center, BC Cancer Agency, Vancouver, BC, Canada, [4]The Princess Margaret Hospital, Toronto, ON, Canada, [5]Fred Hutchinson Cancer Research Center, Seattle, WA, USA

This study details a RNA transcriptome-based method to identify stage-specific cancer stem cells (CSC) in myeloid leukemic disorders. While previous studies have described CSC signatures that can predict disease stage in chronic myeloid leukemia (CML) or in acute myeloid leukemia (AML), these are primarily based on gene expression and/or microarray analysis techniques. In contrast, our refined progression signature identifies the differential expression profile of 1) whole transcriptome isoforms, 2) cluster differentiation (CD) surface antigen isoforms, 3) non-coding (nc) RNA isoforms, and 4) whole gene expression. The signature was developed by analysis of FACS-sorted CSC (CD34+CD38+Lin-) from primary CML samples taken from patients in blast crisis (BC, n=8) versus chronic phase (CP, n=9), and from myelodysplastic syndrome (MDS, n=7) versus secondary AML (n=7). These novel transcriptomics-based signatures allow more precise identification and characterization of CSC corresponding to specific disease progression states. Moreover, these unique expression profiles may be implemented in clinical trials to aid in patient diagnostics, prognostics, and evaluation of therapeutic response. This paves the way for patient-based personalized CSC medicine and could have broader application to other CSC-driven malignancies.

W-1110
DOSE DEPENDENT EFFECT OF OCT4A ON BRAIN TUMOR AGRESSIVENESS

Silva, Patricia B G[1], Rodini, Carolina O.[1], Kaid, Carolini[1], Furukawa, Gabriela[2], Okamoto, Oswaldo[3]
[1]University of São Paulo, São Paulo, Brazil, [2]University of São Paulo, Sao Paulo, Brazil, [3]University of Sao Paulo, São Paulo, Brazil

Medulloblastoma is a common form of embryonic tumor of the CNS in which expression of OCT4 has been recently associated with poor survival. However, the contribution of this transcription factor to medulloblastoma aggressiveness is still poorly understood. In this work, we have investigated the specific effects of the OCT4A isoform on tumorigenic properties of human medulloblastoma cells and underlying molecular alterations. Two retroviral-mediated expression of OCT4A were performed in medulloblastoma DAOY cells, achieving distinct overexpression levels. Real time PCR revealed that native DAOY cells express low levels of OCT4A compared with human embryonic stem cells. Upon overexpression, cells with increments of about 67 and 246 times in OCT4A transcripts were obtained, respectively. Such pattern of OCT4A overexpression was confirmed at the protein level by western blotting. Cells overexpressing OCT4A generated significantly higher amounts of colonies when cultivated in soft agar, indicating enhanced anchorage-independent cell proliferation. Noteworthy, colonies of OCT4A overexpressing cells were larger than those of control cells. Such effect on colony formation was dose-dependent with respect to the level of OCT4A expression. A similar dose-dependent effect was found in a subcutaneous model of medulloblastoma in Balb/C nude mice, in which OCT4A overexpressing cells displayed enhanced in vivo tumorigenesis. Altogether, these results suggest pro-tumorigenic effects of OCT4A in medulloblastoma. To investigate the molecular alterations that could contribute to the OCT4A-induced tumor cell phenotype, a global gene expression analysis was performed and revealed a significant enrichment of differentially expressed genes functioning in tissue and system development, cell differentiation, cell cycle, cell adhesion and immune system. Additionally, enrichment of differentially expressed genes involved in neuron function and development, apoptosis, cell migration, response to hypoxia, blood vessel development, inflammatory response and cytokine-cytokine receptor interaction were detected in the more aggressive tumor cells displaying the highest level of OCT4A overexpression. These findings indicate that expression of OCT4A, an isoform with well characterized function in pluripotency and stem cell self-renewal, contribute to medulloblastoma development and aggressiveness.

W-1111

PITUITARY TUMORS CONTAIN A SIDE POPULATION WITH 'TUMOR STEM CELL'-ASSOCIATED CHARACTERISTICS

Vankelecom, Hugo[1], Gremeaux, Lies[1], van Loon, Johan[2], Bex, Marie[3], Cristina, Carolina[4], Becú-Villalobos, Damasia[5], Mertens, Freya[1]

[1]Development and Regeneration, Cluster Stem Cell Biology and Embryology, Unit Stem Cell Research, KU LEUVEN (University of Leuven), Leuven, Belgium, [2]Neurosurgery, UZ LEUVEN (University Hospital Leuven), Leuven, Belgium, [3]Endocrinology, UZ LEUVEN (University Hospital Leuven), Leuven, Belgium, [4]National University of the Northwest of Buenos Aires Province (UNNOBA), Pergamino, Argentina, [5]Lab. Pituitary Regulation, Biology and Experimental Medicine Institute, National Research Council of Argentina (IBYME-CONICET), Buenos Aires, Argentina

Pituitary tumors represent the most frequent pathology of the gland and cause severe morbidity due to hormone dysregulation and invasion in neighboring brain regions. Little is known on pituitary tumor pathogenesis. In a variety of cancers, tumor-driving 'cancer stem cells' (CSC) represent a mechanistically as well as therapeutically valuable concept. Here, we started to search for CSC (or better, 'tumor stem cells') in pituitary adenomas by applying the side population (SP) technology. SP cells are identified as Hoechstlow cells using dual-wavelength flow cytometry, based on their efficient efflux capacity. In several tumor types the SP has been found enriched in (candidate) CSC. A SP was detected in all pituitary tumors obtained after surgery, irrespective of hormonal phenotype (~2% of the adenoma cells; n=60). Further phenotyping revealed the presence of a varying number of CD31+ endothelial cells (6-95%) and CD45+ hematopoietic cells (0.2-67%) in the SP. Whole-genome expression profiling of the $CD31^-/CD45^-$ SP (purified SP or pSP; 0.5% of all $CD31^-/CD45^-$ adenoma cells) versus the bulk $CD31^-/CD45^-$ main population (pMP) showed upregulation of several 'tumor stemness' markers and, intriguingly, of genes pointing to epithelial-mesenchymal transition (EMT). EMT has recently been identified as a major driver in the generation and activity of CSC. RT-qPCR exposed the EMT markers and regulators VIM, SNAI2 and ZEB2 as most prominently expressed in the pSP, together with the 'tumor stemness' genes CD44, CXCR4, and NOTCH2/HES1. The human pituitary adenomas were found to contain self-renewing sphere-forming cells, considered a general property of CSC/'tumor stem cells'. The sphere-forming cells segregated to the tumor's pSP, although only very few spheres could be obtained in these conditions. Human pituitary adenomas, typically benign, were found incapable to grow in immunodeficient mice. Therefore, in vivo tumorigenic capacity of adenoma (pSP) cells could not be tested. In addition, primary human pituitary adenoma cells do not grow (well) in culture. Hence, we further turned to the well-established (mouse) pituitary tumor cell line AtT20.

The cell line was found to contain a SP (~1%) and to induce tumor growth after s.c. injection in SCID mice. We tested the impact of the EMT-regulatory pathways TGFβ and Cxcr4. AtT20-derived xenograft tumor growth was not affected by TGFβ activation, but was reduced by Cxcr4 inhibition. The above-mentioned microarray analysis also suggested upregulated expression of the pituitary stem cell marker SOX2 in the human pSP. Higher Sox2 expression was also found in the AtT20 SP (versus the MP). The link of Sox2 expression with pituitary tumors was further investigated in the dopamine receptor D2 knock out (Drd2-/-) mouse in which pituitary tumors (prolactinomas) develop de novo. The absolute number of $CD31^-/CD45^-$ SP cells, as well as of colony-forming and Sox2+ cells, is found higher in the tumorous Drd2-/- pituitaries than in control. The Drd2-/- pituitary contains some expanded Sox2+ stem-cell niche regions. In addition, Sox2+ cell clusters occur within the prolactinomas. Lineage tracing is now performed to examine the relationship between the pituitary stem cells and the tumor. In conclusion, we detected a SP in pituitary adenomas and identified 'tumor stem cell'-associated characteristics. Our study may contribute to a better understanding of pituitary tumor pathogenesis, and lead to the identification of new therapeutic targets.

W-1112

TUMOR SUPPRESSOR ROLE OF HIF-1ALPHA IN MYELOID MALIGNANCIES

Velasco, Talia, Hyrenius-Wittsten, Axel, Cammenga, Jorg

Lund University, Lund, Sweden

Acute Myeloid Leukemia (AML) is a hematological disease caused by diverse cytogenetic and molecular alterations. While new therapeutic approaches are desperately needed, the genetic heterogeneity makes difficult to develop targeted therapies for this disease. AML shows a hierarchical cellular organization with some primitive cells known as Leukemia-Initiating Cells (LICs) at the top of the hierarchy. LICs are known to stay in a quiescent state, which makes them resistant to chemotherapy and responsible for the relapse of AML. Hypoxia signaling has been established as one of the main regulators in keeping hematopoietic stem cells (HSCs) in quiescence. Better understanding of the molecular response to hypoxia in LICs could lead to develop new therapies for this complex and highly heterogeneous disease. We used retroviral expression of different leukemic oncogenes in HSCs from conditional HIF-1α knock-out mice, the main protein involved in the hypoxic response. Expression of oncogenes that are regulating HIF-1α(like HoxA9-Meis1 and MLL fusion proteins) and those that have no described effect on HIF-1α signaling (like AML1-ETO9a) have been studied. Additionally, we have investigated how the phenotype of Flt3-ITD-induced myeloproliferative disease (MPD) is influenced by deletion of HIF-1α. Although primary AMLs originated from the different oncogenes showed no differences in disease latency derived from HIF-1α -/- and HIF-1α +/+ cells, we observed a faster myeloproliferation of HIF-1α -/- cells. Secondary leukemias derived from HIF-1α -/- cells showed a shorter latency in the case of AML-ETO9a-induced AML, arguing for an increased proliferation of LICs lacking HIF-1α. However, we did not observe the same effect in HoxA9-Meis1-induced AML, whose latency showed no differences regarding the status of HIF-1α. Flt3 ITD/+ HIF-1α -/- transgenic animals showed a more severe MPD phenotype, indicated by higher white blood cell (WBC) counts, increased myeloid cells in peripheral blood and bigger spleens than Flt3 ITD/+ HIF-1α +/+ mice. This phenotype was transplantable into secondary recipients indicating a cell intrinsic effect. We could neither observe a change in phenotype nor a delay or prevention of leukemia development in hematopoietic cells lacking Hif-1α. In contrary, we found that in the majority of these leukemia models, deletion of Hif-1α resulted in increased proliferation and an enhanced phenotype. These results lead us to conclude that HIF-1α function is not required for the initiation and progression of myeloid malignancies induced by these genetic alterations and that its absence instead could accelerate the progression of the disease mainly by enhancing the cycling of the LICs in a transient way depending on the driver mutation.

W-1113

CHEMOGENOMIC PROFILING OF HUMAN GLIOMA INITIATING CELLS REVEALS A STEMNESS-RELATED SENSITIVITY TO ION FLUXES

Wee, Shimei[1], Niklasson, Mia[2], Marinescu, Voichita[2], Linnarsson, Sten[3], Uhrbom, Lene[2], Nelander, Sven[2], Andäng, Michael[1]
[1]Physiology and Pharmacology, Karolinska Institutet, Stockholm,Sweden, [2]Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden, [3]Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden

Tumor-initiating cells found in aggressive cancers share traits such as the ability of self-renewal and differentiation with embryonic stem cells. In malignant glioma, a subpopulation of cells known as glioma-initiating cells (GICs) exhibits this stem cell-like phenotype, and is more resistant to chemotherapy and radiation therapy than the differentiated tumor cells. Using RNA sequencing, we found that a large number of ion-related and ion channel specific signaling genes were downregulated as GICs began to differentiate. We also showed functional ion signaling sensitivity using a small molecule screen and post-screen dose response analysis, and this ranked the undifferentiated GIC lines according to their expression of stemness markers. The results show that the ion signaling is a target mechanism that may allow efficient eradication of the therapy resistant tumor initiating subpopulation.

W-1114

PODOCALYXIN-LIKE 1 PROMOTES INVADOPODIA FORMATION AND METASTASIS THROUGH ACTIVATION OF RAC1/CDC42/CORTACTIN SIGNALING IN BREAST CANCER CELLS

Wu, Han-Chung[1], Lin, Cheng-Wei[1], Sun, Min-Shiou[1], Liao, Mei-Ying[1], Yu, John[1], Lu, Jean[2]
[1]Institute of Cellular and Organismic Biology, Academia Sinica, Taipei, Taiwan, [2]Genomics Research Center, Academia Sinica, Taipei, Taiwan

The study of stem cell markers that determine the fate of a stem cell is interesting from both fundamental biology and clinical applications in regenerative medicine and cancer therapy perspectives. Recently, we have used hybridoma or phage display technology to identify surface markers of human embryonic stem cells (hESCs), and we have successfully generated more than 10 monoclonal antibodies which recognized undifferentiated hESCs but not differentiated hESCs. Immunoaffinity chromatography and LC/MS/MS were used to identify hESC-Ab-5-22; a monoclonal antibody that recognized specific membrane protein of undifferentiated hESCs and cancer stem cells (CSCs). We further verified the target protein of hESC-Ab-5-22 to be an unique form of highly sialylated podocalyxin-like protein 1 (PODXL). Herein, we demonstrated that PODXL is overexpressed in breast tumor cells and elevated in lymph node metastatic cancer. Mechanistically, we found that the expression of PODXL was associated with cell motility and invasiveness. Suppression of PODXL in MDA-MB-231 cells reduced lamellipodia formation, and FAK and paxillin phosphorylation. Inhibition of PODXL reduced colocalization of F-actin with cortactin, and suppressed phosphorylation of cortactin and N-WASp. Conversely, overexpression of PODXL in MCF7 cells induced F-actin/cortactin colocalization and enhanced invadopodia formation and activation. Suppression of PODXL showed similar effects on invadopodia activity and tumor invasion as those in cortactin-knockdown cells. We further found PODXL to be associated with cortactin. Deletion of the DTHL motif in PODXL abrogated the association with cortactin and inhibited Rac1/Cdc42 activation and invadopodia activity. Suppression of PODXL inhibited Rac1/Cdc42 activity, and blockage of Rac1/Cdc42 diminished cortactin phosphorylation and its association with PODXL. Moreover, inhibition of PODXL in MDA-MB-231 cells suppressed tumor colonization in the lungs and distant metastases, similar to those in cortactin-knockdown cells. DNA microarray analysis data revealed that suppression of PODXL significantly affected subsets of genes associated with extracellular matrix organization, the epithelial-mesenchymal transition, and metastasis-related cytokine expressions. These findings show that overexpression of PODXL enhanced invadopodia formation and tumor metastasis by inducing Rac1/Cdc42/cortactin signaling network.

EMBRYONIC STEM CELL CLINICAL APPLICATION

W-1115

DEVELOPING A HUMAN ES CELL DERIVED DOPAMINE NEURON SOURCE FOR CELL THERAPY IN PARKINSON'S DISEASE - FROM ACADEMIC TO TRANSLATIONAL RESEARCH

Irion, Stefan[1], Tomishima, Mark[1], Riviere, Isabelle[1], Rutishauser, Urs[1], El-Maarouf, Abderrahman[1], Wakeman, Dustin R.[2], Kordower, Jeffrey H.[3], Sadelain, Michel[4], Henchcliffe, Claire[5], Tabar, Viviane[1], Studer, Lorenz[1]
[1]Memorial Sloan Kettering Cancer Center, New York, NY, USA, [2]Rush University, Chicago, IL, USA, [3]Neurological Sciences, Neurosurgery, Rush University, Chicago, IL, USA, [4]Department of Human Genetics, Memorial Sloan Kettering Cancer Center, New York, NY, USA, [5]Weill Cornell Medical Center, New York, NY, USA

The discovery of pluripotent stem cells will change the scope of treatment options for human diseases. Today several stem cell therapies are on the verge of clinical translation and funding agencies, such as New York State and its NYSTEM office, realize the potential of these findings. By providing laboratories with the necessary funds they enable these crucial, translational steps. Our team secured a 4-year contract with the stem cell funding agency for New York State (NYSTEM) with a specific goal to file an 'investigational new drug (IND)' application for Parkinson's disease (PD) by 2017. PD is the second most common neurodegenerative disorder and affects nearly 5 million patients worldwide, a number predicted to more than double by 2030. A fundamental characteristic of PD is the progressive, severe and irreversible loss of specific dopamine-producing neurons in the midbrain, resulting in disabling motor dysfunction. Multiple therapies have been developed for PD but none can restore the function of the lost cells. Despite the challenges encountered in the fetal graft trials, cell therapy for PD remains attractive due to the possibility of restoring actual dopamine (DA) neurons capable of integration into the host circuitry. After extensive efforts in labs across the world, our team has recently made a major discovery that enables the derivation of nearly unlimited numbers of authentic, engraftable midbrain DA (mDA) neurons from hESCs. This discovery has the potential to become the first in human clinical trial for PD using hESC derived mDA neurons. We entered the early stages of this 4-year project and are presenting our current progress towards an FDA approval for this novel therapy for PD. In this presentation we will use a dopaminergic neuron Parkinson's disease therapy as an outline to highlight the unique challenges one faces when moving a promising finding from the bench to the clinic. For our group, this meant assessing the relative risks of hESCs vs. induced pluripotent stem cells (iPSCs). We selected hESCs but then had to do a similar risk analysis on the different hESC lines. We next had to decide on the ideal media formulation for the expansion of hESCs and are now adapting the differentiation process to be compatible with current good manufacturing practices (cGMP).

In parallel, work to set-up a suitable, physical laboratory for on-site manufacturing of the clinical product was started and is now nearing completion. The second large class of experiments is designed to make sure that the product is safe, functional and well tolerated and we have designed a suite of tests that will address the safety, toxicology and efficacy of the differentiated cells. Lastly, we have a team of clinicians that define the target patient population, the surgical procedure and the immunosuppression regimen. For any group attempting this, it will become clear that this effort requires multiple disciplines and broad support from the host institution. This has significant impact on how teams collaborate and requires a step outside the usual academic lab practices. Our presentation will focus on the process of clinical translation, the considerations necessary for cGMP manufacturing and point out the differences between academic and translational research. We believe that sharing our experience with the stem cell community will be of great value to all attendants.

W-1116
DISCRIMINATION BETWEEN HUMAN EMBRYONIC STEM AND HUMAN EMBRYONAL CARCINOMA CELLS USING GLYCAN SIGNATURE ON PLURIPOTENT STEM CELL MARKER, PODOCALYXIN

Itakura, Yoko[1], Kuno, Atsushi[2], Hirabayashi, Jun[2], Umezawa, Akihiro[3], Toyoda, Masashi[1]
[1]Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan, [2]National Institute of Advanced Industrial Science and Technology, Ibaraki, Japan, [3]National Institute for Child Health and Development, Tokyo, Japan

Human embryonic stem cells (hESCs) and human embryonal carcinoma cells (hECCs) have been extensively used for stem cell research. Although these cells are known to share many properties including high developmental capability and cell surface antigens, such as SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81. However, their origins are basically different: hESCs are derived from inner cell mass of blastocysts cultured *in vitro*, while hECCs are from malignant pluripotent stem cell lines of human germ tumor origins. The pluripotent stem cell markers common to hESCs and hECCs have been used for characterization of induced pluripotent stem cells (iPSCs). These high development capable cells have concerns about risk of tumorigenesis in cell transplantation therapy. In this context, development of specific markers to distinguish hESCs from hECCs is of clinical value. In other words, effort to distinguish hESCs and hECCs will helpful for diagnosis of pluripotent stem cells used for regenerative therapy, e.g., ESCs, iPSCs and other pluripotent stem cells. In this study, we focused our glycan analysis on a carbohydrate-rich glycoprotein, podocalyxin, known as a carrier of TRA-1-60 and TRA-1-81 antigens, which represent hESC glycan markers. The target glycoprotein semi-quantified by immunoblotting was enriched from the cell extracts by immunoprecipitation, and the glycosylation differences occurring between hESCs and hECCs were systematically analyzed by an advanced technology of lectin microarray, antibody-overlay lectin profiling (ALP). Profiles of human embryonic bodies (hEBs) differentiated from hESCs were also analyzed. A glycan profile of podocalyxin from hECCs was significantly different from that of hESCs. Lectin signals corresponding to α2-6 linked sialic acid were elevated in the hECCs, and glycosidase digestions further revealed significant difference in the non-reducing terminal and penultimate structures. These results demonstrate that the present procedure with focus on a particular glycoprotein could enhance relatively small but significant differences between closely related cells like hESCs and hECCs at the glycome level. The present finding will be helpful to develop a diagnostic method to distinguish undifferentiated stem cells from differentiated ones used for regenerative therapy.

W-1117
INHIBITION OF PLURIPOTENT STEM CELL-DERIVED TERATOMA FORMATION BY SMALL MOLECULES

Jeong, Hochang[1], Lee, Mi-Ok[2], Moon, Sung Hwan[3], Kim, Kwang-Soo[4], Cha, Hyukjin[1]
[1]Sogang University, Seoul, Republic of Korea, [2]KRIBB, Daejeon, Republic of Korea, [3]Konkuk University, Seoul, Republic of Korea, [4]Harvard Medical School McLean Hospital, Belmont, MA, USA

The future of safe cell-based therapy rests on overcoming teratoma/tumor formation, in particular when using human pluripotent stem cells (hPSCs), such as human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). Because the pre- sence of a few remaining undifferentiated hPSCs can cause un- desirable teratomas after transplantation, complete removal of these cells with no/minimal damage to differentiated cells is a prerequisite for clinical application of hPSC-based therapy. Having identified a unique hESC signature of pro- and antiapoptotic gene expression profile, we hypothesized that targeting hPSC-specific antiapoptotic factor(s) (i.e., survivin or Bcl10) represents an efficient strategy to selectively eliminate pluripotent cells with teratoma potential. Here we report the successful identification of small molecules that can effectively inhibit these antiapoptotic factors, leading to selective and efficient removal of pluripotent stem cells through apoptotic cell death. In particular, a single treatment of hESC-derived mixed population with chemical inhibitors of survivin (e.g., quercetin or YM155) induced selective and complete cell death of undifferentiated hPSCs. In contrast, differentiated cell types (e.g., dopamine neurons and smooth-muscle cells) derived from hPSCs survived well and maintained their functionality. We found that quercetin-induced selective cell death is caused by mitochondrial accumulation of p53 and is sufficient to prevent teratoma formation after transplantation of hESC- or hiPSC-derived cells. Taken together, these results provide the "proof of concept" that small-molecule targeting of hPSC-specific antiapoptotic path- way(s) is a viable strategy to prevent tumor formation by selectively eliminating remaining undifferentiated pluripotent cells for safe hPSC-based therapy.

W-1118
CD13 MODULATION ENHANCES TRANSPLANT OUTCOMES

Kim, Jiyeon, Shapiro, Linda H.
Cellular and Vascular Biology, University of Connecticut Health Center, Farmington, CT, USA

Stem cell based reparative therapies are being introduced in the clinic for patients suffering from diseases that are either refractory to current standards of care or do not have any available treatments. Most of the new studies approved for clinical trials involve the use of autologous adult stem cells which circumvent the problem of immune-mediated rejection, but fall short of tapping into the enormous potential of pluripotent embryonic stem cells which would provide a theoretically unlimited source of any cell type in the body. While the landmark generation of induced pluripotent stem cells has provided the foundation for personalized stem cell therapies, current limitations of cost, efficiency and scalability have hampered transition of these cells into general practice. The discovery of transplant-specific immunoregulatory mechanisms is critical to the expeditious and effective application of non-autologous stem cell treatments. We have identified CD13 as a novel and specific modulator of transplant immunity. Using CD13 transgenic mice and mouse embryonic stem cells, we show that CD13 affects not only the survival of transplants, but also growth and development of teratomas in immune competent mice. As a highly expressed cell surface protein on myeloid cells,

activated endothelial cells and stem cells, CD13 is implicated in a variety of inflammatory disorders, but has yet to be studied in the context of transplant immunity. In this study, we identify and elucidate the role of CD13 as a potent regulator of immune responses and demonstrate its utility by applying ES-derived therapeutic cells to immune competent mice.

W-1119
MOTOR RECOVERY AND PERSISTENT MICROGLIA ACTIVATION AFTER TRANSPLANTATION OF HUMAN DOPAMINERGIC CELLS FROM PLURIPOTENT STEM CELLS IN ANIMAL MODELS OF PARKINSON´S DISEASE

Leal, Maria C[*1], Peng, Jun[*2], Wenker, Shirley[3], Farías, Maria I.[3], Ferrari, Carina C.[4], Depino, Amaicha[5], Nuñez, Myriam C.[6], Fernicola, Marcela[6], Radice, Pablo[3], Cavaliere Candedo, Verónica L.[3], Zeng, Xianmin[2], Pitossi, Fernando[3]
[1*] These authors contributed equally to this work.
Leloir Institute, Buenos Aires, Argentina, [2]Buck Institute for Research on Aging, Novato, CA, USA, [3]Leloir Institute, Buenos Aires, Argentina, [4]Italian Hospital, Buenos Aires, Argentina, [5]Faculty of Exact and Natural Sciences, University of Buenos Aires, Buenos Aires, Argentina, [6]Department of Mathematics, Faculty of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina

Parkinson's Disease (PD) is a neurodegenerative disorder mainly characterized by the degeneration of dopaminergic neurons of the substantia nigra pars compacta (SN), leading to a dysfunctional nigro-striatal pathway. Transplantation of dopamine-producing cells into the striatum has already shown its effectiveness in animal models and clinical trials, although undesired complications in a number of cases and the lack of Good Manufacture Practice (GMP)-grade cell preparation precludes its wide application in the clinic. Another major caveat of this approach is the low level of cell survival after transplantation We sought to test the effects of the striatal transplantation of dopaminergic neurons derived from human embryonic stem cells using a GMP-compatible process into rat models of Parkinson's Disease (PD). Adult rats were denervated by the inoculation of 6-OHDA, tested for motor asymmetry by the cylinder test and transplanted with 8 x 105 dopaminergic neurons or control cells into the striatum. Control cells were human pluripotent stem cells subjected to a similar differentiation protocol as the dopaminergic cells, but harvested 4 days before they start producing dopamine. Animals were immunosuppressed with cyclosporine A one day before transplantation and throughout the experiment. Pharmacological and non-pharmacological tests showed a clear improvement in motor behavior of animals transplanted with dopaminergic cells but not the control cells 12-13 weeks after transplantation. Persistent microglial activation at the site of transplantation was observed by MHC-II, ED-1 and GSA staining 90 days post-transplantation in dopaminergic- and control cells-treated animals. Similar experiments are being conducted in an inflammation-based animal model of PD. We conclude that the transplant of this GMP-compatible preparation of human dopaminergic neurons provides a robust motor recovery in the animals tested. In addition, the chronic microglia activation observed at the site of transplantation opens up the possibility of increasing neuronal survival and differentiation by immunomodulaton.

W-1120
INTERVENTIONAL MAGNETIC RESONANCE IMAGING (MRI)-GUIDED CELL TRANSPLANTATION INTO THE BRAIN WITH RADIALLY BRANCHED DEPLOYMENT (RBD)

Silvestrini, Matthew[1], Yin, Dali[1], Martin, Alastair[1], Coppes, Valerie[1], Zeng, Xianmin[2], Panter, Scott[1], Desai, Tejal[1], **Lim, Daniel**[1]
[1]University of California, San Francisco, San Francisco, CA, USA, [2]Buck Institute for Research on Aging, Novato, CA, USA

Cell transplantation to the brain is being pursued for as a treatment for many neurological diseases, and effective cell delivery is critical for clinical success. Currently, cell transplantation is performed with straight cannulas inserted with frame-based stereotaxy. We have previously demonstrated that radially branched deployment (RBD) of a cell delivery catheter at multiple points along the initial cannula penetration tract facilitates cell distribution. However, RBD performed with traditional frame-based stereotaxy does not provide "real-time" monitoring of cell delivery. Interventional magnetic resonance imaging (iMRI) enables such real-time monitoring of stereotactic procedures. Here we describe an iMRI-guided RBD system for intracerebral cell transplantation. We developed an RBD system with FDA-approved, MRI-compatible materials. Our iMRI-guded RBD system functioned as an "add-on" to a commercially-available stereotactic platform and software system, which can be used in essentially any clinical 1.5T MRI. Catheter deployment was highly precise, and we used iMRI-guided RBD to deliver multiple deposits of human embryonic stem cell-derived dopaminergic (hDA) neurons to swine striatum with "real-time" monitoring and guidance. hDA cells were biocompatible with the RBD system and efficiently delivered with this novel neurosurgical system. Testing in human cadaveric heads demonstrated function of iMRI-guided RBD at the human scale: delivery to the entire human putamen - a target relevant for the treatment of Parkinson's disease - could be achieved via a single initial cannula insertion. In conclusion, iMRI-guided RBD overcomes many of the technical limitations related to the use of straight cannulas and indirect stereotactic targeting, thus addressing a critical bottleneck to clinical translation. This neurosurgical device and approach may facilitate a wide range of clinical trials involving intracerebral cell transplantation.

W-1121
RESCUE OF INJURED MOTONEURONES BY GRAFTED NEUROECTODERMAL STEM CELLS: EFFECT OF THE LOCATION OF GRAFT

Pajenda, Gholam[1], Pajer, Krisztian[2], Marton, Gabor[2], Redl, Heinz[3], Nogradi, Antal[2]
[1]Department for Trauma Surgery, Medical University Vienna, Vienna, Austria, [2]Department of Anatomy, Histology and Embryology, Faculty of Medicine, University of Szeged, Hungary, Szeged, Hungary, [3]Research Centre of the Austrian Workers' Compensation Board, Ludwig Boltzmann Institute for Experimental Traumatology, Vienna, Austria

Purpose: Avulsion of one or more ventral roots from the spinal cord leads to the death of the majority of affected motoneurons. In this study we investigated whether immortalized clonal neuroectodermal stem cells applied to the injured cord in various ways impart neuroprotection on motoneurons otherwise destined to die. Methods: The lumbar 4 (L4) ventral root of Sprague-Dawley rats was avulsed and reimplanted ventrolaterally into the injured cord. Clonal neuroectodermal murine stem cells (NE-GFP-4C) were placed in fibrin clot around the reimplanted root, were injected immediately following avulsion into the reimplanted ventral root or directly into the L4 segment. Three months after the primary surgery the L4 motoneuron pool was retrogradely labelled with Fast blue and the numbers of reinnervating

motoneurons were determined. Functional recovery was tested biweekly through the use of the CatWalk automated gait analysis system. Results: Transplantation of neuroectodermal stem cells into the reimplanted root or into the L4 spinal segment resulted in similarly extensive regeneration of the motoneurons (671 ± 26 and 711 ± 14 L4 motoneurons, respectively). In these groups significant functional recovery was achieved. The negative controls and animals with periradicular stem cell treatment showed poor motor recovery and reinnervation (42 ± 10 and 65 ± 2.5, respectively). Conclusion: This study provides evidence that neuroectodermal stem cell transplantation into the reimplanted ventral root induces as successful regeneration of injured motoneurons as stem cells grafted into the spinal cord.

GERMLINE CELLS

W-2001
EXPANSION OF MOUSE SPERMATOGONIAL STEM CELLS USING A NOVEL FEEDER-FREE CULTURE SYSTEM

Choi, Na Young, Park, Yo Seph, Ryu, Jae-Sung, Lee, Hye Jeong, Ko, Kinarm
Department of Stem Cell Biology, School of Medicine, Konkuk University, Seoul, Republic of Korea

Male spermatogonial stem cells (SSCs, also called germline stem cells) are self-renewing unipotent stem cells that produce differentiating germ cells in the testis. SSCs can be isolated from the testis and cultured *in vitro* for long-term periods in the presence of feeder cells (often mouse embryonic fibroblasts). However, the maintenance of SSC feeder culture systems is tedious because preparation of feeder cells is needed at each subculture. In this study, we developed a Matrigel-based feeder-free culture system for long-term propagation of SSCs. Although several *in vitro* SSC culture systems without feeder cells have been previously described, our Matrigel-based feeder-free culture system is time- and cost-effective, and better preserves self-renewability of SSCs. In addition, the growth rate of SSCs cultured using our newly developed system is equivalent to that in feeder cultures. We confirmed that feeder-free cultured SSCs expressed germ cell markers both at the mRNA and protein levels. Furthermore, the functionality of the established SSCs was confirmed by their transplantation into germ cell-depleted mice. These results suggest that our newly developed feeder-free culture system provides a simple approach to maintaining SSCs *in vitro* and studying the basic biology of SSCs, including determination of their fate.

W-2003
TERT IS DYNAMICALLY REGULATED AND CO-EXPRESSED WITH OCT4 DURING GERM CELL DEVELOPMENT

Garbuzov, Alina[1], Pech, Matthew F.[2], Zhang, Ruixuan J.[3], Artandi, Steven[3]
[1]Genetics, Stanford Univerisity, Stanford, CA, USA, [2]Cancer Biology, Stanford Univerisity, Stanford, CA, USA, [3]School of Medicine, Stanford Univerisity, Stanford, CA, USA

Telomerase plays an important role in the maintenance of highly proliferative organs, including the gonad. Unlike somatic tissues, telomerase activity is sufficiently high in the germline to fully blunt telomere shortening with age. We hypothesized that high telomerase activity would be a feature of self-renewing cells of the germline in the adult and during development. We generated TERT-Tomato transcriptional reporter mice, which enables the robust isolation of telomerase-positive cells. Remarkably, FACS and wholemount analysis of the embryonic gonad revealed TERT-Tomato expression closely follows the OCT4 (POU5F1) profile. Like OCT4, TERT is expressed in the entire epiblast at dpc 7.5 and TERT-Tomato expression is restricted to primordial germ cells (PGCs) by dpc 9.5. During the migratory phase of PGC development, we detect a heterogeneity in TERT expression, with both a TERT+ and a TERT- population present at dpc 9.5-10.5. In contrast to previous reports, we observed TERT-Tomato expression and high telomerase activity in both mitotically active and quiescent PGCs. Mitotically arrested male PGCs continue to express high levels of TERT until their commitment to differentiation and entry into meiosis in the young adult testis. Female PGCs down-regulate TERT at dpc 14.5, also upon entry into meiosis. We propose that high telomerase activity is a hallmark of the undifferentiated cells of the germ cell lineage and has evolved to prevent inter-generational telomere attrition. Our data also suggests that TERT and OCT4 are co-regulated and both act as markers of the totipotency cycle.

W-2004
DNA METHYLTRANSFERASE 1 IS REQUIRED FOR MAINTAINING CYTOSINE METHYLATION DURING PGC REPROGRAMMING

Hargan Calvopina, Joseph[1], Lee, Serena[1], Taylor, Sara[1], Cook, Helene[2], Clark, Amander T.[3]
University of California, Los Angeles, Los Angeles, CA, USA, [2]MCDB, University of California, Los Angeles, Los Angeles, CA, USA, [3]University of California, Los Angeles, Los Angeles, CA, USA

Reprogramming in PGCs is essential for erasing the epigenetic memory of the parental genome in order to establish a new epigenetic landscape based on the sex of the embryo. Failure to reset parental epigenetic memory results in either infertility, or birth defects in the next generation. At the time of specification at embryonic (e) day 7.5, PGCs have high levels of cytosine methylation. From e8.0 to e13.5 cytosine demethylation occurs in two distinct phases. The first phase involves the global loss of cytosine methylation before e9.5, with retention of cytosine methylation at specific loci in the genome including maternally methylated imprinting control centers (ICCs), germline genes that are required at later stages of development and specific retrotransposon sequences. Phase 2 DNA demethylation occurs in PGCs from e10.5 to e13.5 in a time and locus-specific manner that is regulated in part by Tet1. Maintenance of cytosine methylation at specific loci during phase 1 DNA demethylation could involve either protection against demethylation, or maintained activity of de novo methyltransferases. Given that maintenance DNA methyltransterase 1 (Dnmt1) is expressed throughout PGC development, whereas Dnmt3a and Dnmt3b are expressed at or below the limit of detection, we hypothesize that Dnmt1 functions to maintain cytosine methylation in PGCs in a Uhrf1 independent manner. To address this, we created a conditional germline knockout of Dnmt1 using the Dnmt12lox/2lox strain crossed to Blimp1-Cre (BC). Analysis of BC; Dnmt11lox/1lox PGCs revealed that Dnmt1 is not required for PGC specification. Instead we found that loss of Dnmt1 leads to precocious expression of the gonadal stage germline gene VASA. In order to confirm that Dnmt1 is responsible for maintaining methylation in PGCs we crossed Dnmt12lox/2lox to Oct4-GFP and using this strain we will examine cytosine methylation at ICCs using bisulfite sequencing. Taken together, we propose a model in which maintenance of cytosine methylation by Dnmt1 in the absence of Uhrf1 is essential to maintain cytosine methylation in PGCs until the time when 5-methyl cytosine is converted to 5-hydroxymethyl cytosine leading to Tet targeted replication-coupled demethylation.

W-2005

XENOTRANSFER OF ADULT EXOTIC BIRD GERMLINE STEM CELLS TO CHICKEN GALLUS GALLUS HOST EMBRYOS: AVIAN CONSERVATION IN THE 21ST CENTURY

Jensen, Thomas, Roe, Mandi, Durrant, Barbara
San Diego Zoo Institute for Conservation Research, Escondido, CA, USA

As advanced reproductive technologies have become routine in domestic animals over the past decades, it was inevitable they would be applied in the field of endangered species conservation. For avian conservation, techniques such as in vitro fertilization and cloning will not be a viable method for routine germplasm rescue. However, the xenotransfer of germline stem cells from deceased donor gonads to chicken host embryos show potential as a way to rescue valuable germplasm. In this study, adult testes were dissociated and the cells (including spermatogonia) were transferred to stage 14-17 chicken host embryos. Flow cytometry was used to quantify the PKH26 dyed donor cells' colonization of the host gonad. Fluorescent microscopy co-localization of stem cell specific antibodies on PKH dyed cells was used to confirm continual stem cellness of donor-derived cells. Gonadal cells from 16 deceased male birds were xenotransferred to host embryos, either freshly isolated or following culture. No effect of species was observed on the number of positive gonads (Levene's test p=0.92), and all species were combined for further analysis. Of the 84 host gonads analyzed, 5 of 6 (69%) fresh cell injected host embryos and 54 of 78 (83%) cultured cell injected host embryos contained donor-derived cells. The range of donor-derived cells per positive host gonad ranged from 0.05-0.43% (fresh cells) and 0.01-0.78% (cultured cells) of total host gonadal cells. No statistical difference was observed in number of positive gonads (Fisher's exact test p=0.66), or donor-derived cells/gonad (t-test t=0.40, df=57, p=0.69) following fresh or cultured cell transfers. PKH26 dyed donor-derived cells co-stained with stem cell marker antibodies indicates they maintained their stem cellness during host gonad organization. This study suggests it is feasible to rescue exotic adult bird germplasm. By collecting germline stem cells from deceased exotic birds, the reproductive lifespan of individuals from critically endangered species or otherwise genetically valuable individuals can be extended by transfer to an embryonic host. Furthermore, as this study included xenotransfers from 14 species in eight orders it implies germline stem cell migration during the early embryonic development may be highly conserved in birds.

W-2006

EFFECT OF STO FEEDER LAYER ON PROLIFERATION OF AVIAN SPERMATOGONIAL STEM CELLS IN SHORT-TERM CULTURE

Karimi, Rasoul
Animal Science, Tehran University, Karaj, Iran

Spermatogonial stem cells (SSCs) are exceptional adult stem cells that transfer genes to new generations and this unique potential makes them the perfect cells in the production of transgenic chickens. For this purpose, proliferation of SSCs is very important, as there is a few numbers of these cells (0.03%) among testis cells. Using a feeder layer for cell culture is an effective method for SSCs proliferation. The objective of this experiment was to evaluate the effects of STO (SIM mouse embryo-derived thioguanine and ouabain resistant) as a feeder layer on *in vitro* short-time culture of prepubertal chicken testicular stem cells. Testis cells harvested from *Rhode Island Red* chicken were cultured both on STO and non-STO surface for ten days. LIF and bFGF were used as growth factors. Several tests were conducted at the 7th and 10th day of culture, including: immunoflourecent staining, PAS staining (Periodic Acid-Schiff), and colony assay. Detected SSCs markers such as SSEA1, SSEA3 on both treatment were positive. Also, PAS test in both treatment showed proliferation of SSCs like cells during cell culture. The result of the Colony assay at the 7th day revealed a higher colony number as well as higher cell/colony on STO surface. Furthermore, the area of colonies formed on the STO feeder was significantly greater than the non-STO surface on day 7. In contrast the result of colony assay at the 10th day of culture were declined in both treatment. In conclusion the results have shown that STO is a suitable feeder layer for prepubertal *Rhode Island Red* chicken SSCs on *in vitro* culture and proliferation of chicken testicular stem cells.
Keywords: STO, Spermatogonial stem cell, Chicken testicular cell.

W-2007

DNA METHYLATION AND GENE EXPRESSION PROFILES DURING SPERMATOGONIAL STEM CELL GENERATION IN NEONATAL MOUSE TESTIS

Kubo, Naoki[1], Toh, Hidehiro[1], Shirane, Kenjiro[1], Shirakawa, Takayuki[2], Sato, Tetsuya[3], Kamizato, Yoshito[2], Sone, Hidetoshi[2], Sato, Yasuyuki[2], Tomizawa, Shin-ichi[2], Tsurusaki, Yoshinori[4], Kobayashi, Hisato[5], Shibata, Hiroki[6], Saitsu, Hirotomo[4], Matsumoto, Naomichi[4], Kono, Tomohiro[5], Suyama, Mikita[3], Ohbo, Kazuyuki[2], Sasaki, Hiroyuki[1]
[1]Division of Epigenomics and Development, Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan, [2]Department of Histology and Cell Biology, Yokohama City University, Yokohama, Japan, [3]Division of Bioinformatics, Multi-scale Research Center for Medical Science, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan, [4]Department of Human Genetics, Yokohama City University, Yokohama, Japan, [5]Department of BioScience, Tokyo University of Agriculture,, Tokyo, Japan, [6]Division of Genomics, Research Center for Transomics Medicine, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan

DNA methylation has an important role in mammalian germ cell development. In the male germline, the bulk genome is initially hypomethylated in primordial germ cells and becomes progressively methylated during fetal prospermatogonium stage. After birth, prospermatogonia start to differentiate into spermatogonia, which include the stem cell population that supports continuous spermatogenesis in adult life. To understand the epigenetic regulation of spermatogonial differentiation, we have determined the DNA methylation and gene expression profiles of prospermatogonia, undifferentiated spermatogonia and differentiating spermatogonia in neonatal testis. We revealed large partially methylated domains similar to those found in cancer cells and placenta, extremely high levels of non-CG methylation in neonatal prospermatogonia, and stage-specific differentially methylated regions potentially important for regulation of genes involved in stemness and/or differentiation. Our findings reveal unique epigenetic changes that may regulate the spermatogonial stem cell generation and differentiation.

W-2008

EVIDENCE OF STEM CELLS' IMMUNITY TO CELL DEATH IN DROSOPHILA MELANOGASTER

Xing, Yalan[1], Su, Tin Tin[2], Ruohola-Baker, Hannele[3]
[1]Biochemistry, University of Washington, Seattle, WA, USA, [2]Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO, USA, [3]Institute for Stem Cell and Regenerative Medicine University of Washington, Seattle, WA, USA

Stem cells are responsible for regenerating differentiated cells and tissues, especially in response to external stresses, such as injury, wounding, or nutrient deprivation, which frequently cause increasing apoptosis in the corresponding tissues. Some specific cell types, such as

cancer cells, have been demonstrated to be resistant to apoptosis, so that no efficient cell death can be induced to eliminate the deteriorate cells. In *Drosophila Melanogaster* germline and midgut, we have observed striking phenomenon that comparing to the differentiated progenies, the adult stem cells are more resistant to irradiation or chemical induced apoptosis. Furthermore, some well-studied cell survival genes, *pineapple eye (pie)* as an example, function differentially in stem cells--*pie* mutant stem cells are carefully exempted from apoptosis; they rather enter differentiation and leave the stem cell niche. This stem cell-specific role is through negative regulation on protein level of the transcription factor FOXO, by ubiquitin-mediated protein degradation. The potential FOXO target genes regulated in this process is now under investigation. Further exploration of the cell type-specific response to apoptosis and the mechanism underlying will extend knowledge to the understanding of the stem cells' apoptosis-resistant characteristics and future treatments towards cancer cells.

TOTIPOTENT / EARLY EMBRYO CELLS

W-2009
ROLE OF THE SOLUTE CARRIER, SLC2A2, EXPRESSED BY MURINE EMBRYONIC STEM CELLS ISOLATED IN PHYSIOLOGICAL GLUCOSE MEDIA IN EXOGENOUS GLUCOSAMINE TRANSPORT AND ENHANCEMENT OF SELF-RENEWAL AND ANAEROBIC AND ANABOLIC METABOLISM

Loeken, Mary R., Jung, Jin Hyuk
Section on Islet Cell and Regenerative Biology, Joslin Diabetes Center, Boston, MA, USA

The sugar transporter, Slc2a2, is expressed by preimplantation and early postimplantation mouse embryos. Slc2a2 is a high K_M (16 mmol/L) glucose (Glc) transporter as well as a low K_M (0.8 mmol/L) glucosamine (GlcN) transporter. Slc2a2 is unlikely to function as a Glc transporter for embryos, because it is not an efficient transporter at normal Glc concentrations (~5.5 mmol/L), and the embryo also expresses Slc2a1 and Slc2a3, whose K_M's for Glc are near normal Glc concentrations. Whether Slc2a2 functions as a GlcN transporter for the early embryo has not been investigated, however, *Slc2a2*$^{+/-}$ and *Slc2a*$^{-/-}$ embryos are produced at fewer than predicted Mendelian frequency, suggesting that it is important. We previously showed that murine embryonic stem cells (mESC) isolated and cultured in low Glc (5.5 mmol/L) media (referred to as LG-ESC) retain expression of Slc2a2, whereas traditional mESC maintained in conventional high Glc (25 mmol/L) media do not. GlcN-6-PO_4 is an intermediate in the hexosamine biosynthetic pathway, which leads to production of UDP N-Acetylglucosamine for O-glycosylation (O-GlcNAcylation) of proteins. O-GlcNAcylation of many transcription factors that regulate stem cell self-renewal and pluripotency, including Oct4 and Sox2, enhances their DNA-binding and transcriptional activity. GlcN-6-PO_4 can be synthesized from fructose-6-PO_4 + glutamine or it can be obtained from transport of exogenous GlcN and phosphorylation by hexokinases. Here we tested the hypothesis that Slc2a2 is important for exogenous GlcN uptake and enhances stem cell functions by ESC. We employed LG-ESC as well as LG-ESC in which *Slc2a2* mRNA was silenced with a constitutively expressed shRNA vector (Slc2a2-KD ESC). Adding 0.8 mmol/l GlcN to low Glc media increased numbers of alkaline phosphatase-positive colonies and stimulated expression of proliferating cell nuclear antigen (PCNA) by LG-ESC but not by Slc2a2-KD ESC. Whereas total O-GlcNAcylation of proteins in LG-ESC was increased by GlcN, it does not appear that the effects of GlcN were due to increased O-GlcNAcylation because inhibiting O-GlcNAcylation by the enzyme, *O*-GlcNAc transferase (OGT) with alloxan did not inhibit increased formation of alkaline phosphatase-positive colonies. GlcN increased glycolytic activity, as indicated by lactate production, and pentose shunt pathway flux as indicated by glucose-6-PO_4 dehydrogenase activity. This suggests that exogenous GlcN stimulates the anaerobic and anabolic metabolic pathways that promote stem cell function and self-renewal. These studies indicate that GlcN derived from maternal circulation is important for early embryo development. Moreover, they indicate that expression and retention of Slc2a2 by ESC and iPSC lines by isolation and culture in physiological Glc media may be advantageous for stem cell metabolism, self-renewal, and perhaps, clinical applications, over existing culture methods.

W-2010
DYNAMIC MODEL OF MURINE TROPHOBLAST AND EMBRYONIC STEM CELLS INTEGRATING SIGNALLING PATHWAYS, TRANSCRIPTION NETWORK AND EPIGENETIC MACHINERY

Pir, Pinar, Le Novère, Nicolas
Babraham Institute, Cambridge, United Kingdom

Decrypting the regulation of initial cell fate decision in the morula and blastula is important to understand the causes of early miscarriages. The generation, from totipotent cells, of pluripotent embryonic stem cells and trophoblast stem cell, is also a model of epigenetic fate restriction. Cell fate is regulated by a cascade of interactions involving signalling pathways, transcription networks and epigenetic mechanisms. We are building a mathematical model that presents a dynamic view of theses cascades. This model can be simulated to predict the decision towards cell differentiation, proliferation or re-programming for a given set of external stimuli or genetic modifications. The external signals received by the cell are processed by a few signalling pathways involved in initial cell fate decisions. These pathways act together to regulate the core transcriptional network of pluripotency and differentiation in early mouse embryos. The effect of these factors on cell fate is modulated by chromatin condensation and nucleosome positioning, depending on DNA methylation and histone marks, themselves under the control of transcription factors. The epigenetic status of a promoter can be used to predict the availability of the promoter for transcription factor and POLII binding, which regulate the transcriptional activation or inactivation of each transcription factor. To represent interactions of different nature requires different modelling approaches. For example, enzymatic reactions in the cytosol involve many molecules and can be approximated by classical chemical kinetics. However, binding of transcription factors have to be represented as stochastic events, because the small copy numbers of TFs and binding sites. Also, different time scales have also to be taken into consideration, to represent for example cell division, and DNA binding or protein phosphorylation. Therefore, we have built modular models, represented each type of interaction with the appropriate formalism and used simulators supporting each formalism. The simulations of each module were carried out in parallel, with event-driven synchronisation. A Python interface facilitates the interactions between simulators running in parallel and integrates the different simulation results. Our model is able to predict dynamics of the early cell commitment decisions in mouse embryos, as well as dynamic transcriptional profile of cells that are re-programmed by genetic modifications and/or growth in specific niches. Our simulations have indicated that the cross-talk between the external signals together with the complex epigenetic regulation of transcription can lead to a vast variety of transcription and epigenetic profiles in cells with the same initial state. For example forced generation of trophoblast stem cells (TSC) from embryonic stem cells (ESC), a transformation that does

not take place naturally, can follow more than one route. However not all of these routes lead to transcriptional and epigenetic profiles which exactly matches the profile of TSCs derived from trophectoderm. In silico identification of such routes can be used in designing improved experimental conditions for increased efficiency of re-programming.

W-2011
METABOLOMICS IN STEM CELLS - CHANGES FROM NAïVE TO PRIMED STAGE

Sperber, Henrik[1], Mathieu, Julie[2], Shannon, Sandra[2], Hesson, Jennifer[2], Gu, Haiwei[2], Meissen, John K.[3], Fiehn, Oliver[3], Ware, Carol B.[4], Hockenbery, David[5], Raftery, Daniel[2], Ruohola-Baker, Hannele[6]
[1]Biochemistry, University of Washington, Seattle, WA, USA, [2]University of Washington, Seattle, WA, USA, [3]University of California, Davis, Davis, CA, USA, [4]University of Washington, Seattle, WA, USA, [5]Fred Hutchinson Cancer Research Center, Seattle, WA, USA, [6]Institute for Stem Cell and Regenerative Medicine University of Washington, Seattle, WA, USA

Naïve human embryonic stem cells (hESC) have recently been derived and have more robust developmental potential than primed ESCs (Ware et al. 2014, Gafni et al. 2013). Metabolic signatures are highly characteristic for a cell and may act as a leading cause for cell fate changes, preceding changes in cell fate genes. We have previously shown that from naive to primed stage the cells undergo a dramatic transition from metabolically bivalent to highly glycolytic. However, primed state of inert mitochondria rapidly changes to highly potent mitochondria during further differentiation. It is not yet understood how and why the pluripotent cells enter the highly glycolytic, metabolically cancer-like (Warburg effect) stage and how a differentiating cell leaves this stage. To search for critical metabolites that might control those transitions as well as other pathways that change during ESC development, we have now performed metabolomics profiling of naïve and primed ESCs using non-targeted GC-TOF and LC-QTOF mass spec analysis as well as targeted LC, showing a difference in metabolite profile between naïve and primed cells, which is consistent across species for human and mouse. We have verified through this analysis that glucose levels are reduced and lactate levels increase in the primed stage, which is consistent with the cells becoming exclusively glycolytic. Further study of the glycolysis shows a high upregulation of fructose 1,6-bisphosphate (F16BP) due to upregulation of PFK and downregulation of gluconeogenesis gene FBP in primed ESCs indicating that the accumulation of F16BP is due to upregulated glycolysis and downregulated gluconeogenesis. Interestingly glyceraldehyde-3-phosphate (G3P), the downstream metabolite of F16BP, does not increase in primed ESCs. Since G3P can be conserved for biosynthetic purposes in the synthesis of fatty acids and amino acids we tested the upregulation of these pathways. Interestingly we observed a significant increase in long carbon chain lipids in primed ESCs, an indication of increased synthesis and/or decreased beta-oxidation. In addition primed cells show high enrichment of the tryptophan degradation product kynurenine. Kynurenine can act as a ligand for the transcription factor AHR. In cancer cells AHR activation by kynurenine is shown to induce growth while in surrounding T-cells kynurenine based AHR activation inhibits the immune response against cancer cells. Microarray and qPCR data showed a high increase of the tryptophan degrading enzyme IDO1 in primed hESC, which - in combination with the increase in glycolytic products - would explain the mechanism by which kynurenine is accumulated. After peaking in primed ESCs IDO1 levels quickly drop as the ESCs begin to differentiate, indicating that the function of IDO1 is specific for the primed stage. Preliminary data from validation by qPCR show that IDO1 levels are 60 fold higher in primed hESC (H1) compared to naïve human ESC (Elf1). Our working hypothesis is that kynurenine is a key metabolite acting in primed hESC in a manner similar to cancer cells; In primed hESC kynurenine may support stem cell growth and self-renewal while when secreted from hESC kynurenine may inhibit Treg cell proliferation, thereby providing protection to the primed stage embryo by silencing the mother's immune cells through AHR activation.

W-2012
SPECIFICATION OF BIPOTENT NEUROMESODERMAL PROGENITORS FROM PLURIPOTENT EPIBLAST

Wilson, Val, Tsakiridis, Anestis, Huang, Yali
University of Edinburgh, Edinburgh, United Kingdom

During gastrulation, the fate of epiblast cells is thought to be constrained principally by their position. Cell fate is progressively restricted by localised signalling cues from areas including the primitive streak, and yet cells remain pluripotent (able to give rise to derivatives of all three embryonic germ layers) until the end of gastrulation. We previously showed that pluripotency in embryonic epiblast cells is extinguished over a very short period at the beginning of somite formation, accompanied by reduced levels of Oct4, just as a population of bi-potent neural/mesodermal stem cell-like progenitors begin to produce the spinal cord, muscles and skeleton (Osorno et al. 2012 Development 139, 2288-98). Epiblast stem cells (EpiSCs) - pluripotent stem cell lines that are equivalent to the gastrulation-stage postimplantation epiblast - express gastrulation-stage regional markers in self-renewing conditions. We show here that undifferentiated EpiSC cultures contain a major subpopulation of cells with reversible early primitive streak characteristics, which is mutually exclusive to a neural-like fraction. Using in vitro differentiation assays and embryo grafting we demonstrate that primitive streak-like EpiSCs are biased towards mesoderm and endoderm fates while retaining pluripotency. The acquisition of primitive streak characteristics by self-renewing EpiSCs is mediated by endogenous Wnt/β-catenin signalling. Further elevation of Wnt/β-catenin activity in EpiSCs promotes downregulation of Oct4, exit from pluripotency and a transition towards at least two distinct primitive streak-associated lineages with mesendodermal and neuromesodermal characteristics. Thus both the dynamics of pluripotent subpopulations expressing regional markers during gastrulation, as well as the restriction of pluripotency in post-gastrulation embryos in vivo towards neuromesodermal progenitors, are reflected by the behavior of EpiSC cultures.

EMBRYONIC STEM CELL DIFFERENTIATION

W-2014
RHO-ASSOCIATED PROTEIN KINASE (ROCK) INHIBITOR Y-27632 ABROGATES DEFINITIVE ENDODERM COMMITMENT THROUGH DISTINCT P53 SIGNALS IN HUMAN PLURIPOTENT STEM CELLS

Chen, Kevin G.[1], Hoeppner, Daniel H.[1], Park, Kyeyoon[1], Johnson, Kory R.[1], Mallon, Barbara[1], Garfield, Susan H.[2], Chu, Virginia[3], Carpentier, Arnaud[3], Liang, T. Jake[3], Robey, Pamela G.[4], McKay, Ronald D.[1]
[1]National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, USA, [2]National Cancer Institute, NIH, Bethesda, MD, USA, [3]National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, USA, [4]National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD, USA

Human pluripotent stem cell (hPSC)-based therapeutics and

pharmaceutical applications require efficient production of clinical-grade hPSCs with controlled stress responses, homogeneous cellular states, and stable genomic integrity. Molecular modulation of hPSC growth and differentiation by small molecules has provided a powerful approach to achieve the above goals. Currently, small molecule-mediated pathway interventions represent an emerging field in hPSC biology, regenerative medicine, and cellular reprogramming. However, this approach has been constrained by the lack of simple and robust assays that accurately measure the effects and impact of small molecules on key signaling pathways that control hPSC stress response, survival, and multilineage differentiation. In this study, we describe such a generic genome stress-response assay, based on a p21-YFP knock-in reporter at the *CDKN1A* locus. It is known that *CDKNA1A (p21)* is a specific downstream effector gene of the MDM2-p53 pathway. Consistent with this, we demonstrated that the p21-YFP reporter system was highly sensitive to p53 pathway-related stress signals such as MDM2 inhibition by the small molecule, nutlin-3, and MDM2 knockdown by specific shRNAs. Therefore, this reporter system allows us to examine correlative responses of downstream effectors of the p53 pathway and perform single-cell kinetic assays of the effects of pivotal small molecules (e.g. Rho-associated protein kinase inhibitors or ROCKi) on hPSC cellular states and fate determination. Our data revealed previously unknown survival and death mechanisms that involve both the p53 and ROCK pathways. Under dissociated hPSC conditions, the ROCKi, Y27632, promoted single-cell survival by transiently modulating p53 signaling concomitantly with cell cycle arrest at G1 and G2/M phases. However, the same treatment also promoted hPSC growth inhibition and cell death when the cells were grown as condensed colonies or aggregates, likely through a different p53 signaling pathway. Molecular profiling data of 21 hESC and 8 hiPSC lines showed that p53-effector *CDKN1A* (*p21*) mRNA levels were up-regulated in all differentiated hPSC lines, particularly in cells differentiated toward definitive endoderm. Moreover, down-regulation of p21-YFP activity was much reduced in hESCs under single-cell based and Y27632-mediated non-colony type monolayer (NCM) culture compared with that of the same cells grown as colonies. Accordingly, the Y27632-mediated NCM had an impaired capacity to differentiate toward definitive endoderm as indicated by only 5% of cells becoming SOX17 positive cells, as compared to 60-80% SOX17 positive cells generated from colony-type culture (in the absence of ROCKi). Furthermore, laminin isoform-521-mediated NCM, without the use of Y27632, significantly enhanced the generation of SOX17 positive cells (>80%). These data highlight the differential roles of ROCKi in cell survival and differentiation, which depend on the modes of multicellular association and growth-patterns. Our study sheds light on the role of ROCK, p53, and CDKN1A (p21) in influencing the propensity of a specific lineage differentiation. Thus, fine-tuning of p53 and ROCK signaling would enable us to optimize growth conditions suitable for in vitro hPSC maintenance, expansion, and differentiation, which are essential steps for regenerative medicine and drug discovery.

W-2015
A MICRO-ENGINEERED PLATFORM OF ORGAN-DERIVED ECM ISLANDS TO PROBE CELL-ECM INTERACTIONS DURING DIFFERENTIATION

Goh, Saik Kia[1], Bertera, Suzanne[2], Halfter, Willie[1], Banerjee, Ipsita[1]
[1]*University of Pittsburgh, Pittsburgh, PA, USA,* [2]*Children's Hospital of Pittsburgh, Pittsburgh, PA, USA*

Introduction: Extracellular matrix (ECM) components are important physiological regulators of stem cell fate and function. Recapitulating the native ECM microenvironment niche with single purified proteins has proved to be inadequate, hence more complex ECM has also been investigated using cell-derived matrix *in-vitro* or decellularized organ or tissue. However, purified matrix is simply unavailable in sufficient quantity and it remains a challenge to perform high throughput analysis of interactions between stem cells and native ECM in vitro. Here, we describe a novel miniature ECM array combined with quantitative imaging that permits high-throughput screening of the effects of organ-specific ECM on stem cell behavior. We hypothesize that organ-derived ECM will constitute an appropriate milieu suitable for stem cell fate and function. Materials and Method: Organs of interest (Heart, Pancreas, and Liver) were perfusion-decellularized with detergent as previously described [1,2,3]. For ECM extraction, each decellularized organ was digested in protein solubilization buffer (4M guanidine HCl, 8M Urea in PBS) overnight with gentle shaking. We then immobilized the ECM extracts on nitrocellulose coated surfaces to micro-pattern spatially separated ECM islands. We characterized the molecular composition, structural features with antigenic recognition and atomic force microscopy respectively. To quantify specific cellular response to multiple organ-derived ECM extracts, we seeded pancreatic β-cell line (MIN6); human embryonic stem cell derived definitive endoderm cells (hESC-DE) and hESC derived pancreatic progenitor cells (hESC-PP) (10^5 cells/mL) on the ECM array. Seeded cells were cultured for 4-7 days on the ECM array before being evaluated for viability (Live/dead), proliferation (Ki-67), functions (C-peptide) and differentiation (FoxA2 and PDX1) via in-cell western assay (Licor). Results and Discussion: Perfusion-decellularization of organs with detergent resulted in complete removal of cells. AFM characterization verified the immobilization of ECM proteins onto the nitrocellulose coated slide. Quantification from the ECM array identified distinct differences in ECM properties of three organs. Analysis of seeded cells on ECM array demonstrated that cells attached preferentially on the ECM islands and retained high viability (90±5.8%). The cellular patterning remained robust over 7 days of culture, yielding a uniform array of confluent cellular islands. In our studies with hESC-PP cells cultured on the organ-derived ECM islands, in-cell western analysis showed significantly higher number of PDX1+ cells (18.1±3.8% higher, $P<0.05$) on pancreas derived ECM than heart or liver derived ECM. Proliferation marker remained high (>90%) throughout the 7 days culture and showed no significant differences among organs ($P>0.05$). Conclusion: Our methodology enables high throughput analysis of the contribution of native-like multicomponent ECMs toward stem cells fate and function. This miniature bioassay is especially useful to study low amounts of ECM proteins and cells, such as organ derived ECM and stem cells respectively. In addition, this μ-engineered platform is very versatile and easily adaptable to any other cell types and/or tissue or organ derived ECM. The developed ECM array will pave the way for a more rational design of engineered stem cell niches and support the rapidly developing field of regenerative medicine.

W-2016
ANEUPLOIDY ENDOWS EMBRYONIC STEM CELLS WITH FEATURES OF NEOPLASTIC PROGRESSION

Huang, Yue, Zhang, Meili, Jia, Yuyan, Liu, Guang, Cheng, Li
Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

Aneuploidy, an uneven number of chromosomes, leads to severe developmental defects in mammals and is also a hallmark of cancer. Aneuploidy has been observed in more than 90% of human solid tumors with rapid proliferation. However, whether aneuploidy is a driving cause or a consequence of tumor formation remains controversial. Paradoxically, recent studies showed that gains of single chromosomes in yeast, mouse fibroblasts (MEFs) and human Down syndrome (DS) fibroblasts impaired cell proliferation *in vitro*. Here, we examined the

effects of aneuploidy on mouse embryonic stem cells (mESCs). Using a novel genetic scheme, we generated a series of aneuploid mESC lines that each carries an extra copy of single chromosomes and then characterized the traits shared by these cell lines. All cultured aneuploid mESCs morphologically resembled diploid stem cells, and expressed the core transcription factors, Oct4, Sox2 and Nanog, normally. They all had rapid proliferation rates and enhanced colony formation efficiencies. They were less dependent on exogenous growth factors LIF for self-renewal and showed a reduced capacity to differentiate *in vitro*. Moreover, xenografted aneuploid ESCs formed teratomas more efficiently, with features of neoplastic progression. In addition, microarray analysis showed that some differentiation and development related gene expression changes are conserved among these aneuploid cell lines. Our study demonstrates that aneuploidy enhances the self-renewal capacities of stem cells and reduces their differentiation abilities. The findings also reveal that aneuploidy endows stem cells with features of neoplastic transformation by reinforcing the capacity of self-renewal.

W-2017

GENERATION OF ORIGIN SPECIFIC CORONARY-LIKE VASCULAR SMOOTH MUSCLE CELLS FROM HUMAN PLURIPOTENT STEM CELLS AND THEIR ABILITY TO SUPPORT VASCULOGENESIS

Iyer, Dharini, Low, Lucy, Cheung, Christine, Sinha, Sanjay
University of Cambridge, Cambridge, United Kingdom

Cardiovascular regenerative medicine is an emerging field that intersects both developmental biology and clinical research. Human pluripotent stem cells (hPSCs) offer unique value to this field as they can be directed to become specialised cardiovascular cells. Due to its fundamental role in heart development, the epicardium has emerged as a multipotent cardiovascular progenitor source with therapeutic potential in terms of coronary smooth muscle cell (CoSMC), cardiac fibroblast and cardiomyocyte regeneration. We recently reported an *in vitro* strategy for generating lineage-specific vascular smooth muscle cells (VSMCs) from hPSCs. We have now extended this approach to obtain epicardium and epicardium-derived VSMCs. Here we investigated the function of key developmental growth factors such as BMP, FGF, Wnt and retinoic acid in guiding hPSCs to a pro-epicardial/ epicardial lineage through lateral plate mesoderm (LM), under chemically defined conditions. We were able to induce high expression of epicardium specific genes (Tbx18, Tcf21, Wt1 and Basonuclin), comparable to human fetal epicardial explants. The LM-derived epicardial cells, when treated with PDGF-BB and TGF-β1, underwent an epithelial to mesenchymal transition (EMT) and resulted in contractile SMCs that expressed a repertoire of early and late VSMC markers. Our results further suggest that epicardium derived SMC differentiation is regulated by the RhoA and Notch signalling pathways. The epicardium derived VSMCs also support better endothelial network formation when co-cultured with HUVECs in a 3D *in vitro* Matrigel assay compared to neuroectoderm- or paraxial mesoderm-derived VSMC subtypes. We anticipate that this coronary-like VSMC population will serve as a platform for disease modelling and lay the foundation for novel approaches in regenerative cardiovascular medicine.

W-2018

A SHH CORECEPTOR CDO IS REQUIRED FOR NORMAL HEART DEVELOPMENT AND CARDIOMYOGENESIS.

Jeong, Myong-Ho
Samsung Biomedical Research Institute, Suwon, Republic of Korea

Shh signaling is implicated in the early specification stages of cardiomyogenesis and Shh mutant mice show various cardiac abnormalities, such as abnormal heart tube formation and specification of the myocardial and outflow tract. A multifunctional receptor Cdo functions as a Shh coreceptor to fully activate Shh signaling. Cdo mutant mice exhibit defects in CNS development associated with the decreased Shh activity. In this study, we assessed the role of Cdo in cardiomyogenesis and heart development. Cdo is expressed in developing hearts and Cdo-/- hearts are small. Cdo-depleted P19 EC and Cdo-/- ES cells show delayed cardiomyogenesis with decreased expression of cardiac markers, including Gata4, Nkx2.5 and Mef2c, and reduced Shh signaling activities. In addition, Cdo deficiency causes a stark reduction in the formation of beating mature cardiomyocytes. Furthermore forced activation of Shh signaling by purmorphamine treatment restores cardiomyogenesis of Cdo-/- ES cells comparable with Cdo+/+ ES cells. Taken together, Cdo is required for normal heart development and cardiomyogenesis.

W-2019

STEPWISE DIFFERENTIATION OF PLURIPOTENT STEM CELLS INTO OSTEOBLASTS USING FOUR SMALL MOLECULES UNDER SERUM-FREE AND FEEDER-FREE CONDITIONS

Kanke, Kosuke[1], Masaki, Hideki[2], Saito, Taku[3], Komiyama, Yusuke[1], Hojo, Hironori[1], Nakauchi, Hiromitsu[2], Lichtler, Alexander C.[4], Takato, Tsuyoshi[5], Chung, Ung-il[1], Ohba, Shinsuke[1]
[1]Center for Disease Biology and Integrative Medicine, The University of Tokyo, Tokyo, Japan, [2]Division of Stem Cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, Institute for Medical Science, The University of Tokyo, Tokyo, Japan, [3]Department of Bone and Cartilage Regenerative Medicine, The University of Tokyo, Tokyo, Japan, [4]University of Connecticut Health Center, Farmington, CT, USA, [5]Department of Sensory and Motor System Medicine, The University of Tokyo, Tokyo, Japan

Pluripotent stem cells are a promising tool for in vitro mechanistic studies of tissue development, models of human disease, drug screening, and stem cell-based therapies. Here, we developed an efficient, mass-producible, and reproducible culture protocol for directing mouse embryonic stem cells (mESCs), mouse induced pluripotent stem cells (miPSCs), and human iPSCs (hiPSCs) toward osteoblasts under serum-free and feeder-free conditions. The protocol is composed of four phases: the maintenance of pluripotent stem cells in serum-free 2i culture (mESCs and miPSCs) or mTeSR1 media (hiPSCs), mesoderm induction (5 days), osteogenic induction (14 days), and maturation of osteoblasts (4 days). Mesoderm induction was achieved in serum-free media by inhibiting neural differentiation with the Hh signaling inhibitor cyclopamine and by inducing mesoderm differentiation with a high concentration of GSK-3 inhibitor CHIR99021. Cells were then cultured with the Hh signaling activator SAG and the helioxanthin-derivative TH in serum-free osteogenic media for 14 days; the cells spontaneously differentiated into mature osteoblasts without any inducers in the next maturation phase. In the mESCs, the mesoderm markers *T* and *Mixl1* were transiently up-regulated by the mesoderm induction; subsequent osteogenic induction strongly induced osteoblast-related genes including *Runx2*, *Sp7*, *Col1a1*, *Ibsp*, and *Bglap* ($p<0.05$ vs. control) without upregulating ectoderm- (*Sox1*) and

endoderm-lineage genes (*Sox17* and *Gata4*) throughout the culture. The expression levels of these osteoblast-related genes were comparable to or higher than those in the mouse primary osteoblasts and the osteogenic cultures of mESCs obtained by a conventional strategy. Immunocytochemistry revealed that high percentages of the cells expressed RUNX2 (78 ± 3%), SP7 (66 ± 5%), and Col1a1-GFP (45 ± 1%) at the end of the osteogenic induction, which supressed the expression of pluripotency markers (OCT4, 2.3 ± 0.6%; NANOG, 1.5 ± 0.7%). von Kossa staining revealed the uniform formation of calcified cell clusters by the present strategy. These results indicate that the strategy induced both the expression of osteoblast-related genes and the calcification of matrix, two key features of osteoblasts. We next examined whether *Runx2*$^{-/-}$ mESCs cultured using the present strategy could molecularly recapitulate osteoblast phenotypes in *Runx2*$^{-/-}$ mice. The results revealed that the gene expression patterns of *Ibsp*, *Sp7* and *Bglap* in *Runx2*$^{-/-}$ mESCs were similar to those in developing bones in *Runx2*$^{-/-}$ mice. We also applied the protocol to 2i-adapted miPSCs and hiPSCs maintained in mTeSR1 media. Both cells showed the upregulations of osteoblast-related genes (*Runx2*, *Sp7*, *Col1a1*, *Ibsp*, and *Bglap*) at the end of the osteogenic induction ($p<0.05$ vs. control); RUNX2 and SP7 proteins were highly expressed in the periphery of the cell clusters. Thus, we achieved the direct differentiation of pluripotent stem cells into osteoblasts using four small molecules under serum-free and feeder-free conditions. The results obtained in *Runx2*$^{-/-}$ mESCs suggest that the present strategy will be useful for analyzing development of mutant osteoblasts. The strategy will be a novel platform for biological studies of osteoblast development, patient-oriented research regarding skeletal disorders, the screening of bone augmentation drugs, and the regeneration of skeletal tissues.

W-2020
CALRETICULIN MODULATES CELL-SUBSTRATUM ADHESION IN CARDIAC DIFFERENTIATION BY REGULATING EXTRACELLULAR MATRIX RECEPTORS AND CADHERIN SWITCH DURING EMBRYOID BODY FORMATION

Karimzadeh, Fereshteh[1], Pilquil, Carlos[1], Opas, Michal[2]
[1]*Medicine and Pathology, University of Toronto, Toronto, ON, Canada,* [2]*University of Toronto, Toronto, ON, Canada*

Calreticulin (CRT) is a multifunctional endoplasmic reticulum-resident protein that plays a critical role in epithelial to mesenchymal transition (EMT), a key event in cardiogenesis. The EMT is characterized by a switch in the class of cadherins on the cell surface of cardiac progenitors, from E-cadherin in the pluripotent stage to N-cadherin in the differentiated stage. Changes in cell-cell adhesion („the cadherin switch") are likely to affect cell-substratum (extracellular matrix; ECM) adhesion, both critical during cell commitment and subsequent differentiation. Calreticulin modulates adhesions: cell-substratum via focal contacts and cell-cell via zonulae adherens, thus being uniquely positioned to affect global adhesiveness of a differentiating cell community. CRT-deficient (CRT-KO) mouse embryonic stem (ES) cells are less adhesive to the substratum than wild-type (WT) ES cells and express lower levels of proteins involved in focal contact-based adhesion mediated by integrins. Integrins regulate mouse ES cell self-renewal and, consequently, differences in pluripotency exist between WT and CRT-KO cells. A change in expression of isoforms of laminin receptor alpha subunits 6 ("the alpha integrin flip") occurs as ES cell exit from the pluripotency, alpha 6B being replaced by alpha 6A as the predominant isoform. The integrin flip is suppressed in the absence of calreticulin in CRT-KO ES cells. Another form of cell-substratum adhesion receptors are discoidin domain receptors (DDRs)-1 and -2, which are responsible for adhesion to collagen. Interestingly, changes in expression of DDR variants 1 and 2 that occur in WT cells are suppressed in the absence of CRT in the CRT-KO ES cells. By manipulating intracellular calcium homeostatsis in the presence or the absence of CRT in our ES cell lines, we could specify the effect of CRT on transmembrane receptors of ECM proteins such as collagen, laminin, and fibronectin, cadherin expression profile and thus cell-cell contact preferences which affect dynamics of embryoid body formation and, ultimately, cardiomyocyte differentiation.

W-2021
TLR5 IS INVOLVED IN NEURONAL DIFFERENTIATION THROUGH NF-KAPPA-B ACTIVATION IN MOUSE EMBRYONIC STEM CELL

Kim, Won-Jae
Chonnam National University, Gwangju, Republic of Korea

Toll-like receptor 5 (TLR5) is pattern recognition receptors characteristic of immune cells and crucial for inducting and immune response to pathogens. TLR5 is widely expressed in multiple tissues and organs including innate immune cells such as dendritic cells and macrophages and regulates cellular growth and differentiation. However, the roles of TLR5 in regulating neurogenesis from mouse embryonic stem cell (mESC) have not been elucidated. Here, this study demonstrated that TLR5 is involved in the process of neurogenesis from mESC. In the present study, TLR5 was gradually up-regulated in mESC during neuronal differentiation in a time-dependent manner. To verify role of TLR5 in neural differentiation from mESC, the transfections of TLR5 overexpression vector or TLR5 shRNA to mESC were performed. The down-regulation of TLR5 significantly reduced the expression of NeuroD1 (a neural marker), β-III tubulin (a neural marker)-positive cells and increased SOX2 expression (a stem cell marker), whereas overexpression of TLR5 increased expression of NeuroD1 and β-III tubulin-positive cells and decreased SOX2 expression in mESC during neuronal differentiation. Down-regulation of TLR5 decreased the phosphorylation of IκBα and expression of NeuroD1, whereas overexpression of TLR5 increased p-IκBα and expression of NeuroD1 in mESC during neuronal differentiation. Inhibition of NFκB using BAY11-7082 decreased the expression of NeuroD1 and β-III tubulin-positive cells, and decreased generation of embryoid body (EB) as well as interleukin-6 (IL-6) expression during neuronal differentiation of mESC. Neutralization of IL-6 also decreased the expression of NeuroD1 and β-III tubulin-positive cells, and decreased generation of EB and neuronal outgrowth during neuronal differentiation of mESC. In addition, translocation to nucleus of pCREB was reduced by inhibition of NFκB activation or neutralization of IL-6 during neuronal differentiation of mESC. Taken together, these data suggest that TLR5 enhances neuronal differentiation from mESC through NFκB mediated IL-6 signaling.

W-2022
TALEN MEDIATED KNOCK-IN OF EGFP INTO OCT4 LOCUS OF HUMAN EMBRYONIC STEM CELLS DOES NOT EFFECT DIFFERENTIATION POTENTIAL: A PROMISING STRATEGY FOR GENERATING CELL SPECIFIC REPORTER LINES.

Krentz, Nicole AJ, Lynn, Francis C.
University of British Columbia, Vancouver, BC, Canada

Human embryonic stem cells have great promise as a source of unlimited transplantable tissues for regenerative medicine, including the production of insulin-producing beta cells for diabetes therapy. However, producing efficient differentiation protocols is currently limited due to both an insufficient understanding of the developmental

processes that govern the differentiation of these cell types as well as a lack of tools to study differentiation in vitro. Potential tools to aid in understanding the in vitro differentiation process are hESC reporter lines. The traditional method of altering the genome of hESC was homologous recombination, which proved highly challenging and inefficient. However, advances in genome editing technologies has greatly improved the efficiency and allowed for the rapid generation of novel hESC lines. In this study, we outline a strategy using Transcription Activator Like Effector Nucleases (TALENs) to generate OCT4-eGFP-2A-Puro CyT49 hESC lines. Electroporation of the OCT4-eGFP-2A-Puro donor plasmid with TALENs resulted in 16/52 (31%) correctly targeted clones. Further analysis of three of these lines, OCT4-2, OCT4-3, and OCT4-28, using immunofluorecence staining and qRT-PCR for OCT4 determined that the insertion of eGFP downstream of the last exon of OCT4 did not impact native OCT4 expression compared to the parental CyT49 cells. Moreover, eGFP was co-expressed in all OCT4+ cells, highlighting the specificity of this reporter strategy. Upon differentiation, the number of eGFP+ cells rapidly declined and mirrored the number of immunopositive OCT4 CyT49 cells. Using a directed differentiation strategy towards the definitive endoderm germ layer, there was similar formation of CXCR4+ cells between the OCT4 hESC lines and parental CyT49. As a proof of principle that this reporter strategy would be amenable to creating lines specific for differentiation markers, we differentiated the OCT4-eGFP lines towards pancreatic progenitors and found a similar proportion of NKX6.1+/SOX9+ pancreatic progenitor cells by immunofluorescence. In conclusion, these findings highlight the feasibility of creating cell specific reporter hESC lines using TALEN mediated genome-editing techniques. Generation of these lines will greatly improve our understanding of human developmental biology and our progress towards making desired cell types for regenerative medicine.

W-2023
DIRECTED DIFFERENTIATION OF PATIENT SPECIFIC INDUCED PLURIPOTENT STEM CELLS TOWARDS OVARIAN GRANULOSA-LIKE CELLS

Lan, Chen-wei
National Taiwan University, Taipei, Taiwan

Ovarian granulosa cells are vitally important for the development and maturation of oocytes. Recent studies have suggested that the dysfunction of granulosa cells may lead to ovarian diseases, such as polycystic ovary syndrome (PCOS), premature ovarian failure (POF), and granulosa cell tumors. Induced pluripotent stem cells (iPSCs) can differentiate into all the specialized cells. These features make iPSCs potentially valuable for the study of early embryo development, establishment of disease models, and applications in regenerative medicine. Through multi-step approaches comprising in vitro treatments with cocktails of growth factors, gene expression analyse the progress of iPSCs from patients with POF or PCOS to primitive streak-mesendoderm, intermediate plate mesoderm, and to functional granulosa-like cells that expressed the granulosa cell-specific marks FOXL2, CYP19A1, anti-Müllerian hormone (AMH), the type 2 AMH receptor (AMHR2), and the follicle stimulating hormone receptor (FSHR). Using a flow cytometry, we have identified cell-surface markers AMHR2 and FSHR for the separation of granulosa-like cell types derived from iPSCs. Gene expression analyses showed that FOXL2, CYP19A1 and AMH levels in AMHR2+/FSHR+ population cells were significantly higher than in AMHR2-/FSHR- population cells from iPSCs-derived granulosa-like cells. These enriched cells will aid investigations that use granulosa-like cells generated from pluripotent stem cells to study folliculogenesis and steroidogenesis and in the creation of novel treatment modalities for ovarian diseases in future. Furthermore, it may aid in the creation of novel treatment modalities for ovarian diseases in future.

W-2024
MONOCARBOXYLATE TRANSPORTER 8 IS EXPRESSED ON OLIGODENDROCYTE PROGENITORS DERIVED FROM HUMAN EMBRYONIC STEM CELLS.

Lee, Jae Young[1], Kim, Min Joung[1], Stanley, Ed[2], Elefanty, Andrew George[2], Petratos, Steven[1]
[1]Department of Medicine, Central Clinical School, Monash University, Prahran, Melbourne, Australia, [2]Murdoch Childrens Research Institute The Royal Children's Hospital, Parkville VIC, Australia

Thyroid hormone (TH) plays a significant role in brain development, responsible for critical cell cycle events during oligodendrogenesis. Specifically, tri-iodothyronine (T3) is an essential regulator of terminal oligodendrocyte differentiation and myelination in vivo. Transport of thyroid hormone into neural cells is not simply a passive diffusion process but rather an active process involving specific thyroid hormone transporters. The monocarboxylate transporter 8 (MCT8) has recently been defined as a TH-specific transporter that is crucial for brain development. In humans, mct8-deficiency results in the X-linked-inherited psychomotor retardation disorder, known as Allen-Herndon-Dudley syndrome (AHDS). Because delayed myelination is a feature of this disease, we hypothesised that MCT8 may play a significant role in oligodendrocyte development and myelination. We have established a novel method of deriving pre-myelinating O4-positive oligodendrocytes from human embryonic stem cell (hESC) lines by utilising the NKX2.1-GFP as reporters. By live cell imaging and flow cytometry, expression profile of NKX2.1-GFP at the early differentiation stage was determined and GFP+ and GFP- cells were separated by fluorescence-activated cell sorting (FACS). By immunocytochemistry, flow cytometry and quantitative real-time polymerase chain reaction (qRT-PCR), GFP+ cells differentiated towards oligodendrocyte precursor cells (OPCs) (CD140a+/NG2+) then immature oligodendroglia (O4+) whereas GFP- cells either stayed as neural progenitors (Nestin+) or differentiated towards astroglia (GFAP+) or neurons (βIII-tubulin+). From GFP+ sorted cells, we have demonstrated that MCT8 protein is expressed on subsets of OPCs, and immature oligodendroglia by immunocytochemistry, flow cytometry and western blot. Through qRT-PCR, their mRNA levels during differentiation showed an increasing trend as cells differentiate towards immature oligodendroglia. Moreover, we have identified the expression of the type 2 and 3 deiodinases, the enzymes that regulate intracellular TH concentration, on these oligodendroglial lineage cells by immunocytochemistry and their mRNA levels were measured during differentiation by qRT-PCR. Our results highlight the possible role of MCT8 in TH transport for oligodendrocyte development and may implicate this TH-transporter as a central co-determinant in the promotion of myelinating oligodendrocyte.

W-2025
GENETIC DISSECTION OF ES CELL DIFFERENTIATION USING HAPLOID ES CELLS

Leeb, Martin[1], Smith, Austin G.[2]
[1]Wellcome Trust-Medical Research Council Stem Cell Institute, University of Cambridge, Cambridge, United Kingdom, [2]Wellcome Trust - Medical Research Council Cambridge Stem Cell Institute, Cambridge, United Kingdom

The genetic circuitry that governs self-renewal of embryonic stem cells is relatively well defined. In contrast, mechanisms that dissolve this

network at the onset of differentiation to enable cell fate transition and entry into a lineage specific programme are less appreciated. We followed a large scale loss of function approach in haploid ES cells to identify key players in the transition from self-renewal to the commitment to differentiation. Suppression of differentiation by blocking mitogen activated protein kinase signalling and partial inhibition of Gsk3 in 2i medium is sufficient to allow ES cell self-renewal. Importantly, the capacity for proliferation in 2i is rather specific for undifferentiated ES cells and is lost early in the commitment transition. Thus, the ability to self-renew in 2i after a period of exposure to differentiation conditions provides a powerful means to identify and quantify delayed exit from the ground state. We combined this functional assay together with haploid ES cell mutagenesis in a genetic screen for differentiation inducers. Mutagenesis of the haploid genome allows recessive phenotypes to be directly unmasked. Furthermore, haploid ES cells are pluripotent and maintain full developmental potential. Therefore haploid ES cells provide a uniquely powerful system for elucidating the genetic circuitry of mammalian developmental processes. We have developed both a clonal and a high throughput approach that allow the comprehensive identification of all insertions of a piggyBac based gene trap cassette in a complex pool of mutants. These efforts provided a list of more than 200 candidate genes potentially involved in the exit from pluripotency, containing multiple members of the Fgf pathway and the β-catenin destruction complex. Importantly, in secondary siRNA validation assays, knockdown of 19 out of 24 novel candidate genes showed a clear delay in ES cell differentiation. Among those were the conserved small zinc finger protein Zfp706 and the RNA binding protein Pum1. Pum1 targets several mRNAs for naive pluripotency transcription factors and accelerates their downregulation at the onset of differentiation whereas Zfp706 appears to be required for efficient downregulation of Klf4 expression. Our results provide an example of the power of haploid ES cells for the genetic interrogation of developmental processes.Currently, we aim to achieve saturation mutagenesis using hyperactive piggyBac transposase, alternative mutagenesis vectors and an enhanced method of insertion site mapping. With these experiments we want to generate a comprehensive list of factors that direct the first cell fate transitions during embryonic differentiation.

W-2026
ONTOGENY OF IN VITRO DERIVED HUMAN NEURAL CREST CELLS

Leung, Wai Lun, Garcia-Castro, Martin
Yale University, New Haven, CT, USA

Introduction:The neural crest (NC) is a recent phenomenon in evolution, appearing only in vertebrate embryos. These cells are identified adjacent to the neural plate border in early embryos, later migrate throughout the embryo via stunning stereotypic patterns, and generate an unexpected and ample array of derivatives. Multiple induction scenarios have been proposed to regulate NC formation, including conflicting contributions from mesoderm, neural, and non-neural ectoderm that interact through a classic induction mechanism and provide distinct signaling molecules such as Wnt, FGF, BMP, and Notch. Result: We have developed a novel, fast and consistent protocol to generate NC based on Wnt activation and, unlike existing protocols, does not include BMP and/or Nodal antagonism. Our human ES cell cultures rapidly form NC cells expressing SOX10 and PAX3/7, along with other crest markers, and differentiate into NC derivatives. Wnt activation quickly drives loss of pluripotency markers OCT4 and NANOG, and neither neuroepithelial markers nor mesodermal markers are substantially induced. Instead, transcripts associated with neural plate border are sequentially upregulated during early differentiation preceding the appearance of migratory SOX10+ cells. Conclusion: Our data reveals that Wnt activation is sufficient for *in vitro* human NC induction. NC formation appears to pass through an intermediate stage not expressing definitive neural markers, and without mesodermal contributions. This suggests an early NC specification -preceeding neural and mesoderm formation- in agreement with our observations in chick embryos.

W-2027
QUANTITATIVE CHARACTERIZATION OF MOUSE EMBRYONIC STEM CELL STATE TRANSITION

Lu, Xibin, Huang, Wei
The University of Hong Kong, Hong Kong

It is known that ES cell differentiation or reprogramming processes are determined by highly regulated on and off gene regulation of core transcription factors (TFs) which determines the final cell fate. And it is shown that most of the cells, if not all, can be converted into another cell type given sufficient and appropriate perturbations such as from fibroblasts to embryonic stem cell like cells (iPS) or from pancreatic exocrine cells to β-cells etc. Here, we propose to develop a multidisciplinary method to engineer the core gene networks that control ES cell self-renewal, differentiation or dedifferentiation. The attractor landscape concept will be incorporated in our project and together we hope to have a deeper insight into these highly complicated processes. We first generated a dual reporter ES cell line in which EGFP was controlled by Nanog promoter and a constant EF1a promoter drive H2B-mCherry expression. Using a simple ES cell differentiation process (RA induced ES cell differentiation), we observed the differentiation and dedifferentiation of Nanog based on flow cytometry and time-lapse imaging. Mathematical models (drift-diffusion-growth equation) are also introduced based on experimental data which in turns leads to new experiments and hypotheses. Based on the quantitative cellular dynamics studies both on the population level and single cell level, the landscape based on Nanog distribution was carried out and this would provide more clues for future ES cell differentiation. To expand this method into more complex processes, we generated multiple ES cell reporters in which Nanog, Oct4 and Rex1 were all labeled with different fluorescence proteins. We differentiated the reporter ES cell line specifically into epiblast stem cell based on previous protocols. Based on flow cytometry and time-laspe imaging, we found, during the transition from ES cell to EpiSC state, all the three marker genes showed heterogeneous expression pattern, three sub-populations can be found at the later steady state. The cell fate identity and potential converting ability into other identities like ES cell or inter-converting ability among three populations were being conducted based on synthetic biology combined with chemical factors. By using fluorescent protein reporters, we can track the key interested marker gene expression pattern from time to time, and based on the quantitative studies of key marker genes, we can observe and design synthetic circuits to tune gene expression by which we hope to control the cell state transition finally.

W-2028
NOTCH SIGNALING CONFERS ENHANCED LYMPHOID POTENTIAL IN MURINE ESC/IPSC-DERIVED HSC

Lu, Yi-Fen[1], Cahan, Patrick[1], Hadland, Brandon[2], Bernstein, Irwin D.[2], Daley, George[1]
[1]Boston Children's Hospital, Boston, MA, USA, [2]Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Transplantation of hematopoietic stem cells (HSCs) from bone marrow, cord blood, or mobilized peripheral blood can be curative for a variety of malignant and genetic blood diseases, but the lack of

optimal tissue-matched donors and the immunologic complications that accompany hematopoietic reconstitution limits wider application and causes considerably morbidity and mortality. One strategy for enhanced transplantation outcomes is the creation of autologous HSCs via cellular reprogramming, as it is now feasible to generate induced pluripotent stem cells (iPSCs) from virtually any patient, and to repair gene defects via genome editing. The challenge now is to direct the differentiation of these customized iPSC into engraftable HSC. Our laboratory has previously reported the derivation of murine HSC from ESC (ESC-HSCs) through ectopic expression of HoxB4 and Cdx4 during embryoid body (EB) differentiation, but these ESC-HSC manifest a strong myeloid bias in their differentiation potential. By comparison to the transcriptional profiles of embryonic and fetal HSCs, we found that ESC-HSC closely resemble the definitive HSC that emerge in the developing fetal liver, but are deficient in gene expression programs induced by activation of Notch signaling, which is known to play critical roles in lymphoid development. We thus have employed novel differentiation strategies to enhance Notch pathway activation, including inducible expression of a Notch intra-cellular domain transgene during EB formation, co-culture of the ESC-HSCs on OP9 stroma bearing the Notch ligand DL-1, as well as stromal-free culture in the presence of a tethered Notch ligand. We found that Notch induction within the early hematopoietic specification stage of EB formation diverted cells to a lymphoid-biased fate, whereas titrating the dose of Notch during post-EB in vitro culture enabled the isolation of ESC-HSCs with enhanced in vivo reconstitution of mixed populations of myeloid and lymphoid cells in immune-deficient NSG mice. Reconstituting T lymphocytes displayed a diverse V-beta usage, indicating functional recombination activity in these ESC-derived cells. Clonal analysis of sorted lymphoid and myeloid repopulating cells at 16 weeks post-transplantation demonstrated that Notch-enhanced ESC-HSC possess long-term repopulating multi-potentiality. Interestingly, a small subset of engrafted ESC-HSC within bone marrow obtained the $Lin^{-}Sca\text{-}1^{+}cKit^{+}$ (ESC-LSK) surface marker expression, phenotypically resembling embryo-derived definitive HSC. Microarray analysis shows that ESC-LSK cluster closer to definitive HSC than do ESC-HSC. We applied CellNet, a network biology algorithm, to expression profiles of ESC-LSK and observed a significant increase of similarity to hematopoietic stem/progenitor cells (HSPC). Lastly, evaluation of NSG mice engrafted with Notch-enhanced ESC-HSC revealed antigen-specific immune-reconstitution and evidence for B and T cell-dependent immunity. Our data suggest that the timing and signal strength of Notch plays a pivotal role in deriving *bona fide* definitive HSC from ESC/iPSC in vitro.

W-2029
A HUMAN PLURIPOTENT STEM CELL MODEL OF FSHD-AFFECTED SKELETAL MUSCLE

Caron, Leslie, Kher, Devaki, Gubernator, Miriam, **Main, Heather**, Schmidt, Uli
Genea Biocells, Sydney, Australia

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common forms of muscular dystrophy affecting up to 1:7000 people. This enigmatic genetic disorder is characterized by a progressive weakness and atrophy of facial, shoulder, and upper arm muscles, eventually affecting the trunk and lower extremities. Currently, our knowledge of the pathogenesis of the disease is very limited, and the molecular and biochemical changes linked to progressive muscle degeneration remain unknown. To date, no treatment is available specifically for FSHD. Cellular models for FSHD are critical not only for understanding the molecular mechanism of the disease but can also have a significant impact on the development of new therapies. With a lack of suitable animal models, a major challenge in drug development for FSHD is the identification of model systems that accurately recapitulate normal and diseased human muscle physiology in vitro. Due to their plasticity and unlimited capacity to proliferate, human embryonic stem cells (hESCs) potentially represent a renewable source of skeletal muscle cells (SkMCs) and provide an alternative to invasive biopsies from affected patients. To date, we have derived 4 embryonic stem cell (hESC) lines carrying the chromosomal deletion causing FSHD type 1. To generate a cellular model of FSHD, we developed a robust and efficient monolayer system to differentiate hESC into mature skeletal muscle cells (SkMCs), achieving 70% differentiation efficiency. Briefly, in our novel protocol, hESCs are induced to form MyoD+ myoblasts using a combinatorial application of growth factors and small molecules. This intermediate population is further differentiated into a population of mature and multi-nucleated myotubes, which display contractile ability and express the muscle specific markers myogenin, MF20, SkMHC and dystrophin. Using our optimised protocol, FSHD-affected hESC lines have been differentiated to SkMCs with similar efficiencies to control cell lines, and their phenotypic and molecular characterization is currently in progress. This includes cell proliferation and viability, RNA profiling, responses to oxidative stress, functional testing using multi electrode arrays, and expression levels of pathogenic markers of FSHD including, Dux 4 and PITX1. We believe that these unique cells will be a useful resource for FSHD and other muscular dystrophy research, will help to better understand the disease mechanism and ultimately assist in the development of effective treatments.

W-2030
DIFFERENTIATION CAPACITY OF CHROMOSOMALLY ABNORMAL HUMAN EMBRYONIC STEM CELLS

Markouli, Christina, Geens, Mieke, Nguyen, Ha Thi, Dziedzicka, Dominika, Sermon, Karen D., Spits, Claudia
Research Group Reproduction and Genetics, Vrije Universiteit Brussel (VUB), Brussels, Belgium

Gains of 20q11.21 and, to a lesser extent, losses of 18q, are chromosomal aberrations commonly found in hESC. We aimed at evaluating the spontaneous and lineage-directed differentiation capacity of three hESC lines carrying a 20q11.21 gain and two with an 18q deletion and compared them to six control lines with balanced genetic content. The differentiation efficiency was evaluated by gene-expression analysis of lineage specific and pluripotency markers. First, cells were differentiated towards mesoderm lineage using a 42-48 days-long protocol. In this assay, all lines seemed to equally differentiate. Residual undifferentiated cells were more present in the 18q group but still significantly reduced compared to the initial undifferentiated population. For endoderm differentiation, a 4-days protocol was used, to which the 18q lines responded less efficiently than the control and the 20q11.21 lines, with a significant residual expression of pluripotency markers. Next, we assayed *in vivo* spontaneous differentiation capacity using embryoid bodies. The 18q deletion lines were not capable to spontaneously differentiate into embryoid bodies, whilst the 20q11.21 lines showed a fast loss of pluripotency and induction of lineage-specific markers. This absence of spontaneous differentiation in 18q deletion lines was confirmed by the lack of epithelial-mesenchymal transition at the colony borders during culture, suggesting that this mutation compromises the differentiation capacity of the cells. In the case of the lines with gains of 20q11.21, there was remarkable inter-line variability, but no loss of differentiation capacity to a particular lineage, suggesting that this mutation does not affect differentiation capacity, and the observed differences are line-specific.

W-2031

POTENTIAL OF EPITHELIAL EPIBLAST-LIKE PLURIPOTENT STEM CELLS FOR INCORPORATION AND INTEGRATION INTO THE MOUSE EMBRYO

Mascetti, Victoria L.[1], Mendjan, Sasha[1], Ortmann, Daniel[1], Ferrer Vaquer, Anna[2], Tomishima, Mark[2], Hadjantonakis, Anna-Katerina[3], Studer, Lorenz[4], Pedersen, Roger A.[1]

[1]Laboratory for Regenerative Medicine, Wellcome Trust-MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, United Kingdom, [2]Memorial Sloan-Kettering Cancer Center, New York, NY, USA, [3]Sloan-Kettering Institute for Cancer Research, New York, NY, USA, [4]Sloan-Kettering Institute for Cancer Research, New York, NY, USA

Pluripotent stem cells (PSCs) are defined by their ability to differentiate into all body tissues and to renew themselves through unlimited proliferation. The pluripotency of mouse embryonic stem cells (mESCs) has traditionally been assayed by formation of chimeras by blastocyst injection, demonstrating that mESCs can contribute to all tissues of the resulting mouse. Mouse epiblast stem cells (mEpiSCs), established by culturing post-implantation mouse epiblast layers, have limited capacity for incorporation into mouse blastocysts. Importantly, the epithelial epiblast-like pluripotent nature of mEpiSCs is shared with human PSCs, and this distinguishes them from the inner cell mass-like pluripotency of mESCs. The similarity among epithelial epiblast-like PSCs is all the more compelling because mEpiSCs can thus be used to model the clinical suitability of human PSCs by determining mEpiSC's capacity for incorporation, survival and integration into normally developing tissues and thereby ensuring that they are not predisposed to developmental abnormalities. Our experimental objective was to determine the capacity of epithelial epiblast-like mEpiSCs to participate in early mouse embryo development. Early post implantation mouse embryos were dissected and mEpiSCs were transplanted prior to culture. Subsequent, in vitro analysis of lineage-specific contribution by fluorescence confocal microscopy was used assess integration and function of the stem cells and their progeny. mEpiSCs transplanted into post-implantation stage mouse embryos were able to integrate and contribute to each of the three primary germ layers, endoderm, ectoderm and mesoderm. We found high potential of mEpiSCs for targeted incorporation, survival and integration following injection at the presumptive locations of organ rudiments. These findings, together with the similar nature of epithelial epiblast-like mEpiSCs and human PSCs, provide a predictive model for the functional participation of human PSCs in normal development following transplantation into the post-implantation human embryo, which due to practical and ethical reasons cannot otherwise be determined. In sum, mEpiSCs can incorporate into mouse embryos in a stage- and location-specific (synchronous, orthotopic) manner. This novel approach enables the study of cell fate decisions and plasticity of tissue specific progenitors during normal development. Faithful recapitulation of tissue-specific fate post-transplantation is the ultimate clinically relevant functional validation for PSCs.

W-2032

SYSTEMS ANALYSIS OF REGULATORY INTERACTIONS IN SIGNAL TRANSDUCTION PATHWAYS DURING SELF-RENEWAL AND EARLY ENDODERM DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

Mathew, Shibin[1], Sundararaj, Sankaramanivel[1], Mamiya, Hikaru[1], Banerjee, Ipsita[2]

[1]University of Pittsburgh, Pittsburgh, PA, USA, [2]University of Pittsburgh, Pittsburgh, PA, USA

Motivation: Signaling pathways controlling human embryonic stem cell (hESC) self-renewal and differentiation are regulated at the systems level by complex regulatory interactions. While current experimental techniques have established significant correlations between states of signaling pathways and the end fate of hESCs, it is restrictive to analyze the dynamics of the interactions using a purely experimental approach. Here, we present a complete systems level analysis of the regulatory interactions in the PI3K/AKT pathway (during self-renewal) and TGF-β/SMAD pathway (during endoderm differentiation) by integrating efficient computational tools with targeted experimental perturbations. Methods:*Experimental analysis:* H1 hESCs were maintained on matrigel-coated plates in mTeSR1. Endoderm differentiation of H1 hESCs was performed using 100 ng/ml Activin A (to activate TGF-β/SMAD2,3) prepared in DMEM/F12 supplemented with B27 and 0.2% BSA for 24 hr. The phosphorylation dynamics of key molecules of the signaling pathways were measured using MagPix Luminex xMAP technology. Mathematical analysis: Detailed ordinary differential equation based mathematical model was developed for the PI3K/AKT and TGF-β/SMAD pathway using literature based model with additional novel hESC specific mechanisms. The models were calibrated to experimental dynamics in hESCs using replica exchange Markov Chain Monte Carlo strategy. To identify sensitive nodes from the network of interactions, a computationally efficient global sensitivity approach (GSA) called Random-Sampling High Dimensional Model Representation was adopted which identifies the global sensitivity and non-linear contributions of competing reactions for systems with significant uncertainty. Results: *Self-renewal:* Here, sensitivity of nodes in the insulin mediated PI3K/AKT pathway was analyzed to purport strategies to support self-renewal. Application of GSA on the detailed mathematical model and combinatorial perturbation experiments showed that the mean levels and variations in self-renewal molecules like p-AKT were under the control of negative feedback on IRS1 (via PKCζ). Negative feedback also modulated the sensitivity of upstream PI3K and PIP3 mediated reactions. Therefore, inhibition of negative feedback can promote self-renewal conditions; a strategy based on PKCζ inhibition was recently observed to boost viability and growth of naïve pluripotent cells. *Early endoderm differentiation:* Our preliminary experiments showed that the effector molecules of the TGF-β pathway, p-SMAD2 and p-SMAD3 showed divergent dynamics under Activin A treatment, a novel result observed in hESCs. Using hESC specific model, it was observed that the sensitivity of reactions for p-SMAD2 and p-SMAD3 were of similar ranking but of varying strengths resulting in the divergent dynamics as well as different downstream (gene level) effects. Among the most sensitive reactions were, phosphorylation and de-phosphorylation of SMADs, while SMAD nucleo-cytoplasmic shuttling rates were associated with modulation of the aforementioned reactions. Conclusion: Our results have successfully explained the importance of feedback structures playing homeostatic roles in self-renewal and those resulting in divergent responses to endoderm signals. Identification of such sensitive mechanisms under experimental variability will enable the rational design of defined chemical media for modulating hESC fate.

W-2033

SOX17-MEDIATED CONVERSION OF MOUSE EMBRYONIC STEM CELLS (ESCS) INTO FUNCTIONAL EXTRAEMBRYONIC ENDODERM STEM (XEN) CELLS IDENTIFIES DYNAMIC NETWORKS CONTROLLING CELL FATE DECISIONS

McDonald, Angela C.H.[1], Biechele, Steffen[1], Rossant, Janet[1], Stanford, William L.[2]
[1]SickKids, The Hospital for Sick Children Research Institute, Toronto, ON, Canada, [2]Ottawa Hospital Research Institute, Ottawa, ON, Canada

The extraembryonic endoderm (ExEn) of the mammalian conceptus is important for patterning of the embryo proper, gives rise to support 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 ESC. 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 ESCs can induce ExEn gene expression. However, the ability of Sox17 to convert ESCs to functional XEN cells has not been explored. To address this, we overexpressed Sox17 in ESCs using a doxycycline-inducible system (Sox17-ESCs), and generated cells with cell morphology indistinguishable from embryo-derived XEN cells. Sox17-ESCs rapidly induce ExEn genes including Col4a1, Lama1, Gata4 and Gata6 and subsequently repress pluripotency genes Oct4 and Nanog. 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. Single-cell Sox17-mediated conversion is highly efficient with over 90% of cells converting by day 30. Transgene silencing is observed following Sox17 induction and stable Sox17-XEN cells can be maintained independent of transgene expression for greater than 30 passages. Upon injection into host blastocysts, Sox17-XEN cells integrate and proliferate in the parietal endoderm of E8.5 mouse 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. Using the Dynamic Regulatory Events Miner algorithm, we generated a dynamic regulatory map of gene expression throughout Sox17-mediated XEN conversion. Mapping of gene expression bifurcation points revealed 39 dynamic gene expression paths throughout the conversion process. By overlaying transcription factor binding data on top of our dynamic regulatory map, we have identified novel putative regulators of ExEn cell fate. Based on this analysis, we have drafted three classes of ExEn cell fate regulators including ExEn cell fate repressors in ESCs, activators of ExEn cell fate in XEN cells and transcription factors active in both ESCs and XEN cells, acting to either repress or activate ExEn genes respectively. Taken together, our findings suggest that Sox17-mediated XEN conversion is a robust system to study cell fate changes and can be used to identify novel transcriptional network modules regulating these changes. To confirm predicted ExEn regulators, we are currently perturbing the expression of transcription factors in ESCs and XEN cells to induce cell fate changes.

W-2034

BIOMECHANICAL STRESS INDUCED CHONDROGENIC DIFFERENTIATION OF ESCS

McKee, Christina[1], Yao, Donggang[2], Roth, Brad[1], Perez-Cruet, Mick[3], Chaudhry, G. Rasul[1]
[1]Oakland University, Rochester, MI, USA, [2]Georgia Institute of Technology, Atlanta, GA, USA, [3]Beaumont Health System, Royal Oak, MI, USA

While stem cell fate in vivo is determined by complex interactions with the native three-dimensional (3-D) microenvironment, including the organization, composition and mechanical properties of the extracellular matrix (ECM), in vitro differentiation is often achieved by addition of inductive growth factors to adherent culture. Recent studies have indicated that mechanical microenvironments are one of the major epigenetic factors regulating proliferation, survival and differentiation of stem cells in vivo. To mimic these in vivo conditions, 3-D scaffold culturing conditions capable of transducing mechanical signals have attracted a great deal of interest. We hypothesized that understanding the mechanobiology of differentiation could help lineage specific differentiation of embryonic stem cells (ESCs). In this study, we used scaffolds composed of a biocompatible and highly elastic polymer, polydimethylsiloxane (PDMS) to understand the biomechanical control of differentiation. ESCs seeded in the PDMS scaffolds and subjected to mechanical loading, either compressive or tensile forces, underwent significant morphological changes when compared to the control scaffolds. Cell growth and morphological analysis showed selected differentiation into chondrogenic lineage. Further analysis of the cells seeded into PDMS scaffolds subjected to mechanical stress revealed high levels of chrondrogenic markers as judged by immunological and quantitative real time chain reaction (qRT-PCR) studies. Selective up-regulated chondrogenic markers were observed in cells seeded in PDMS scaffolds subjected to mechanical stress and cultured in non-chondrogenic induction medium. A significant increase in collagen type 2 (Col2) and Sox9 was observed following a one-time application of a static compressive or tensile force for 24 hours. Additionally, an increase in RhoA was observed concurrent with up-regulation of chondrogenic markers, signifying a role in RhoA-ROCK signaling in the induction of specific chondrogenic differentiation upon mechanical stress. The results of this investigation suggest a role of mechanical forces in differentiation and may provide basis for translational studies to regenerate skeletal tissues using cell therapy.

W-2035

RAPID AND QUANTITATIVE ASSESSMENT OF HUMAN PLURIPOTENT CELL DIFFERENTIATION POTENTIAL

Meissner, Alexander, **Tsankov, Alexander,** Akopian, Veronika
Harvard University, Cambridge, MA, USA

Human pluripotent stem cells can give rise to all cell types in the body and therefore hold enormous potential for regenerative medicine and for studies in developmental biology and pharmacology. Recent advances in transforming somatic cells directly into pluripotent stem (iPS) cells provide an attractive avenue for generating patient-specific stem cells. However, frequently observed variation between pluripotent stem cell lines is a practical concern for both basic research as well as clinical applications and therefore requires efficient and accurate ways of screening lines for their differentiation potential. Current characterization generally includes immune fluorescence for selected surface markers such as SSEA3/4 or TRA1-60/81, in vitro differentiation or teratoma formation. We have previously shown that a genomic approach that utilizes gene expression signatures can

provide high-dimensional, informative results that correlate well with established measures of differentiation potential. Here we describe a new qPCR-based platform that would be accessible to most labs and enables a faster more quantitative assessment. We provide an in-depth characterization of the new signature panel as well as a direct comparison with the teratoma assay. We also demonstrate the utility of this platform for screening purposes that could be readily scaled and/or adopted to other cell types beyond pluripotent stem cells. In summary, our study provides detailed insights into the power of gene expression signatures as a way to rapidly characterize cell populations in a quantitative fashion.

W-2036
FAS SIGNALING CASCADE DURING THE DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

Miura, Taichi[1], Hirano, Kazumi[1], Sato, Chikara[2], Nishihara, Shoko[1]
[1]*Department of Bioinformatics, Faculty of Engineering, Soka University, Hachioji, Japan,* [2]*Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan*

Fas is a membrane protein, which works as a major signal for the regulation of apoptosis. Activation of the Fas signaling cascade is induced even by the redistribution of Fas into lipid rafts. We have previously reported that the activation of Fas signaling was required for the differentiation from mouse embryonic stem (ES) cells to both primitive endoderm and primitive ectoderm. Fas was recruited into lipid rafts from intracellular pool by up-regulated 3-O sulfated heparan sulfate during differentiation. The activated Fas signaling induced the degradation of Nanog protein by caspase-3. In human T cells, during the redistribution of Fas into lipid rafts, Fas interacts with actin fiber (F-actin) via ezrin, the cytoplasmic peripheral membrane protein. Then Fas is internalized in a clathrin-dependent manner and forms receptosomes, the vesicles including receptors. Subsequently Fas-receptosome activates caspase-8 and caspase-3. However, detailed molecular mechanisms involved in the activation of the Fas signaling during the differentiation are still unclear. To observe the localization of Fas during differentiation to primitive endoderm in detail, we used atmospheric scanning electron microscopy (ASEM), which can observe the sample in culture condition and estimate the distance of the proteins from the cell surface. Then we found that Fas was expressed at low level in mouse ES cells, whereas Fas was significantly increased in primitive endoderm cells. Furthermore, Fas at the edge of the cells was co-localized with F-actin and intracellular Fas was co-localized with clathrin. These results suggest that F-actin and clathrin regulate the recruitment of Fas into lipid rafts on cell surface and its internalization, respectively, during the differentiation. These findings contribute to the clarification of the molecular mechanism of Fas signaling cascade during the differentiation of mouse ES cells.

W-2037
IDENTIFICATION OF TCF7L1-PROXIMAL PROTEINS IN MOUSE EMBRYONIC STEM CELLS BY USING ENGINEERED CELL LINES EXPRESSING BIOTIN-LIGASE-TCF7L1 KNOCK-IN ALLELES

Moreira, Steven[1], Ng, Deborah[2], Fung, Vincent[1], Raught, Brian[2], Doble, Bradley[1]
[1]*Biochemistry and Biomedical Sciences, McMaster University, Hamilton, ON, Canada,* [2]*Medical Biophysics, University of Toronto, Toronto, ON, Canada*

Activation of the Wnt/β-catenin signaling pathway can assist in sustaining mouse embryonic stem cells (mESCs) in a pluripotent state in specific cell culture conditions. It also plays important roles in proper lineage specification throughout different stages of embryonic development. The primary outcome of Wnt/β-catenin signalling is the stabilization, nuclear translocation and direct binding of β-catenin to one of the four members of the T-cell/Lymphoid enhancer factor (TCF/LEF) family of transcription factors. In general, the result of this interaction is the displacement of co-repressors such as transducin-like enhancers of split (TLEs) and the recruitment of co-activators that upregulate the expression of Wnt target genes. Of particular interest in embryonic stem cells is TCF7L1 (formerly known as TCF3), which is a negative regulator of pluripotency, repressing pluripotency-associated genes to promote rapid exit from the pluripotent state. It has been reported that Wnt/β-catenin-mediated enhancement of mESC self-renewal and pluripotency results from β-catenin-mediated dissociation of TCF7L1 from DNA, which results in TCF7L1 proteasomal degradation. Although all TCF/LEF family members share conserved β-catenin, TLE and High Mobility Group (DNA-binding) motifs, TCF7L1 functions as a repressor of reporter activity, even in the presence of elevated β-catenin levels. However, a TCF7L1 mutant lacking a large portion of the context-regulatory domain (CRD) or possessing the substituted CRD of LEF1 stimulates reporter activity in mESCs, demonstrating that CRDs of TCF/LEF factors have unique functions, which may be due to unique protein-protein interactions. In this study, we aimed to identify unique TCF7L1 protein interactions utilizing a promiscuous, Escherichia coli biotin protein ligase (BirA*) knock-in at the TCF7L1 locus in the E14TG2a mESC line. Transcription Activator-Like Effector Nucleases (TALENs) in conjunction with a BirA*-TCF7L1 targeting vector were used to derive 3 independent clones expressing a BirA*-TCF7L1 fusion from a single allele. BirA*-TCF7L1 fusion proteins biotinylate neighboring and/or binding protein partners in a proximity based fashion, in cells supplemented with biotin. Biotinylated TCF7L1 protein interactors were isolated using streptavidin affinity purification and subsequently identified by using mass spectrometry. TCF7L1 protein interactors were identified in cells maintained for 48 hours in the following media conditions: 1) Serum-free medium supplemented with Wnt3a + LIF medium and 3) standard and defined media lacking LIF. This approach termed, BioID is advantageous over conventional co-immunoprecipitation as it identifies weak, transient and proximate interactions such as those that occur with modifying enzymes. Here we provide data in which we describe TCF7L1-proximal proteins and their potential involvement in controlling the function of this key regulator of embryonic stem cell biology.

W-2038
EPIGENETIC BARRIER IN DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS TOWARDS TROPHOBLAST LINEAGE

Ng, Ray
The University of Hong Kong, Hong Kong

The first cell differentiation event in mammalian embryo development is the formation of trophoectoderm (TE) and inner cell mass (ICM). It is now clear that the diversity of cell differentiation and the corresponding gene expression pattern is mediated by means of epigenetic mechanisms, such as DNA methylation and covalent histone modifications. With a global comparison of promoter DNA methylation patterns between mouse embryonic stem (ES) cell and mouse trophoblast stem (TS) cell, we have identified a transcription factor, Elf5, which regulates the determination of trophoblast cell fate through its DNA methylation status. However, the mechanism which directs the setup of differential Elf5 methylation in embryonic precursor cells remains to be elucidated. Recent studies demonstrated that both H3K9 methylation and DNA methylation can be established

at the same loci by a repressor protein complex. We therefore hypothesize that H3K9 methylation is involved in the regulation of trophoblast lineage differentiation, potentially associated with the setup of DNA methylation at Elf5 promoter. To manipulate the cellular H3K9 methylation level, we have constitutively overexpressed Kdm4 (Jmjd2) family histone demethylases in mouse ES cells. While overexpression of Kdm4 family members in ES cells demonstrated no significant induction of trophoblast differentiation under normal TS culture condition, the addition of 5-aza-deoxycytidine triggered significant increase of Elf5 expression, with the exception of Kdm4b overexpressing ES cells. Further analysis of the Kdm4b overexpressing ES cells indicated persistent expression of Nanog under 5-aza-deoxycytidine treatment. Our results thus suggest a role of Kdm4b in the maintenance of pluripotent state of ES cells, and prevent the differentiation to trophoblast lineage. Investigation of the functions of Kdm4 members using the ES cell differentiation system enables us to unveil the details of epigenetic mechanism as well as identify novel lineage determination genes in trophoblast development. Studies on the epigenetic regulation in trophoblast differentiation can provide valuable information on the formation of the placenta, which is crucial for the survival and normal development of an embryo.

W-2039
BMP SIGNALING ARE REGULATED THE DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS INTO EPENDYMAL-LIKE CILIATED CELLS

Ninomiya, Naoto[1], Nishimura, Yusuke[1], Nakanishi, Mio[1], Ohnuma, Kiyoshi[1], Komazaki, Shinji[2], Ishiura, Shoichi[1], Asashima, Makoto[1], Kurisaki, Akira[3]

[1]University of Tokyo, Tokyo, Japan, [2]University of Saitama, Saitama, Japan, [3]Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki, Japan

Motile cilia play crucial roles in the maintenance of homeostasis in vivo. Defects in the biosynthesis of cilia cause immotile cilia syndrome, also known as primary ciliary dyskinesia (PCD), which is associated with a variety of complex diseases. In this study, we found that inhibitory Smad proteins, Smad7 and Smad6, significantly promoted the differentiation of mouse embryonic stem (ES) cells into ciliated cells. Moreover, these Smad proteins specifically induced morphologically distinct Musashi1-positive ciliated cells. These results suggest that inhibitory Smad proteins could be important regulators not only for the regulation of ciliated cell differentiation, but also for the subtype specification of ciliated cells during differentiation from mouse ES cells.

W-2040
SYNERGISTIC EFFECTS OF FOXA2 AND NURR1 ON DOPAMINERGIC NEURONS SURVIVAL VIA MULTIPLE MECHANISMS

Oh, Sangmin[1], Rhee, Yong-hee[2], Yi, Sang Hoon[3], Lee, Sang-Hun[4]

[1]Hanyang Univ., seoul, Republic of Korea, [2]Graduate School of Biomedical Science and Engineering, Seoul, Republic of Korea, [3]Hanyang University, Seoul, Republic of Korea, [4]Department of Biochemistry and Molecular Biology, College of Medicine, Hangyang University, Seoul, Republic of Korea

Parkinson' disease (PD) is regarded as an intractable disorder. Without fundamental therapeutic methods, medical treatment has been commonly done using L-DOPA, a precursor form of dopamine (DA). Current treatment methods have encountered various difficulties of their own. The nuclear receptor related 1 protein (Nurr1) and Forkhead box protein a2 (Foxa2) are the transcription factors of which specific roles in the development and maintenance of the midbrain dopamine(mDA) neurons have been suggested. I herein demonstrate that these factors determine the therapeutic roles in PD by protecting mDA neurons from toxic insults. I showed in this study that forced expression of Nurr1 and Foxa2 protect DA neurons derived from ventral midbrain tissues from toxic insults induced by the general oxygen free radical inducer H_2O_2 and the Parkinsonian toxin MPP+. Co-expression of Nurr1 and Foxa2 showed further dramatic effects on mDA neuron survival and resistance in a synergistic manner. Interestingly diffusible factors released from Nurr1+Foxa2-transduced astrocytes and neighboring non-dopaminergic neuronal cells exerted the protective roles, suggesting a paracrine mode of the Nurr1+Foxa2 actions. I further revealed that SHH, GDNF, BDNF, and NT3 are the responsible paracrine factors. Furthermore, levels of inflammatory cytokines such as TNFα, interleukin-1β released from glial cells were decreased by Nurr1+Foxa2 overexpression. This study is the first demonstration for the therapeutic potentials of Nurr1 and Foxa2 in PD, and thus can offer promise for the treatment of PD.

W-2041
MICROCARRIER-CELL AGGREGATE DIMENSIONS MARSHAL EFFICIENT AND RELIABLE PLURIPOTENT CELL EXPANSION AND CARDIOGENESIS IN BIOREACTORS

Oh, Steve K.W.

Bioprocessing Technology Institute, Singapore

The expansion of human pluripotent stem cells for biomedical applications such as cardiac regeneration compels a defined, reliable and scalable platform in suspension bioreactors which can generate cell densities in the multi-million cells/ml range. This study describes a special technology whereby 100 micron diameter polystyrene microcarriers are layered with a cationic charge followed by either defined Vitronectin or Laminin coatings to provide cell attachment, spreading and growth. This coating combination critically enables formation and evolution of microcarrier-cell aggregates which generate high cell yields reaching over 3.5 million cells/ml in 7 days. The numbers of these microcarrier-cell aggregates increase from 20/ml to ~80/ml at a quazi-constant size of ~300 micron indicating that growth occurs within a self-regulating microenvironment. Importantly, this paired coating enables single cell seeding and cell growth under continuous agitation. Different cell lines (HES-3, H7, IMR90) show highly reproducible cell responses to these surface properties. Post expansion, these microcarrier-cell aggregates were further directed towards the cardiomyocyte lineage with Wnt signalling modulators (CHIR 99021 and IWP2) in 4 different conditions. The continuous agitation bioreactor process yielded the highest numbers of cardiomyocytes of 1.9 million cells/ml compared to the conventional embryoid bodies (EB) method which generated 0.1 million cells/ml - a significant 19 fold improvement. Cardiomyocytes expressed high levels of cardiac troponin (>50%) compared to the EB method (13%). Cardiomyocytes exhibited normal responses to drug -induced QT prolongation assays against E-4031 and Verapamil. This superior technology is ready to be translated to larger bioreactor scales for integrated expansion and differentiation of pluripotent stem cells to cardiomyocytes.

W-2042

LIVE-IMAGING OF THE DIOXIN RESPONSIVE GENE DURING NEURONAL OR HEPATIC CELL DIFFERENTIATIONS FROM HUMAN EMBRYONIC STEM CELLS

Ohsako, Seiichiroh
The University of Tokyo, Faculty of Medicine, CDBIM, Tokyo, Japan

In the course of research to establish an effective alternative developmental toxicity test using human pluripotent stem cells, we have previously reported a human embryonic stem (hES) cell line that carries a mouse Cyp1a1 driven EGFP promoter gene to enable real-time monitoring of dioxin biomarker induction during various phases of differentiation. After sequential passage, responsiveness to TCDD as displayed by EGFP fluorescence in this ESC disappeared, consistent with the non-inducible endogenous human CYP1A1 gene. On the contrary, the EB formed from KhESCCYPEGFP showed fluorescence responsiveness to TCDD in a dose-dependent manner. The TCDD responsive cells were mainly distributed over the EB surface. After outgrowth on matrix-coated plate and neural Rosetta formation, the MAP2-positive cells differentiated in the neuronal differentiation media showed no response to TCDD, and were also EGFP negative. However, in the hepatocyte differentiation culture system, CXCR4- and ALB-positive cells exhibited significant TCDD responsiveness. These results demonstrate that the cell population at the early stage of embryo and endodermal cell lineages may be very susceptible to xenobiotics, but ectodermal cells including neurons are relatively resistant in comparison to other germ layer cells. By applying KhESCCYPEGFP to identify the chemical susceptibility of specific human cell populations, various differentiation culture systems constructed using KhESCCYPEGFP will become an effective test battery for evaluation of chemical and drug safety.The research was supported by Grant-in-aid from the Ministry of Health, Labour and Welfare of Japan.

W-2043

LITHOGRAPHY-TEMPLATED HYDROGEL MICROARRAYS AND PATTERNING INDICATE NONUNIFORM COMPLEX MORPHOLOGIES INHERENT IN FORMATION AND DIFFERENTIATION USE OF LARGE MULTI-CYSTIC HUMAN PLURIPOTENT EMBRYOID BODIES

Tomov, Martin, Olmsted, Zachary, **Paluh, Janet L.**
Nanobioscience, SUNY College of Nanoscale Science and Engineering, Albany, NY, USA

The differentiation of human pluripotent stem cells through embryoid body (EB) formation is increasingly relied upon to evaluate pluripotency potential in vitro and as a precursor for lineage differentiation and organoid formation. EBs formed spontaneously in solution are by nature nonuniform in size and shape. Varied procedures exist for generating uniformity including spin cultures, hanging drop, microwells, and lithography templated arrays, but a convincing need to control the EB size range has not been made. Lithography is a common tool of the semiconductor industry and a desirable choice for generating microwells of customized dimensions. We generated lithography-templated SU-8 moulds for 200 and 500 micron hydrogel microarrays of polydimethylsiloxane (LTA-PDMS grids) as an optically clear seeding platform for formation of human embryonic stem cell (hESC) EBs. Independent of how EBs were formed, that is seeding hESCs as single cells, 2D cell clusters or as one to two day pre-formed 3D early aggregates, or by differentiation protocol, we found that larger EBs generate and retain a complex multi-cystic internal core structure during differentiation that contributes to delayed 3D/2D transition during monolayer formation and the retention of polarized cell features from the multi-cystic core. These characteristics make larger 500 micron EBs unsuitable for high throughput statistical differentiation since cell-cell contact is variable and disrupted by pockets from mixed 3D/2D character that persists longer in early monolayer formation and generates non-uniform culture characteristics. Our studies reveal greater uniformity with 200 micron monocystic EBs, and further demonstrate the usefulness of transparent hydrogel microwells in EB cell formation and follow-up analysis. Our new findings on EB formation indicate the liability of using nontemplated EBs or templated EBs of incorrect size. By adding a subsequent post-patterning step we are also able to ensure uniform settling of EBs, an additional parameter that can affect statistical analysis of differentiation when optimizing conditions in a high throughput platform. Combined microarrays and post-formation patterning control multiple parameters in EB formation and analysis to provide uniform evaluation important for optimizing differentiation protocols with EBs. We are extending the application of these lithography-based tools to in vitro platforms for tumorsphere formation and reprogramming studies in cancer research and for use in multi-cellular mixed co-cultures. Our approach offers an exciting new strategy for studying cell aggregates by enabling better control of formation of multi-cellular 3D environments and their monitoring. This work is supported in part by NYSTEM Award C026189.

W-2044

THE TRANSPLANTATION OF NEURAL PRECURSOR CELLS OVEREXPRESSING ARGININE DECARBOXYLASE ALLEVIATES TISSUE DAMAGE AND IMPROVES LOCOMOTOR ACTIVITY IN CONTUSED SPINAL CORD INJURY

Park, Yu Mi, Han, Sun Hyup, Park, Kyung Ah, Lee, Won Taek, Lee, Won Taek, Lee, Jong Eun
Department of Anatomy, Yonsei University College of Medicine, Seoul, Republic of Korea

Agmatine, an endogenous primary amine and a novel neuromodulator synthesized through the decarboxylation of L-arginine by arginine decarboxylase (ADC), has been reported to exert neuroprotective effects both in the *in vitro* and *in vivo* models. Recently, neural precursor cells (NPCs) with transgenes have been suggested to be a promising alternative for the treatment of CNS injuries. In this study, we employed NPCs expressing ADC to explore the potential application in spinal cord injury (SCI) mice model. The followings include the experimental groups chosen for the study 1) SCI model transplanted with control NPCs, 2) SCI model transplanted with empty vector carrying NPCs, 3) SCI model transplanted with ADC gene overexpressed NPCs. In brief, the vertebra thoracic level 9 of mice was injured with a bilateral micro clamp clip. PKH-26 labeled NPCs, LXSN-NPCs and ADC-NPCs were transplanted at 7th day following SCI. Two injections of 0.5ul (1*10^5 cells/μl) were made using a micro syringe into bilateral part from the midline and both rostral and caudal part to the injury epicenter. Then, mice were sacrificed at 1st, 2nd and 5th week and spinal cords were isolated for further analysis. We found that ADC-NPCs transplanted mice demonstrate the improved motor function compared to LXSN-NPCs or NPCs alone transplanted mice. On the other hand, glial scar formation was significantly reduced in ADC-NPCs group. The higher number of PKH-26 and Ki-67 double positive cells was seen in ADC-NPCs transplantation group, where MAP-2 and Olig-2 expressions were robust around the lesion sites, suggesting the implication of ADC in proliferation and differentiation in terms of the locomotion improvement. Collectively, the present data suggest that ADC-NPCs transplantation significantly enhanced motor activity, induced proliferation and differentiation of neuron and oligodendrocyte, and attenuated the glial scar formation following SCI.

W-2045

CONTROLLING HUMAN EMBRYONIC STEM CELL FATE IN THREE-DIMENSIONS USING BIOCHEMICALLY AND BIOMECHANICALLY TUNABLE MATRICES

Petrie, Timothy A.[1], Leung, Matthew[2], Cooper, Ashleigh[2], Jana, Soumen[2], Zhang, Miquin[3], Moon, Randall T.[4]

[1]Department of Pharmacology, Institute for Stem Cell and Regenerative Medicine, University of Washington-Seattle, Seattle, WA, USA, [2]Department of Materials Science and Engineering, University of Washington-Seattle, Seattle, WA, USA, [3]Department of Materials Science and Engineering, Department of Neurological Surgery, University of Washington-Seattle, Seattle, WA, USA, [4]HHMI, Department of Pharmacology, Institute for Stem Cell and Regenerative Medicine, University of Washington-Seattle, Seattle, WA, USA

Stem cell therapy has the potential to dramatically change the course of treatment for tissue repair and human disease due to stem cell robust self-renewal and pluripotency. The success of this therapy relies on the ability to generate a large number of stem cells with high purity in vitro while being able to attain directed differentiation down specific germ lineages. Recapitulating the in vivo microenvironment with respect to stiffness, extracellular matrix, and growth factors in vitro is critical toward developing translational stem cell therapies. Current methods of expansion utilize either two-dimensional animal-sourced feeder layers, which bears risk of viral transmission and is highly labor intensive, and three-dimensional (3-D) feeder-free systems, including ECM-coated Matrigel scaffolds, which suffer from long-term structural integrity and poorly defined mechanical and biochemical parameters. Moreover, a fundamental understanding of how 3-D material, biomechanical, and molecular events regulate ESC self-renewal and differentiation is critical to the design of a more ideal biomaterial-based microenvironment for large-scale undifferentiated ESC ex vivo maintenance. In this study we have developed a feeder-free 3-D chitosan-alginate (CA) scaffold that provides a highly tunable platform within which key biochemical (ECM protein covalent tethering) and biomechanical parameters (stiffness: 20 kPa-20 MPa, porosity: 30-250 μm) can be precisely, independently controlled in physiologically-relevant ranges. Moreover, both chitosan and alginate are biocompatible, biodegradable, and non-immunogenic. Thus, unlike most other 3-D systems, this scaffold populated with renewed stem cells is implantable for direct tissue engineering and generative medicine applications. We seeded human embryonic stem cells (hESCs, H1) in CA scaffolds of varying stiffness, porosity, and ECM protein (fibronectin, collagen I, IV, elastin, laminin) combinations with and without Wnt3a ligand stimulation, which we have previously shown to enhance hESC differentiation. We also utilized a Venus-based Wnt/β-catenin reporter in this H1 line in which Venus expression is induced when the cell is undergoing Wnt/β-catenin signaling, in order to be able to directly link microenvinromental parameters with cell signaling within hESCs. Using flow cytometry, immunohistochemistry, and rt-PCR, we show that stem cell self-renewal and lineage-specific differentiation are robustly differentially affected by the combination of Wnt stimulation, ECM, and biomechanical parameters after just 3 days within seeding. Specifically, higher stiffness matrices promoted more mesodermal character in hESCs in all conditions, while porosity did not affect stem cell fate significantly in the absence of Wnt stimulation. However, greater pore size matrices promoted faster and more expansive differentiation into all 3 germ lineages under Wnt stimulation, establishing a clear biomechanical link between stem cell signaling and fate. Fibronectin-functionalized matrices promoted the highest degree of differentiation, although the directed lineage was highly dependent on the stiffness and dose of Wnt stimulation. In summary, we have developed an innovative and translationally-relevant 3-D stem cell culture system within which we have conducted a combinatorial study to determine the integrated effects of critical biochemical and mechanical parameters on hESC fate.

EMBRYONIC STEM CELL PLURIPOTENCY

W-2046

REPORT OF SPONTANEOUS TRISOMY RESCUE IN HUMAN EMBRYONIC STEM CELLS

Lee, Jeoung Eun, Shim, Sung Han, Park, Ji Eun, Shim, Myung Sun, Lee, Sung-Geum, Lee, Dong Ryul

CHA Stem Cell Institute, CHA University, Seoul, Republic of Korea

Human Embryonic Stem Cells (hESCs) offer a renewable source for cell based therapy and regenerative medicine by their self-renewal ability and differentiation ability to all kinds of cell type in the body. For clinical application, hESCs must have normal karyotype and then genetic stability of hESCs should be proven during cultivation and differentiation in the chromosomal level at least. Several reports showed that sometimes, hESCs in early passage had normal karyotype and then changed abnormal karyotype after many passages in vitro cultivation. Rarely, it also reported that hESCs were showed karyotypically abnormal in early passage but normal at late passage. Trisomy rescue is the phenomenon in which a fertilized ovum initially contains 47 chromosomes, but loses one of the trisomic chromosomes in the process of cell division such that the resulting daughter cells and their descendants contain 46 chromosomes. Here, we described two cases of spontaneous trisomy rescue in hESCs during maintenance. CHA-hES 42 and 45 were established from donated frozen embryo in CHA Stem Cell Institute, after obtaining informed consent and approval by the Institutional Review Board of CHA Gangnam Medical Center. CHA-hES 42 was 47,XY,+5 at passage 4 and 46,XY at passage 24. CHA-hES 45 was 47,XX,+16 at passage 4 and 46, XX at passage 32. In STR marker expression data of CHA-hES 42, the peak size of one allele(10) of D5S818 loci on chromosome 5 was a half of the other allele(12) at passage 4 and then changed to similar peak size at passage 38. In STR marker expression data of CHA-hES 45, the peak size of one allele(10) of D16S539 loci on chromosome 16 was a half of the other allele(12) at passage 3 and then almost disappeared at passage 20. After trisomy rescue, CHA-hES 42 and 45 have been maintained normal karyotype more than 70 passages. Based on these two cases, trisomy rescue in abnormal hESC line could be spontaneously happened during cultivation, and this phenomenon could be confirmed by regular check of STR marker expression analysis and karyotype analysis.

W-2047

SCREENING OF UP-REGULATED GENES ON CHROMOSOME 12Q IN TRISOMY 12 HUMAN EMBRYONIC STEM CELLS

Seol, Hye Won[1], Baek, Jin Ah[1], Jung, Juwon[1], Yoon, Bo Ae[1], Kim, Hee Sun[2], Oh, Sun Kyung[2], Ku, Seung Yup[2], Kim, Seok Hyun[2], Moon, Shin Yong Yong[1], Choi, Young Min[2]

[1]Institute of Reproductive Medicine and Population, Medical Research Center, Seoul National University, Seoul, Republic of Korea, [2]Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Republic of Korea

Human embryonic stem cells (hESCs) represent important cell sources and hold tremendous promise for cell-based therapies and pharmaceutical application in regenerative medicine as well as basic research field. However, several reports described nonrandom genetic changes involving addition of the whole or parts of chromosome

12, 17, 20 and X in hESCs. Because germ cell tumor (GCT) always contain amplified regions of chromosome 12p, it is important to verify the relationship between these specific genetic changes in hESCs and tumorigenesis. SNUhES3 (S3) and SNUhES4 (S4) were derived and maintained on mitotically inactivated STO feeder layers with DMEM/F12 medium including 20% KSR using mechanical passaging. In prolonged culture, we acquired normal (S3, S4) or trisomy 12 (S3v12, S4v12) cells simultaneously and these cells were analyzed the gene expression profiling using the Illumina HumanRef-6 BeadChip, which included 48,095 probe sets. From these analyses, 55 up-regulated genes and 28 down-regulated genes were identified in S3v12 versus S3. In S4v12, 87 up-regulated genes and 61 down-regulated genes were identified. 21 (38.2%) out of 55 up-regulated genes in S3v12 and 24 (27.6%) out of 87 up-regulated genes in S4v12 are located in chromosome 12. Interestingly, 18 genes on 12q (out of 21 up-regulated genes on chromosome 12) were highly expressed in S3v12 compared to S3 and 22 genes on 12q (out of 24 up-regulated genes) were highly expressed in S4v12 compared with S4. In particular, we observed that 8 genes on 12q, including SOCS2, PUS7L, NTS, INHBE, KRT18, GLTP, FAM113B, and GALNT9 were up- or down-regulated in both S3v12 and S4v12 cells when compared to normal hESCs. Thus, genetic aberrations of hESCs, such as trisomy 12, may not resemble a model of GCT malignancy. Our results may provide insights for exploring the mechanisms that control the fate decisions of pluripotent cells between self-renewal, apoptosis and differentiation. This research was supported by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (2012M3A9C6049722).

W-2048
HUMAN PLURIPOTENT STEM CELL RESPONSE TO A 720 CONDITION LIGAND AND SMALL MOLECULE HIGH-THROUGHPUT SCREEN REVEALS REGULATORS OF PLURIPOTENCY, NEUROECTODERM, AND PRIMITIVE STREAK

Nazareth, Emanuel J.P.[1], Ostblom, Joel E.E.[1], Zandstra, Peter W.[2]
[1]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [2]University of Toronto Institute of Biomaterials and Biomedical Engineering, Toronto, ON, Canada

Populations of cells create local environments that lead to emergent heterogeneity. This heterogeneity in population context is increasingly recognized as a cause of cell-cell variability in assays, and has been shown to lead to inconsistencies in large pharmacological studies. The importance of population context is particularly evident in human pluripotent stem cells (hPSCs), which reside in complex microenvironments, or "niches". As in the embryo, these niches have heterogeneous spatial organization and distinct subpopulations of factor secreting cells. Currently, assays do not account for this microenvironmental heterogeneity, which can limit cell fate control, confound results, reduce assay robustness, and hinder the ability to gain mechanistic insight into stem cell fate processes. To address this, we have developed a micro-contact printing based high throughput (μCP-HTP) platform, consisting of hPSC colonies arrayed in 96-well plates. We have systematically optimized microenvironment parameters to achieve rapid and robust cell fate responses to cues, including colony size, colony spacing, cell density, media composition, and substrate. End-point analysis of Oct4 and Sox2 co-staining enables simultaneous tracking of pluripotent, neuroectoderm, primitive streak, and extraembryonic cell fates in response to cues. We have now applied this platform to screen hPSC (H9 cell line) response to a library of 400 small molecule inhibitors at two doses (1 μM and 0.2 μM). Additionally, we have screened 320 combinations of ligands and their small molecule inhibitors, including all 1 and 2 factor combinations of 24 factors modulating early development signalling pathways. Enrichment analysis (hypergeometric test) of the small molecule library data revealed key targets regulating pluripotency, neuroectoderm, and primitive streak. Conditions with reduced pluripotency were enriched for compounds targeting insulin-like growth factor receptor (IGFR, p=0.018), mitogen-activated protein kinase (MAPK, p=0.037), Src (p=0.030), and IRE-1 (p=0.038). Neuroectoderm promoting conditions were also enriched for compounds targeting MAPK (p=0.002), IRE-1 (p=0.011), and Src (p=0.016), and additionally ALK2/4 (p=0.009). Conditions with enhanced primitive streak were enriched for inhibitors of glycogen synthase kinase 3 (GSK-3, p=0.017). Next, we used the μCP-HTP assay with live-cell calcein staining for endpoint analysis as a hPSC toxicity assay and confirmed high viability in 66 out of 67 top responding compounds. We followed up with dose curves of 30 compounds, validating several compounds including novel regulators of pluripotency, neuroectoderm, and primitive streak. We are currently establishing the effect of these compounds in larger-scale studies and investigating their mechanisms of action. Using the combinatorial screen data, we have clarified the role of individual signalling factors in stem cell fate decisions, and identified synergistic and antagonistic relationships between factors. Lastly, we are integrating these data sets to develop a quantitative mechanistic model of how specific signaling pathways are integrated to determine hPSC fate decisions. Ultimately, the μCP-HTP platform will enable the improved screening of stem cell lines and their derivatives, allowing for step-wise optimization of differentiation an accelerating the pace towards a system-level understanding of development.

W-2049
HUMAN EMBRYONIC STEM CELLS SHOW LOW GRADE MICROSATELLITE INSTABILITY

Nguyen, Ha Thi, Markouli, Christina, Geens, Mieke, Barbé, Lise, Sermon, Karen D., Spits, Claudia
Research Group Reproduction and Genetics, Vrije Universiteit Brussel, Brussels, Belgium

It is well known that human embryonic stem cells (hESC) frequently acquire recurrent chromosomal abnormalities, very reminiscent of those found in cancerous cells. Given the parallels between cancer and stem cell biology, we set out to investigate the occurrence of a common form of genome instability in tumors, namely microsatellite instability (MSI), in hESC. MSI is caused by a deficiency in mismatch repair (MMR) genes, which leads to the accumulation of mutations during DNA replication. In this study, we analyzed over 90 microsatellites in a total of ten hESC lines, for two to seven different passages. In two lines, this revealed that two microsatellites had altered alleles. Small-pool PCR for several microsatellites and testing of the Bethesda panel microsatellites (commonly used in cancer studies) revealed that, whilst MSI is common in all tested lines, it occurs at a very low and variable frequency. In cancerous cells, MSI leads to multiple large shifts in allele sizes within the majority of the cells, while hESC show small changes in a minority of the cells. Since these genetic alterations do not take over the culture, we assume that they are not concurrent with a selective advantage as it is in tumors. Finally, the MMR genes showed a very variable gene-expression that could not be correlated to the variable (low) levels of MSI in the different hESC lines.

W-2050

GATA6 DIRECTLY REGULATES PLURIPOTENCY AND EXTRAEMBRYONIC ENDODERM GENES TO REPROGRAM MOUSE EMBRYONIC STEM CELLS

Wamaitha, Sissy[1], Cho, Ting-yin[2], del Valle, Ignacio[1], Wei, Yingying[3], Fogarty, Norah[1], Sherwood, Richard I.[4], Hongkai, Ji[3], **Niakan, Kathy**[1]
[1]Division of Stem Cell Biology and Developmental Genetics, MRC National Institute for Medical Research, London, United Kingdom, [2]Department of Surgery, University of Cambridge, Cambridge, United Kingdom, [3]Department of Biostatistics, Johns Hopkins University, Baltimore, MD, USA, [4]Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Transcription factors regulate the allocation of cells to specific lineages, and ectopic expression of certain transcription factors can reprogram otherwise differentiated cells to alternative fates. To investigate mechanisms of reprogramming, we engineered mouse embryonic stem (mES) cells that can be induced to overexpress endoderm-associated genes and followed transcription factor binding and global gene expression dynamics over time. In contrast to other endoderm transcription factors, we find that the GATA factor Gata6 uniquely reprograms mES cells by rapidly and directly inhibiting the transcription of pluripotency factors Nanog, Esrrb and Nr5a2, and activating an extraembryonic endoderm gene network. Despite persistent expression, loss-of-function analysis suggests that Oct4 is not required to drive this lineage switch. In addition, we identify significant overlap between Gata6 and Esrrb, Prdm14, Klf4 and Nanog bound genes, suggesting competition for gene regulation. We demonstrate reciprocal Esrrb and Gata6 protein expression dynamics in in vivo blastocysts suggesting antagonism, which precedes Nanog and Gata6 segregation during lineage specification. We propose that the ability to both activate and inhibit genes defines Gata6 as a master regulator capable of single factor reprogramming, and our findings provide important insights into how the pluripotency gene regulatory network is disengaged during lineage specification.

W-2051

CONTEXTEXPLORER: EXPOSING SPATIAL ORGANIZATION OF PHENOTYPIC HETEROGENEITY IN HUMAN PLURIPOTENT STEM CELL COLONIES

Ostblom, Joel E E, Nazareth, Emanuel, Rahman, Nafees, Tewary, Mukul, Zandstra, Peter W.
Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada

A growing body of evidence highlights population context as a major contributor to cellular phenotype and heterogeneity within cell populations. This phenotypic heterogeneity can strongly influence experimental outcomes, as has been shown in both mouse and human pluripotent stem cells (PSCs). However, the emergence of spatial heterogeneity is poorly studied and may contain novel insights to microenvironmentally regulated cell fate control mechanisms relevant to the stem cell niche in vivo. Given the strong relationship between population context and cellular phenotype, we believe accessibility to user friendly software to measure microenvironmental factors is paramount. To address this, we have developed ContextExplorer, a standalone application for post-processing data from cell image analysis software, with a focus on measuring population context dependent variables, such as a cell's position within a colony and its number of neighbors. ContextExplorer is open source, cross-platform compatible and has its full functionality accessible through a graphical user interface. It identifies colonies in both patterned and unpatterned wells based on cell density, with optional filters for marker intensity, colony size and colony shape. Both cell level (position, local cell density, marker expression, etc.), colony-level (colony size, shape, etc.) and well level (media condition, total cell number, colony distribution, etc.) parameters are then quantified for each cell. ContextExplorer can be used to expose correlations between these factors through a number of output analytics: superimposition of colony images within the same condition, robustness measurements between conditions, scatter plot matrices of measured parameters and cellular phenotypes and quality control indicators of pattern fidelity. Analysis of patterned colony data has revealed cells at different developmental stages to display spatially organized patterns of protein expression. The pluripotency markers Oct4 and Sox2 show the highest expression at the center of human PSC colonies, and expression gradually decreases towards the colony border. By classifying cells into three groups depending on their position within the colony ("center", "ring" and "edge"), the differences in protein expression between the three groups can be quantified. The Sox2 expression is on average is 93 % higher in the center compared to along the edges of the colony and 36 % higher than among cells located in a ring surrounding the center. Oct4 expression is 41 % higher in the center versus at the perimeter and 12 % higher compared to the ring group. The non-parametric Kruskal-Wallis rank test and Tukey's post hoc test show that 95 % confidence intervals of ranked means are not overlapping for any of the conditions, indicating they all have statistical significance. The phenomena of colony organization appears generalizable, as under other conditions (i.e. targeted differentiation) we observe similar phenotypic patterns. In conclusion, by using ContextExloprer to analyze data from patterned human PSC colonies, we observe spatial heterogeneity in expression of key pluripotency markers, which seem to be organized in a population context dependent pattern of gradually decreasing expression towards the edge of the colony.

W-2052

DESMOGLEIN 2, A NOVEL SURFACE MARKER, PLAYS A ROLE IN MAINTENANCE OF SELF RENEWAL AND PLURIPOTENCY IN PLURIPOTENT STEM CELLS.

Park, Jongjin
Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea

Systematic identification and characterization of the cell surface molecules of pluripotent stem cells(hPSCs) provide simple tools to identify and analyze specific cell populations and has practical applications in the purification of cells for cell transplantation therapy. Here, we generated monoclonal antibodies (MAbs) by immunizing mice with human embryonic stem cells (hESCs) clumps. Among these, we selected and characterized a new MAb, 6-1 that binds specifically to hESCs but not to mouse ESCs and identified its target antigen as the desmoglein 2 (Dsg2). Dsg2 colocalized with hPSC-specific cell surface markers, and its expression was markedly downregulated upon differentiation, similar to the SSEA-3 and SSEA-4 antigens. Stable Dsg2 depletion markedly decreased hESC proliferation. In addition, expression of Oct4, Nanog, and Sox2 was significantly downregulated in Dsg2-depleted hESCs compared to that in control shRNA-transfected cells. Moreover, Dsg2-depleted hESCs exhibited accelerated expression of the lineage markers and showed reduced time expenditure for developing into specific lineage commitment during embryonic body formation. Notably, depletion of Dsg2 in PSC significantly reduced single PSC colony forming ability and increased fibroblast like cells in basement membrane Matrigel coated feeder free culture. Taken together, our results suggest that Dsg2 is closely related to the maintenance of hPSC self-renewal and pluripotency and could be useful for characterizing specific populations in hPSC.

W-2053
ROLE OF SEROTONIN IN MAMMALIAN PLURIPOTENT CELLS

PB, Megha
Lab-5, National Centre for Biological Sciences, Bangalore, India

Though serotonin is more widely recognized as a neurotransmitter, majority of serotonin in the body is found outside the central nervous system where it is attributed with various functions. it plays multiple roles from liver regeneration to insulin secretion. Serotonin is also shown to play a role in the left-right axis pattern formation (in Xenopus and Chicken). The presence of serotonin in mouse embryonic stem cells (mESCs) has been reported earlier. We now report that serotonin is present in human ESCs and iPSCs. Some of the serotonin co-localizes to the mitochondria as shown by dual immuno-staining experiments in both ES and iPS cells. Exogenous addition of serotonin also causes a decrease in the reactive oxygen species (ROS) levels in both cell types. However, addition of serotonin shows a significant difference in its effect on mitochondrial potential - mESCs show an increase while hESCs show no change. This may reflect the inherent differences in the mitochondria of these two cell types - mESCs i.e. 'naïve' cells and hESCs i.e. 'primed' cells which has been reported earlier. To investigate this difference in the effect of serotonin on mitochondrial potential between the two cell types, I further attempted to convert typical 'primed' hESCs to the 'naïve' state and characterize them. Human ESCs being converted to the 'naïve' state show differences in various mitochondrial properties and their mitochondria now show an increase in potential with exogenous serotonin. 'Naïve' cells seem to respond to serotonin unlike 'primed' cells. This may be based on inherent metabolic differences between the two states and could be of potential use in isolating 'naïve' cells from 'primed' pluripotent cells and somatic cells. We are at present trying to determine the mechanism involved.

W-2054
PLURIPOTENCY EVALUATION OF EMBRYONIC STEM CELLS ISOLATED FROM EMBRYO STAGE X IN COMPARISON TO ADULT CELLS OF CHICKEN (GALLUS GALLUS)

Pourasgari, Farzaneh[1], Jahanpanah, Maryam[2], Mohammadi-Sangcheshmeh, Abdollah[3], Soleimani, Masoud[4]
[1]*Molecular Biology and Genetic Engineering, Stem Cell Technology Research Centre, Tehran, Iran,* [2]*Department of Biology, Kharazmi University, Karaj, Iran,* [3]*Department of Animal and Poultry Sciences, College of Aburaihan, University of Tehran, Pakdasht/Tehran, Iran,* [4]*Tarbiat Modares University, Hematology Dept, Tehran, Iran*

Chicken as a model is particularly desirable when studying developmental biology or making animal models of human diseases. In this regard, it has been reported that embryonic stem cells derived from stage X chicken embryo are an appropriate source of stem cells to differentiate into the somatic tissue and also germ line cells. This work is aimed to isolate and characterize the chicken Embryonic Stem Cells (cESCs) and compare their genes expression with primary adult chicken and cell line cultures. To that end, the cells were isolated from area of pellucida from stage X chicken embryo. Then the cells were cultured on inactivated mouse SNL feeder cells in the presence of LIF, IGF-1, bFGF, CNTF, OSM, SCF, Il-6 and Il-11 growth factors. The cESCs colonies were picked up and subsequently passaged. To characterize the cells, they were analyzed for alkaline phosphatase, SSEA-4 and TRA-1-60 as embryonic-specific markers in the level of proteins. Furthermore, the expression of Cvh, Brachyury, Gata6, Sox2, Nanog and cPouV genes as markers of developed chicken tissues were compared to a chicken adult tissue and a chicken cell line, MDCC, in the level of mRNA using Quantitative RT-PCR. After one week culture of cES cells, the morphology of the cells changed, became smaller in size with big nuclei and detectable nucleus. Isolated cells were passaged repeatedly and successfully. The stemness of embryonic cells has been validated by alkaline phosphatase and ICC staining and expression of above mentioned molecular markers. Quantitative RT-PCRs showed expression of cPouV, Nanog and Sox-2 increased in comparison to the tissue and the cell line. Our observation showed that chicken embryonic stem cells can be isolated successfully from stage X of chicken embryo and maintain their stemness properties during multi-passages.

W-2055
ANALYSIS OF DIFFERENTIATION DEPENDENT CONNEXINS IN MOUSE ES CELLS

Saito, Mikako, Asai, Yuma, Tanaka, Kento, Matsuoka, Hideaki
Department Biotechnology and Life Sciences, Tokyo University of Agriculture and Technology, Tokyo, Japan

Pluripotent embryonic stem (ES) cells are capable of maintaining a self-renewal state and have the potential to differentiate into derivatives of all three embryonic germ layers. Despite their importance in cell therapy and developmental biology, the mechanisms whereby ES cells remain in a proliferative and pluripotent state are still not fully understood. Gap junctional intercellular communication (GJIC) has been suggested to be necessary for cellular proliferation and differentiation. We would like to investigate the function of GJIC in mouse embryonic stem (ES) cells using a genetic approach to inhibit the expression of connexins, that is, the subunit proteins of gap junction channels. For this purpose, we have analyzed all known connexin genes in mouse ES cells. All 15 known connexins (Cx26, Cx29, Cx30, Cx30.2, Cx30.3, Cx31, Cx31.1, Cx32, Cx33, Cx37, Cx40, Cx43, Cx45, Cx46, Cx57) were detected by RT-PCR. The expression level of three connexins increased under differentiation process. On the other hand, the expression level of two connexins decreased under same condition. The connexin expression patterns in mouse ES cells may provide indicator in cellular differentiation and the maintenance of pluripotency.

W-2056
PLURIPOTENCY OSCILLATIONS IN CELL CYCLE - IMPLICATIONS FOR CANCER STEM CELLS

Sekyrova, Petra, Sramkova, Zuzana, Omelyanenko, Anna, Andäng, Michael
Department Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Glioblastoma multiforme is a particularly devastating type of brain cancer with poor survival prognosis. High degree of glioblastoma recurrence could be explained by existence of subpopulation of cells in the tumor that posses "stemness" features. To eradicate cancer stem cells successfully we need to understand mechanisms, which maintain/generate stemness properties in cancer cells. Recent publications suggest that stemness is tightly connected to cell cycle progression in embryonic stem cells. Embryonic stem cells in the G2 phase are most effective in inducing pluripotency by somatic cell fusion(1). On the other hand, highest differentiation rate is achieved in embryonic stem cells, which entered G1 cell cycle phase(2). We aim to understand the mechanisms how cell cycle regulates pluripotency in embryonic stem cells and if same mechanisms exist in cancer stem cells. We recently developed a flow cytometry based technique to isolate stem cells in different cell cycle phases in large quantities without need for synchronization. We will analyze transcriptomes and proteomes of cells sorted in different cell cycle phases and search for pluripotency genes that are regulated

by cell cycle. We aim to identify common denominators of stemness in embryonic stem cells and glioblastoma stem cells and manipulate them to eradicate cancer stem cells by dedifferentiation. Our data will bring deeper understanding to mechanisms underlying pluripotency and its connection to proliferation and cell cycle. Targeting stemness via cell cycle might open possibilities for novel therapeutic anti-cancer strategies.

W-2057
REGULATION OF PLURIPOTENCY BY NAT1 IN MOUSE EMBRYONIC STEM CELLS

Sugiyama, Hayami[1], Yamanaka, Shinya[2]
[1]Department of Reprogramming Science, The Center for iPS Cell Research and Application, Kyoto, Japan, [2]Center for iPSC Cell Research and Application, Kyoto, Japan

NAT1 was identified as a candidate RNA modified by the RNA editing enzyme,APOBEC-1, and has amino acid sequence similar to eukaryotic translation initiation factor (eIF) 4G. eIF4G binds and coordinates cap-dependent translation with eIF4E, eIF3 and eIF4A. Human (h) NAT1 is homologous to the carboxyl two-thirds of eIF4G and binds to eIF3, eIF4A but not to eIF4E. It was also reported that hNAT1 regulates the translation of C-MYC, XIAP, BCL-2, CDK1. Mouse (m) NAT1 shares high homology (99%) with hNAT1 and is ubiquitously expressed. In our previous study, to analyze the function of NAT1, we generated $NAT1^{-/-}$ mice and $NAT1^{-/-}$ mES cells. While $NAT1^{+/-}$ mice did not show any detectable phenotypic changes, $NAT1^{-/-}$ mice showed embryonic lethality at the gastrulation stage. In addition, $NAT1^{-/-}$ mES cells exhibited an impaired ability to differentiate into cells of all three germ layers and in cell culture, have a rounded, dome-like morphology without differentiated cells. However, the relationship between NAT1 function and those phenotypes is not clear. In this study, we examined whether NAT1 regulates pluripotency in mES cells. We revealed that $NAT1^{-/-}$ mES cells upregulated several pluripotency-associated genes compared with WT mES cells. After reintroduction of NAT1, rescued mES cells exhibited their gene expression and morphology similar to WT mES cells. In addition, NAT1 was localized in the cytoplasm but not in the nucleus, of mES cells and we figured out NAT1-binding-proteins. These results indicate that NAT1 influences pluripotency through cooperation with NAT1-binding-proteins in cytoplasm and indirect transcriptional regulation of the pluripotency-associated genes.

W-2058
BIOSYNTHESIS OF RIBOSOMAL RNA REGULATES PLURIPOTENCY AND DIFFERENTIATION ABILITY OF PLURIPOTENT STEM CELLS

Takada, Hitomi, Watanabe-Susaki, Kanako, Miwata, Kyoko, Sugino, Hiromu, Asashima, Makoto, Kurisaki, Akira
National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki, Japan

Pluripotent stem cells have large and condensed nucleoli. Although it is well known that the characteristic morphology of nucleoli is one of the hallmarks of ES cells, the contribution of nucleoli to pluripotency of ES cells have not been explored. Here, we examined the function of nucleoli in ES cells by modulating the expression of FBL, a critical regulator of ribosomal RNA (rRNA) processing in nucleoli. Stable expression of FBL in ES cells prolonged the pluripotent state of mouse ES cells cultured in the absence of leukemia inhibitory factor (LIF). Knockdown of FBL induced a delay of rRNA processing and apoptosis in ES cells. Interestingly, partial knockdown of FBL promoted differentiation of ES cells even in the presence of LIF and caused morphological change of nucleoli. Furthermore, we observed that the reduction of FBL expression induced differentiation of ES cells via p53 signaling pathway. These results suggest that biosynthesis of ribosomal RNA in nucleoli is a key regulatory factor for pluripotency and differentiation ability of ES cells.

W-2059
CADHERIN-SWITCHING IS IMPORTANT FOR PLURIPOTENT STEM CELL BETWEEN NAïVE AND PRIMED STATE

Takehara, Toshiyuki[1], Teramura, Takeshi[2], Onodera, Yuta[1], Fukuda, Kanji[1]
[1]Kinki University, Osaka, Japan, [2]Kinki University, Osaka-Sayama, Japan

Recently, because of the development of stem cell biology, pluripotent stem cell has the biphasic state: naïve and primed sate. These stem cells have different characteristics (i.e. morphology, mechanism of maintenance of undifferentiated state and differentiation potency). Understanding and elucidating the mechanisms/factors involved in the differentiation may enable us to switch these two pluripotent states, i.e. the shuttle between mESC- and mEpiSC-states can lead to increasing the value of the hESCs. For example, it may provide a basis for generating more ideal types of mESC-like human pluripotent cells from conventional hESCs. However, the factors that affect the pluripotent state conversions have not been fully elucidated. Interestingly, we have previously demonstrated that differentiation from mouse Embryonic Stem Cells(mESCs) to mouse Epiblast Stem Cells (mEpiSCs) is associated with a cadherin-switching, from E-cadherin to N-cadherin. However, there is still little knowledge about the influence that it has on the difference of revelation of cadherin protein and the character of a pluripotent stem cell. In this study, we tried to elucidate cadherin-switching effect on the state of pluripotent stem cell. To clarify the influence of two type of cadherin expression on the pluripotent state, we used conventional and E cadherin overexpression EpiSC. The analysis was performed by Realtime-PCR, Westernblot, Immunofluorescence and Co- immunoprecipitation. Firstly, we showed cadherin type in mESCs and mEpiSCs. The mEpiSCs express significant N cadherin but not E cadherin, and by the treatment of N cadherin siRNA, mEpiSC decreased pluripotent associated gene such as Nanog and Klf4. Also, in the Ecadherin overexpression mEpiSC, siRNA treatment similarly decreased that. Furthermore, the existence of N cadherin were showed the activation of Akt and Erk signal. It has been found that inhibition of N cadherin suppressed the FGF signal cascade as associated pluripotency. The present study provides evidence that the differentiated expression of cadherin type is important factors for the promotion and stabilization of mEpiSC-state. Determination of the cadherin functions and signal cascade enhancing the conversion or increasing mESC- or mEpiSC-state stabilities in future studies are essential to correctly understanding the developmental stages and properties of pluripotent stem cells, which may lead to a more ideal model system for studying human development.

W-2060
RELB-IMP3-LIN28A COMPLEX CONTROLS CELL CYCLE AND DIFFERENTIATION IN HUMAN PLURIPOTENT STEM CELLS

Thakar, Nilay Y.[1], Ovchinnikov, Dmitry[1], Hastie, Marcus[2], Gorman, Jeffrey[2], Wolvetang, Ernst[1]
[1]Australian Institute for Bioengineering and Nanotechnology, Brisbane QLD, Australia, [2]Protein Discovery Centre, QIMR Berghofer Medical Research Institute, Brisbane QLD, Australia

The molecular mechanisms that orchestrate exit from pluripotency, cell

cycle and lineage-specific differentiation in human pluripotent stem cells (hPSCs) remain to be completely understood. RELB, a key protein in non-canonical NFκB signaling pathway, was previously implicated in controlling the switch between human embryonic stem cell (hESC) proliferation and differentiation. Here we show that RELB maintains pluripotency of hESCs and hiPSCs via suppression of differentiation, and enhances proliferation of these cells. We demonstrate that RELB does this by a) forming a tri-partite complex with LIN28A and IMP3 (IGF-2 mRNA binding protein 3), b) that this interaction controls mRNA levels and protein expression of IGF-2 and key cell-cycle genes and c) that following stress these proteins co-localize in stress granules in hESCs and iPSCs. Our data identify RELB as a novel regulator of hPSC proliferation and differentiation, and reveal a potential mechanism that involves RELB-mediated recruitment of IMP3 and LIN28 to cytosolic mRNA translation-control domains. We are currently performing RNA-Seq to identify novel NFκB interacting RNA molecules and shift the paradigm from more than 25 years of study into NFκB proteins by demonstrating its regulation of RNA metabolism.

W-2061
CONSTRUCTION OF ERK1 AND ERK2 NULL MOUSE EMBRYONIC STEM CELLS WITH THE CRISPR/CAS SYSTEM

Wang, Bo[1], Zhang, Qian[1], Chen, Lingyi[2]
[1]Nankai University, Tianjin, China, [2]Nankai University, Tianjin, China

MAPK signaling plays an important role in regulating the self-renewal and differentiation of mouse embryonic stem cells (ESCs). To better understand the function of Erk1 and Erk2 in mouse ESCs, we applied the CRISPR/Cas system to knockout Erk1 and Erk2 in mouse ESCs. Erk1-/- and Erk2-/- ESCs were obtained with high efficiency. However, we failed to construct Erk1 and Erk2 double null ESCs by sequential disruption of Erk1/2, implying that MAPK signaling is indeed indispensable for mouse ESCs. Next, a doxycycline-inducible Erk1 transgene (iErk1) was introduced into Erk1-/- ESCs. In the presence of doxycycline, we were able to disrupt the Erk2 gene, and obtained the iErk1, Erk1-/-, Erk2-/- ESCs. We demonstrated that 48 hours after doxycycline withdrawal, Erk1 protein becomes undetectable. With these cell lines, we will investigate the role of MAPK signaling in mouse ESC self-renewal and differentiation.

W-2062
MICRORNA CLUSTER 302-367 IS A KEY MEDIATOR FOR CHEMICAL INDUCED SELF RENEWAL PROGRAM IN HUMAN EMBRYONIC STEM CELLS

Wu, Wen-shu
Department of Medicine, University of Illinois at Chicago, Chicago, IL, USA

Human embryonic stem cells (hESCs) and iPS cells hold great potential for regenerative medicine because of their unlimited self-renewal capacity and ability to differentiate into all the cell types in the body. hESC self-renewal is intrinsically regulated by pluripotency-associated transcription factors (e.g., Oct4, Sox2, and Nanog) and ESC-specific microRNAs. On the other hand, extrinsic factors such as bFGF and some small molecules promote self-renewal of hESCs. Especially, histone deacetylase inhibitors (HDACi) such as sodium butyrate (NaB) and TSA can induce self-renewal program in hESCs in medium without bFGF. However, the molecular mechanisms underlying the HDACi-induced self-renewal programs in hESCs remain largely unknown. In the present study, we found that NaB upregulated expression of the miR-302/367 cluster in hESCs. By designing transcription activator-like effector (TALE)-based transcriptional repressors specific for the miR-302/367 cluster promoter, we showed that the miR-302/367 cluster dually regulates cell cycle and cell death in hESCs. Interestingly, the modulation of these two pathways is dictated by the dosage of the miR-302/367 cluster. We performed PCR array to screen target genes of the miR-302/367clsuter, and identified two putative targets- BNIP3L/Nix and BCL-xL. Our studies indicate that BNIP3L/Nix is a direct target gene of the miR-302/367 cluster. Furthermore, we showed that over-expression of BCL-xL could rescue hESC from apoptosis caused by knockdown of endogenous miR-302/367 cluster. Our ongoing experiments suggest that knockdown of the miR-302/367 cluster impaired the NaB-induced pluripotency in hESCs in the absences of bFGF. Based on our data, we proposed a mechanistic action model in which the miR-302/367 cluster mediates the NaB-elicited self-renewal program by concurrently promoting cell cycle progress and inhibiting apoptosis of hESCs.

W-2063
ZFP322A REGULATES MOUSE ES CELL PLURIPOTENCY AND ENHANCES REPROGRAMMING EFFICIENCY

Wu, Qiang
Biochemistry, National University of Singapore, Singapore

Embryonic stem (ES) cells derived from the inner cell mass (ICM) of blastocysts are characterised by their ability to self-renew and their potential to differentiate into many different cell types. Recent studies have shown that zinc finger proteins are crucial for maintaining pluripotent ES cells. Mouse zinc finger protein 322a (Zfp322a) is expressed in the ICM of early mouse embryos. However, little is known regarding the role of Zfp322a in the pluripotency maintenance of mouse ES cells. Here, we report that Zfp322a is required for mES cell identity since depletion of Zfp322a directs mES cells towards differentiation. Chromatin immunoprecipitation (ChIP) and dual-luciferase reporter assays revealed that Zfp322a binds to Pou5f1 and Nanog promoters and regulates their transcription. These data along with the results obtained from our ChIP-seq experiment showed that Zfp322a is an essential component of mES cell transcription regulatory network. Targets which are directly regulated by Zfp322a were identified by correlating the gene expression profile of Zfp322a RNAi-treated mES cells with the ChIP-seq results. These experiments revealed that Zfp322a inhibits mES cell differentiation by suppressing MAPK pathway. Additionally, Zfp322a is found to be a novel reprogramming factor that can replace Sox2 in the classical Yamanaka's factors (OSKM). It can be even used in combination with Yamanaka's factors and that addition leads to a higher reprogramming efficiency and to acceleration of the onset of the reprogramming process. Together, our results demonstrate that Zfp322a is a novel essential component of the transcription factor network which maintains the identity of mouse ES cells.

W-2064
PMF, A KLF4 DOWNSTREAM E3 LIGASE CONTRIBUTES TO PLURIPOTENCY VIA MODULATING ERK PATHWAY

Wu, Mian
School of Life Science, University of Science and Technology of China, Hefei, China

Embryonic stem cells (ESCs) have great potential for regenerative medicine. Increasing evidence suggests that the pluripotency state of ESCs is accurately regulated by an intrinsic signaling network. The underlying mechanisms, however, remain elusive. Here, we apply a retroviral insertion vector, which can cause insertional mutagenesis, to screen and identify novel host genes that are requisite for pluripotency maintenance of mouse ESCs. We identify that pluripotency maintaining factor (PMF), an E3 ubiquitin ligase, is involved in the mESCs identity maintenance. Knockdown of PMF leads to decreased expression

of pluripotent markers and impaired clonogenicity of mESCs, and lower somatic reprogramming efficiency. Meanwhile, we show that PMF is transcriptionally upregulated by klf4. PMF is able to catalyze K63-linked poly-ubiquitination of PKR and activate PKA, thereafter inhibiting Raf/MEK/ERK pathway. Functionally, ectopic expression of PMF partially replaces the MEK/ERK inhibitor for mESCs propagation in the serum-free culture conditions. Altogether, our study uncovers a novel klf4-PMF-ERK pathway that plays an important role in the stemness maintenance of mESCs.

W-2065
A CUSTOM METHYL-SEQ STEM CELL PANEL TO DETERMINE THE EPIGENETIC SIGNATURES OF CSCS

Poulin, Matthew[1], Alexander, Jessica[1], Giorda, Kristina[2], Meredith, Gavin[2], Timp, Winston[3], **Yan, Liying**[1]
[1]EpigenDx, Hopkinton, MA, USA, [2]Thermo Fisher Scientific, San Francisco, CA, USA, [3]Johns Hopkins University, Baltimore, MA, USA

Embryonic stem cells (ESCs) are pluripotent, self-renewing cells. The physiological properties of pluripotency in stem cells and the processes of cell specialization are governed by epigenetic mechanisms. One of the best known epigenetic processes is DNA methylation, which is a major mechanism for regulation of gene expression. Differential DNA methylation of pluripotence-associated genes such as Nanog and Oct4/Pou5f1 has been observed between pluripotent and differentiated cells. As more associations are discovered between aberrant DNA methylation and cancer stem cells (CSCs), there is an increasing demand for high-throughput approaches for DNA methylation analysis. In order to develop a stem cell panel for the determination of a DNA methylation pattern in cancer stem cells (CSCs), we started with a candidate gene approach, in contrast to genome-wide methylation screening. Genes from different functional groups were selected. The stem cell panel includes pluripotency-associated genes (Oct4, Nanog, Sox2, cMyc, and Klf4), DNA methyltransferases (DNMTs and MGMT), DNA repeats (Line1 and Sat2), imprinted genes (IGF1, H19, IG-DMR), genes involved in x-chromosome inactivation (FMR1 and XIST), and cell differentiation regulators (Notch genes, PPARG, RB1, RUNX1). A total of 30 genes containing 77 PCR amplicons were selected and assays for these regions were developed and validated using PCR/Pyrosequencing, individually. The development of a custom assay for targeted Methyl-Seq using an Ion Torrent PGM™ system, requires that the 77 PCR amplicons be grouped into 5 different pools based on the amplicon size, amplicon GC contents, and PCR conditions. DNA methylation controls (0%, 5%, 10%, 25%, 50%, 75%, and 100%) were sequenced on an Ion 318 Chip to validate each target region. The correlation between the calculated methylation levels and the detected methylation levels were examined. Additionally, 10 pairs of ovarian cancer DNA samples (tumor vs adjacent normal) were further tested using both an Ion Torrent PGM™ system and Pyroseqencing. The comparison of the results obtained by Pyrosequencing and Next-Gen Sequencing using PGM shows that PCR pools with common amplicons gave linear correlation. PCR pools with extreme conditions, i.e. AT-rich (less than 20% GC contents) or GC-rich (greater than 70% GC contents) required further optimization. In summary, high-throughput Methyl-Seq using an Ion Torrent PGM™ system has the capability to generate similar results as Pyrosequencing with the potential of using less DNA for the analysis. It is also a more cost-effective and a rapid data generation method by reducing the number of PCR reactions from 77 down to 5 and permits combining and sequencing ampicon pools on a single sequencing chip. These results support future use of a custom Methyl-Seq panel for efficient and sensitive epigenetic detection and analysis of CSCs.

W-2066
ROLES FOR TETS AND 2I IN TELOMERE MAINTENANCE OF ES CELLS

Yang, Jiao
Nankai University, Tianjin, China

TET proteins play diverse roles in epigenetic regulation, germ cell and embryonic development, stem cell function and tumorigenesis, but the underlying mechanisms remain to be defined. We report that Tet1 and Tet2 are required for telomere maintenance and chromosomal stability of mouse ES cells. Either knockdown or knockout of Tet1 and Tet2 leads to telomere shortening and dysfunction, and eventually abrogates pluripotency. Dnmt3b is implicated in Tets role in telomere maintenance by regulating DNA methylation levels at subtelomeres of specific chromosomes. Notably, 2i down-regulate Dnmt3a/b and effectively elongate telomeres, compensating for Tet1 and Tet2 deficiency in mouse ES cells. Together, Tets(Tet1 and Tet2) play novel roles in telomere maintenance by epigenetic modification, implying that telomeres also may link Tets function to ES pluripotency, development, aging and cancer.

W-2067
LIVE-CELL IMAGING FOR THE ANALYSIS OF CELLULAR HETEROGENEITY IN MOUSE EMBRYONIC STEM CELL MUTANT CLONES IDENTIFIED BY FORWARD GENETIC SCREENING

Yoshida, Junko[1], Horie, Kyoji[2]
[1]Osaka University Graduate School of Medicine, Suita, Japan, [2]Nara Medical University, Kashihara, Japan

Forward genetics is a powerful method for the discovery of novel genes involved in the regulation of various biological processes in an unbiased manner. However, recessive genetic screening is difficult in mammalian cells due to the diploidy of the genome. Generating homozygous mutant cells through two rounds of gene targeting is labor-intensive. Therefore, a more streamlined high-throughput approach is anticipated in order to achieve forward genetics in mammalian cells. Embryonic stem cells (ESCs) are attractive for forward genetics because various biological processes can be studied using in vitro differentiation protocols. We recently reported a method to rapidly generate homozygous mutant mouse ESCs from heterozygous mutant ESCs by conditional regulation of the Bloom's syndrome gene (Blm). This method takes advantage of the high rate of loss of heterozygosity associated with Blm-deficient condition. We have so far generated ~200 homozygous mutant ESC clones. To accelerate phenotype analyses of the homozygous ESC clones in various culture conditions, we devised a system to conduct phenotype screening by pooling homozygous ESC clones. To keep track of the behavior of each homozygous mutant ESC clone, we introduced synthetic short nucleotide barcode sequences into homozygous ESC clones. Barcode-labeled homozygous ESC pool was cultured in various conditions and populations of undifferentiated and differentiated ESCs were quantified by counting barcode reads using the Illumina GA2 sequencer. As a result, we identified mutant ESCs that can be grouped into following categories: (1) differentiation-resistant clones, (2) differentiation-prone clones, (3) mutant clones showing both undifferentiated and differentiated states in the absence of LIF. Heterogeneity of the undifferentiated and differentiated states in category (3) strongly suggests that the genes mutated in category (3) play important roles in orchestrating pluripotency-inducing factors and differentiation-inducing factors. We therefore focused on phenotype analyses of a representative clone of category (3) (hereafter designated as "Clone A"). To distinguish undifferentiated and differentiated states in live cells, we knocked-in the EGFP reporter into the Nanog locus of

Clone A as well as wild-type (Wt) ESCs. Upon withdrawal of LIF, EGFP signal dropped rapidly in Wt ESCs. In contrast, Clone A exhibited continuous expression of EGFP. However, the intensity of EGFP signal was substantially heterogeneous in Clone A and EGFP-negative population was always observed in the same culture. We FACS-sorted EGFP-negative and EGFP-positive populations, and then cultured separately in the absence of LIF. Only EGFP-negative cells were observed from EGFP-negative population. In contrast, EGFP-positive population gave rise to both EGFP-positive and EGFP-negative cells, suggesting that the cellular state is fluctuating in undifferentiated state while a portion of the cells go beyond the threshold and reach to an irreversibly differentiated state. We consider that the dynamics of this fluctuating state implies a unique role of the mutated gene in the regulation of pluripotency. To further elucidate the role of the mutated gene, we are currently conducting live-cell imaging with single cell resolution and trying to quantify dynamic alteration of the cellular state upon LIF depletion.

W-2068
REGULATION OF PLURIPOTENCY BY WDR5 AND ITS BINDING PARTNERS

Yuan, Ye[1], Ding, Junjun[1], Waghray, Avinash[1], Chang, Betty[1], Lee, Dung-Fang[1], Pereira, C. Filipe[1], Faiola, Francesco[1], Graham, Robert[2], Williamson, Andrew[3], Wang, Jianlong[1], Lemischka, Ihor R.[1]
[1]Icahn School of Medicine at Mount Sinai, New York, NY, USA, [2]University of Manchester, Manchester, United Kingdom, [3]Paterson Institute for Cancer Research, Manchester, United Kingdom

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are capable to proliferate indefinitely in vitro (self-renewal) while maintaining the potency to generate all types of cells in a mammalian body (pluripotency), thus holding great promise for regenerative medicine. While numerous molecular components of the cell fate regulatory machinery have emerged, more in-depth knowledge is required to facilitate future ESC- or iPSC-based clinical applications. In particular, it remains largely unclear how regulatory networks are wired and how they process information to maintain or change a cellular state. Pluripotent stem cells share the same genomic DNA sequences with lineage-specified cells. Thus epigenetic regulation of gene expression lies at the heart of how a single zygote or ESC develops into a complex organism consisting of multiple tissue types. To date it is largely unclear how epigenetic regulators cooperate with each other and with transcriptional machinery in pluripotent stem cells. Here, we present a protein-protein interaction network centered on Wdr5, an important scaffolding protein in the Set/Mll histone H3 lysine 4 methyltransferase complexes. In this network, we identified several groups of proteins interacting with Wdr5, including histone acetyltransferase complexes, NuRD complex, chromatin remodeling complexes, transcription factors as well as Set/Mll complexes. Among the interacting partners are novel proteins together with known interactors. This study greatly facilitates our understanding of the functional cooperation between several epigenetic regulating complexes in mouse ESCs and the integration of the epigenetic regulatory network with the transcriptional machinery.

W-2069
INACTIVATION OF AN INTEGRATED ANTIBIOTIC RESISTANCE GENE IN MOUSE MOUSE EMBRYONIC STEM CELLS TO RE-ENABLE ANTIBIOTIC SELECTION

Zhikang, Liu[1], Ni1, Peiling[1], Chen, Lingyi[2]
[1]Nankai University, Tianjin, China, [2]Nankai University, Tianjin, China

Removing antibiotic resistance gene allows re-use the same antibiotic in the next round of genetic manipulation. Here we applied the CRISPR/Cas system to disrupt puromycin resistance gene in mouse embryonic stem cells, and used puromycin selection in the resulting cells to establish stable reporter cell lines. With the CRISPR/Cas system, no pre-engineered sequences, such as loxP or FRT, are required. Thus, this technique is applicable to disrupt antibiotic resistance gene which cannot be removed by the Cre-loxP and Flp-FRT systems.

W-2070
RAPID CONVERSION OF HUMAN PLURIPOTENT STEM CELLS INTO A NAÏVE GROUND STATE WITH ENHANCED DIFFERENTIATION CAPACITIES

Zimmerlin, Ludovic, Verma, Karan, Park, Tea Soon, Naler, Lynette, Zambidis, Elias
Pediatric Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

While human and mouse pluripotent stem cell (PSC) cultures share many molecular similarities, they display a number of phenotypic and functional differences that are reminiscent of developmentally distinct pluripotent states. While standard human PSCs are presumed to embody the primed post-implantation epiblast-like state, mouse PSCs can attain a naïve ground state of pluripotency using defined medium with kinase (GSK3β and MEK/Erk) inhibitors commonly referred as 2i. Recent reports have described the conversion of human PSCs into naïve-like cultures using complex cocktails of inhibitors with 2i and have successfully reached a phenotype resembling preimplantation inner cell mass. Here, we present novel culture conditions that ultimately allowed human PSCs to attain mouse-like ground pluripotency with only WNT and MEK/ERK modulation, and which led to a dramatic increase of their differentiation capacity. We initially performed a small molecule screening on a panel of human PSCs (3 ESC lines and several blood-derived and fibroblast-derived iPSC lines) to identify minimal combinations tolerating efficient transition to clonogenic cultures. We identified two molecules modulating protein kinase activity, as well as a conventional 2i-based (i.e. additional modulation of sHH, protein kinase A and WNT; 5i medium) culture condition that readily allowed human PSCs to acquire distinct mouse ESC-like features (e.g. dome-shaped morphology) and tolerate single cell dissociation. Unlike recent reports, effective transition of human PSC did not require survival-enhancing small molecules. We adapted our previously reported efficient bulk reprogramming of human myeloid progenitors and converted de novo human PSC lines to transgene-free clonogenic mouse ESC-like cultures. Converted naïve-like PSC lines rapidly reached a stable phenotype (SSEA-4+ TRA-1-60+ TRA-1-81+) with robust growth kinetics and uniform morphology for up to 30 passages. Uniform retention of NANOG and E-cadherin expression was observed by immunofluorescence throughout small compact colonies and expression of pluripotency-associated genes was confirmed by Q-RT-PCR at levels matching or superior to standard cultures. Our data indicate that most established or de novo human PSC cultures can be readily (<2 passages) and stably adapted into 2i medium with sole supplementation with a beta-catenin modulator (3i medium). Converted and neo-established lines rewired their regulatory circuitry to adopt mouse ESC-like signaling pathways (i.e. sensitivity to JAK/STAT inhibition). Teratoma formation assays revealed remarkably robust tri-lineage differentiation. Converted human PSCs also demonstrated superior hemato-endothelial differentiation efficiencies in vitro. While we previously demonstrated that our hematovascular differentiation system produced consistently efficient hematopoietic and vascular progenitors, converted naïve hPSC lines yielded up-to-7-fold greater amounts of hemangioblast, endothelial and pericytic progenitors. In summary, we have developed novel culture conditions

that can robustly and efficiently sustain clonal growth of human PSC in interchangeable pluripotency states and with augmented differentiation capacities. We are currently specifying the identity of these states in syngeneic lines, to determine molecular pathways, epigenomic and transcriptomic signatures, as well as their capacity for gene targeting by homologous recombination.

REGENERATION MECHANISMS

W-2071
IN VIVO TISSUE INJURY ACTIVATES PROLIFERATION AND MOBILIZATION OF QUIESCENT BONE MARROW-DERIVED STEM CELLS WITH EMBRYONIC-LIKE FEATURES

Zuba-Surma, Ewa[1], Labedz-Maslowska, Anna[1], Karnas, Elzbieta[1], Berdecka, Dominika[1], Ratajczak, Mariusz Z.[2], Madeja, Zbigniew[1]

[1]Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland, [2]Stem Cell Biology Institute, James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA

The adult murine bone marrow (BM) harbors several stem cell (SC) populations including non-hematopoietic Sca-1+/Lin-/CD45- cells exhibiting several features corresponding to embryonic-like cells. Such embryonic-like stem cells (VSELs) have been shown to differentiate into several lineages, including cardiac and vascular lineages in vitro. However, in opposite to ESCs, such adult BM- derived SC represents quiescent, non-proliferating cells. Importantly, they have been shown to be mobilized into blood following tissue injury and to participate in tissue regeneration including in heart repair in vivo. However, their activation and proliferation during such injury/ repair events in vivo have not been reported. In this study, we examined whether acute ischemic tissue injury may stimulate both proliferation of quiescent VSELs in BM and their mobilization into peripheral blood (PB). Thus, wild-type C57BL/6 mice (9-week old) underwent a hind limb ischemia (LI) by permanent proximal femoral artery occlusion. Mice were administrated with bromodeoxyuridine (BrdU, 1mg/mouse/i.p.) every two days and were scarified at 2, 7, 14 and 28 days following ischemia (N=5 per group). PB and BM from non- and ischemic limbs were collected from individual animals. Healthy non-ischemic mice were used as control groups in each time point. The presence of proliferating (BrdU+) VSELs (CD45-/Lineage-/Sca-1+), endothelial progenitor cells (EPCs; CD45-dim/Lin-/Sca-1+/Flk-1+) and hematopoietic stem/ progenitor cells (HSPCs; CD45+/Lineage-/Sca-1+) in PB and BM was evaluated by multiparameter flow cytometry (LSR II; BD Bioscience) and ImageStream system (Amnis Corp.). The expression of genes related to the presence of VSEL and EPC fractions including Oct-4A, Nanog, Rex-1 and Tie-2, VE-cadherin, respectively - was examined by real-time RT-PCR (Applied Biosystems). Moreover, we examined the change in expression of 53 angiogenesis-related proteins in plasma (RandD Systems). We established that the content of BrdU+ proliferating CD45-/Lineage-/Sca-1+ cells was significantly increased in BM of ischemic mice after 7 days post injury. We observed elevated number of BrdU+ VSELs in BM and circulating in PB of the injured animals indicating vast impact of acute ischemia on activation of these cells. Similar results were obtained for BM- derived and circulating EPCs. We confirmed, the presence of activated BrdU+ embryonic-like SC in BM of injured animals by employing both imaging cytometry and transmission electron microccopy (TEM) following immunogold staining for incorporated BrdU. Increased number of BrdU+ VSELs in BM and circulating in PB of the injured animals was accompanied with change in expression of genes guiding their proliferation (H19 and Igf2) and loss of expression of pluripotency- related genes such as Oct-4A and Nanog. We also found increase in expression of angiogenesis-related mRNA and proteins which may stimulate proliferation and mobilization of VSELs. We conclude that acute tissue injury such as limb ischemia may provide stimulatory agents to activate proliferation of rare quiescent embryonic-like SC residing in BM followed by their mobilization into PB. Thus, the ischemic injury may recruit the normally quiescent pluripotent stem cell pools to enhance endogenous mechanisms of tissue repair including stem/ progenitor cell- dependent angiogenesis which we further investigate.

W-2072
DIRECT ADMINISTRATION OF SECRETOME DERIVED FROM UMBILICAL CORD MESENCHYMAL STEM CELLS ATTENUATES PROGRESSION OF HEPATIC FIBROSIS

An, Su Yeon[1], Han, Jiyou[1], Lim, Hee-Joung[1], Jang, Yu Jin[1], Son, Jeong Sang[1], Lee, Jae Hun[1], Kim, Ji Hyang[2], Do, Byung-Rok[2], Kim, Jong-Hoon[1]

[1]Laboratory of Stem Cells and Tissue Regeneration, Korea University, Seoul, Republic of Korea, [2]Laboratory of Stem Cells and Tissue Regeneration, HurimBioCell Inc., Seoul, Republic of Korea

Liver fibrosis is a result of excessive accumulation and reorganization of the extracellular matrix (ECM) and mediated by numerous fibrogenic cytokines. Activated hepatic stellate cells (HSCs), a key source of the excessive production of ECM, may be a potential target for therapeutic intervention to prevent the development of chronic liver disease. We investigated potential therapeutic effects of secreted proteome (secretome) derived from human umbilical cord mesenchymal stem cells (UCMSC) and UCMSC-derived hepatocyte-like cells (HLC) in hepatic fibrosis. HLC differentiated from UCMSC showed multiple features of hepatocytes. However, transplantation of HLC induced only limited improvement of liver functions and caused embolism in the intrahepatic vascular system. In contrast, direct intraperitoneal injection of secretome obtained from UCMSC or HLC reduced fibrotic tissues without cell grafting in a mouse model of fibrosis. Secretome of HLC more effectively and significantly decreased the activation of HSCs in vivo and in vitro compared to that of UCMSC. Using LC-MS/MS and functional network analyses, we identified 701 and 813 proteins secreted from UCMSC and HLC and found three candidate molecules which may play key roles in the control of HSCs activation. The potential anti-fibrotic activity of three candidate molecules was confirmed in in vitro model of HSCs activation using recombinant proteins and activity-blocking antibodies. In summary, we demonstrate that secretome of HLC derived UCMSC attenuates activation of HSCs and progression of liver fibrosis. We also suggest three candidate proteins which may play a key role in anti-fibrotic effects of the secretome. This research was supported by the Bio and Medical Technology Development program of the National Research Foundation (NRF) funded by the Korean government (MEST) (2012M3A9B4028636) and Industrial Core Technology Development Program funded by the Ministry of Knowledge Economy. (No.10033594, Establishment of Therapeutic Stem Cells and Development of Global Cell Based Therapy Using Novel Cell Differentiation Techniques).

W-2073

SUPERIOR REGENERATIVE POTENTIAL OF HUMAN GLIAL RESTRICTED PROGENITORS COMPARED TO MOUSE ALLOGRAFTS IN IMMUNODEFICIENT DYSMYELINATED MOUSE MODEL

Arnold, Antje, Agatha, Lyczek, Miroslaw, Janowski, Bulte, Jeff W.M., Zhang, Jiangyang, Walczak, Piotr
Johns Hopkins University School of Medicine Department of Radiology, Baltimore, MD, USA

A wide range of neurological disorders result in loss or dysfunction of myelin. Recent advances in regenerative medicine raise the hope that the transplantation of glial-restricted progenitor cells (GRPs) may be an effective approach to restore brain function in patients who suffer from myelin disorders. Primary fetal-derived GRPs have a high potential to proliferate, migrate, and remyelinate axons after transplantation into dysmyelinated shiverer mouse brain. Application of immunodeficient animals as graft recipients facilitates successful testing of both, mismatched allografts and human cell xenografts without the interference of immune rejection. In this study, we performed head to head comparison of therapeutic properties of human and mouse GRPs in myelin deficient, immune deficient shiverer rag2-/- mice. msGRPs were derived from transgenic mice expressing eGFP under oligodendrocyte-specific proteolipid protein (PLP) promoter (msGRPeGFP+). Human GRPs were provided by Q Therapeutics, Inc. The cells were transplanted bilaterally (2x2µl; 4x105 cells) into the lateral ventricles of neonatal rag2-/- shiverer mice (P1-3). Transplanted mice (msGRPs n=6; hGRPs n=19) were followed with longitudinal MR imaging (T2, MTR, DTI) for up to 450 days. Individual animals were sacrificed at 360 and 440 days after transplantation for assessment of grafted cells with immunohistochemistry and electron microscopy. We have found that survival of mice transplanted with mGRPs was between 180 and 200 days and was not significantly longer compared to non-transplant controls. In contrast, survival of mice grafted with hGRPs was dramatically extended with 57% of mice surviving for over 400 days. In MRI, evidence of myelination with improvement of imaging parameters in the corpus callosum has been observed at 200 days postgrafting for mGRP transplanted mice and after 360 days in mice grafted with hGRPs. Histological analysis revealed grafted cell survival and myelination in both mouse and human GRPs; however, mouse cells engrafted and myelinated only in the vicinity of the ventricles in the corpus callosum and fimbria while for human cells the effect was much more robust with cells and myelin present throughout the entire neuraxis. Electron microscopy has shown axons with increased number of myelin wraps in comparison to non-grafted mice for both GRP types, but the myelin thickness was lower compared to wildtype mice. The lack of therapeutic effect in msGRP-grafted mice is possibly due to their limited potential to migrate to distant brain regions. Delay in MRI-detecteble myelination for hGRP compared to mGRP-grafted mice may be due to developmental differences. In conclusion, transplanted human GRPs showed superior therapeutic potential compared to mouse cells including more extensive migration, myelination and most importantly extended survival of animals. Further detailed analysis and comparison of both GRP cell types is expected to reveal features that can be exploited for improved therapy of myelin diseases.

W-2074

PDGFRα-POSITIVE PROGENITOR CELLS FORM MYELINATING OLIGODENDROCYTES AND SCHWANN CELLS FOLLOWING CONTUSION SPINAL CORD INJURY

Assinck, Peggy[1], Duncan, Gregory J.[1], Plemel, Jason R.[1], Bandesha, Sundeep[1], Moosvi, Sahir[1], Liu, Jie[1], Tetzlaff, Wolfram[2]
[1]UBC/ICORD, Vancouver BC, BC, Canada, [2]University of British Columbia - ICORD, Vancouver, BC, Canada

Contusive spinal cord injury (SCI) results in considerable demyelination of spared axons, which impairs signal transduction and may leave axons vulnerable to degeneration. Both oligodendrocytes (OL)s and Schwann cells remyelinate denuded axons in the subsequent weeks and months following SCI. NG2 cells, characterized by the near ubiquitous co-expression of platelet derived growth factor receptor α (PDGFRα) in the uninjured central nervous system (CNS), are oligodendrocyte progenitors (OP)s which may serve as a source of new OLs following SCI. PDGFRα-CreERT mice were crossed with Rosa26-YFP mice and administered tamoxifen to label OPs two weeks prior to contusive thoracic spinal cord injury. In the uninjured spinal cord we found that YFP was expressed in NG2+ OPs at very high efficiency, as well as αSMA+ pericytes and fibronectin+ fibrocytic cells in the spinal roots. Following injury, many recombined cells continue to express the PDGFRa+, Olig2 and NG2, indicative they have remained as OPs, but substantial differentiation into new mature oligodendrocytes (CC1+) was observed, particularly in the spared ventral and lateral white matter. Strikingly, the majority of P0+ Schwann cells in the spinal cord expressed YFP, suggesting they originated from central nervous system PDGFRα+ OPs. However, further work is required to characterize if other YFP+ populations like αSMA+ pericytes or the peripheral fibrocytic-like cells can contribute to the formation of myelinating Schwann cells or OL's in the injured CNS. Overall, this work reveals enormous phenotypic plasticity of PDGFRα precursors following spinal cord injury as a source of the new remyelinating Schwann cells and oligodendrocytes in the injured spinal cord. This work is supported by the Canadian Institute of Health Research, and the Multiple Sclerosis Society of Canada.

W-2075

INTERLEUKIN-4/ SIGNAL TRANSDUCER AND ACTIVATORS OF TRANSCRIPTION6 SIGNALLING PATHWAY IN MUSCLE REGENERATION

Babaeijandaghi, Farshad[1], Perona-Wright, Georgia[2], Rossi, Fabio M.V.[3]
[1]Biomedical Research Centre, The University of British Columbia, Vancouver, BC, Canada, [2]Department of Microbiology and Immunology, The University of British Columbia, Vancouver, BC, Canada, [3]University of British Columbia, Vancouver, BC, Canada

We previously identified a new population of bipotent muscle-resident Fibro-Adipogenic Progenitor cells (FAPs) that are quiescent in intact muscle but proliferate efficiently in response to damage to provide a transient source of pro-differentiation signals for proliferating myogenic progenitors. A recent in vitro study suggested that interleukin 4 (IL-4) signals through signal transducer and activators of transcription 6 (STAT6) to promote proliferation of FAPs. To further characterize this signaling axis in vivo, we assessed FAPs proliferation following sterile muscle injury in STAT6 knockout (K/O) mice compared to wild type (WT) controls. Before damage, the number of FAPs per Tibialis Anterior muscle was comparable in both control and transgenic groups. Four days after Notexin injection proliferation of FAPs, assessed by EDU incorporation assay, was significanty impaired in STAT6 K/O mice (16.75 ± 4.66 vs. 31.26 ± 3.46, p-value <0.05).

This phenotype could result from intrinsic loss of IL-4 signaling in FAPs or it could be an indirect consequence of impaired macrophage polarization to M2 phenotype (which is also mediated by IL-4 signals). Further investigations of the effects of such impairment on the overall regenerative process are ongoing and will be presented.

W-2076

PROMOTING SELF-REPAIR: THE RAPID AND DIRECTED MIGRATION OF ADULT NEURAL PRECURSOR CELLS IN ELECTRIC FIELDS IS DEPENDENT ON CALCIUM INFLUX

Babona-Pilipos, Rob[1], Mok, Alex[2], Popovic, Milos R.[3], Morshead, Cindi M.[4]

[1]IBBME, University of Toronto, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]IBBME, Toronto Rehabilitation Institute, University of Toronto, Toronto, ON, Canada, [4]Department of Surgery, Division of Anatomy, IBBME, University of Toronto, Toronto, ON, Canada

The existence of mammalian neural stem and progenitor cells (together termed neural precursor cells or NPCs) in the adult brain has generated interest in utilizing these cells for regenerative medicine strategies. Following injury such as stroke, NPCs within the adult forebrain subependyma become activated to proliferate and migrate toward the lesion where they differentiate into neural cells, albeit with limited efficacy. This is in part due to insufficient migration of NPCs toward the injury site. With the goal of enhancing self-repair, our work has focused on enhancing and directing the migratory behaviour of NPCs using electric fields. We have previously shown that undifferentiated adult NPCs undergo rapid and directed migration toward the cathode in the presence, but not the absence, of direct current electric fields (dcEFs), a phenomenon known as galvanotaxis. We have demonstrated that adult NPC migration occurs with the same velocity, directedness, and tortuosity irrespective of the matrix and the region of isolation (i.e. spinal cord versus brain). It remains unclear exactly how NPCs sense and transduce an external electric field into cellular motility. We hypothesized that calcium signaling played a role in directed migration. Using PCR, we determined that NPCs express voltage dependent calcium channel subtypes CaV1.2 (L-type) and CaV3.1, CaV3.2, CaV3.3, (T-type channels). Using time-lapse live cell imaging microscopy and cell tracking software, we determined the effects of manipulating both intracellular and extracellular calcium concentrations on NPC galvanotaxis. NPCs retracted their cellular processes and cell migration was completely inhibited in the presence of cell membrane-permeable calcium chelator BAPTA-AM. In conditions of reduced external calcium (low-calcium media) (0.46 mM), NPCs exhibit a >50% reduction in migration velocity and eventually cease migrating. Importantly, we demonstrate that cells are not undergoing cell death as we can rescue the migration by restoring conditions back to normal calcium levels (3.20 mM). Using the specific blocker to L-type channels (Nifeipine) we were able to block galvanotaxis in a dose dependent fashion revealing the influx of extracellular calcium is necessary for NPC galvanotaxis. Notably, our preliminary findings suggest the surprising finding that cell migration is unaffected by blocking the more abundant T-type channels on NPCs. We purport that understanding the cellular mechanisms underlying the rapid and directed migration of adult NPCs will aid in the development of novel therapeutics to promote brain repair.

W-2077

SMALL MOLECULE SCREEN FOR INDUCERS OF RETINAL REGENERATION IN ZEBRAFISH

Brady, Colleen Ann, Peterson, Randall

Harvard Medical School and Massachusetts General Hospital, Boston, MA, USA

Unlike mammals, zebrafish have the remarkable capacity to regenerate many different tissues including the neural retina. This retinal regeneration is of particular interest in the search for therapeutic strategies to treat retinal degenerative disease. In response to injury, regeneration in the zebrafish retina occurs through dedifferentiation of resident Müller glia, which then proliferate to generate new photoreceptors. While some of the drivers of this regeneration process have been uncovered, many details remain uncertain. We sought to identify small molecules that could initiate retinal regeneration. Such molecules would provide insight into the signals that activate Müller cells and provide a means to study activation of the regeneration process without injury. We designed a whole-mount *in situ* hybridization screen for compounds that could activate expression of pro-neurogenic bHLH transcription factor *Ascl1a* in Müller cells, which is one of the earliest transcriptional changes indicative of activation of these cells. Positive controls of either intense light injury or treatment with γ-secretase inhibitor compound E were used to induce expression of *Ascl1a* in the Müller glia. We identified and confirmed 7 hits from libraries of 1100 FDA-approved drugs and 2600 known bioactive molecules, and we are currently further analyzing these hits. As injury is a potent inducer of regeneration in the fish eye, we assessed cell death by TUNEL staining in the retina after compound treatment. Several of the hits we identified that activate *Ascl1a* expression also promote cell death of photoreceptors or retinal pigmented epithelium. Interestingly, in whole-mount TUNEL staining, the cell death seemed fairly well restricted to the retina, suggesting that we could use these compounds as a tool for studying retinal injury in a non-genetic system. We are currently investigating the different modes of cell death from different compound treatments. Additionally, we are pursuing further screening and alternate screening methods to identify compounds that modulate Müller glia cell fate.

W-2078

PRO-TISSUE REPAIR CAPACITY OF MYELOID ANGIOGENIC CELLS (MACS) IS IMPAIRED WHEN EXPOSED TO HIGH GLUCOSE

Chambers, Sarah Elizabeth Jane[1], Medina, Reinhold[2], O'Neill, Christina[3], Stitt, Alan[1]

[1]Centre for Experimental Medicine, Queen's University, Belfast, United Kingdom, [2]Centre for Experimental Medicine, Queen's University Belfast, Belfast, United Kingdom, [3]Centre for Experimental Medicine, Belfast, United Kingdom

Endothelial progenitor cells (EPCs) promote angiogenesis, maintain and regenerate the vasculature. We have recently demonstrated that early EPCs, which promote vascular repair in ischaemic tissues, actually represent myeloid angiogenic cells (MACs). They express haematopoietic markers CD45 and CD14, and are molecularly and functionally similar to alternatively activated M2-macrophages. MACs-based cytotherapy significantly enhances vascular repair in the murine ischaemic retina and is mediated by paracrine release of angiogenic factors, such as IL8. This pro-angiogenic potential of MACs could be harnessed as a novel cellular therapy for the treatment of diabetic retinopathy. However, since MACs are akin to M2-macrophages, a switch between their pro-angiogenic phenotype and towards a pro-inflammatory phenotype is possible and will be dictated

by their environment. Therefore, the plasticity and pro-angiogenic function of MACs needs to be assessed within the context of a diabetic milieu. *In vitro* diabetic milieu was achieved by 4-day exposure to 25mM D-glucose (high DG). An *in vitro* 3D-angiogenesis assay demonstrated that high DG significantly reduced the pro-angiogenic potential of MACs and their conditioned medium on retinal microvascular endothelial cell (RMEC) tube formation ($P<0.0001$), compared to the untreated control (5mM DG) and the osmotic control (5mM DG + 20mM L-Glucose LG). Analysis of conditioned media by an angiogenesis protein array highlighted that DG-treated MACs secreted reduced levels of pro-angiogenic cytokine IL8. Gene expression analysis from high DG-treated cells were compared to M1 and M2-macrophages, cultured from CD14+ve sorted cells. DG exposure induced a more M1 pro-inflammatory profile, with higher levels of IL1α, IL6, ICAM-1 and lower levels of M2 markers CD209, CD163 and angiogenic promoter IL8. Before observing MACs *in vivo*, a STZ-induced diabetic retinopathy model was characterised for pathological changes in vascular density and retinal thickness over a period of 4-9months diabetes using Fluorescence Angiography and Optical Coherence Tomography. A reduction in retinal thickness ($P<0.0001$) and vascular density ($P<0.01$) was only observed after 9months of diabetes. This study shows that although MACs are capable of inducing angiogenesis and act as M2 macrophages, they have a reduced capacity to promote angiogenesis when exposed to high DG *in vitro*. This significant decrease in vasoreparative properties is coupled to changes in their secretome profile, which suggest a phenotypic change towards a more M1 profile. This switch in phenotype is an important consideration when delivering MACs as a cell therapy into a diabetic environment, and highlights that more research is required to understand how MACs behave *in vivo*, and the plasticity of their M2 phenotype, before these cells can be used successfully as a cellular therapy.

W-2079
NEUROVASCULAR REPAIR AND FUNCTIONAL RECOVERY BY INTRANASAL DELIVERY OF INDUCED-PLURIPOTENT STEM CELLS IN A WHISKER BARREL STROKE MODEL

Deveau, Todd Carter[1], Yuengling, Katharine[1], Douhne, Timothy[2], Yu, Shan Ping[1], Wei, Ling[1]
[1]*Anesthesiology, Emory University, Atlanta, GA, USA,* [2]*Lee University, Cleveland, TN, USA*

Induced Pluripotent Stem Cells (iPS cells) are a useful tool for tissue repair following stroke. The present study investigates the potential of intranasal administration of induced pluripotent stem cell-derived neural progenitor cells (iNA-iPS-NPCs) to improve functional recovery following ischemic stroke. Mouse iPS cells were differentiated using standard differentiation methods in tandem with a novel rotary suspension technique we developed to improve neuronal differentiation (>80-90% NeuN positive). Semi-quantitative PCR analysis shows iPS-NPCs also express growth factors VEGF and BDNF, as well as the anti-inflammatory cytokine IL-6, demonstrating their potential not only for cell replacement but also for providing growth factors for neurovascular repair. Our data shows that iPS cells can be efficiently differentiated into neurons. In vivo, we employ a permanent distal middle cerebral artery occlusion in tandem with temporary bilateral common carotid occlusion to produce a focal ischemic insult located primarily in the rodent whisker barrel cortex. Twenty-two mice were randomly assigned to two groups: vehicle (n=11) and transplant (n=11). We created a transgenic line of mouse iPS cells that express GFP to track cells after transplant. Using a novel, non-invasive delivery method, vehicle or iPS-NPCs were administered to mice 1-3 hours after stroke through the nose. We observed increased local cerebral blood flow in the barrel cortex of animals that received iPS-NPCs compared to vehicle at day 3 after transplant. Animals who received iPS-NPCs showed significant improvements in functional recovery compared to vehicle as assayed by the adhesive removal test at day 7 ($p<0.05$). Animals were sacrificed at 28 days after transplantation. Microvessel staining data revealed improved angiogenesis and neurogenesis in the transplantation group. Our results suggest that intranasal administration of iPS-dervied neural progenitors can improve neurovascular repair and functional recovery following ischemic stroke.

W-2080
BONE MARROW CELLS RESTORE THE STATUS EPILEPTICUS-RELATED ALTERATION IN LONG-TERM POTENTIATION PROBABLY MEDIATED BY INFLAMMATION

Ferro, Zaquer Suzana Munhoz Costa[1], Salamoni, Simone Denise[2], Simão, Fabricio[1], Borelli, Wyllians Vendramini[2], Heldt, Fagner Henrique[2], Machado, Denise Cantareli[2], daCosta, Jaderson Costa[1]
[1]*Instituto do Cérebro – InsCer, Porto Alegre, Brazil,* [2]*Instituto de Pesquisas Biomédicas, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil*

Status epilepticus (SE) induced in rats by the systemic injection of pilocarpine, a non-subtype-specific partial muscarinic agonist, promotes spontaneous chronic seizures and pathological abnormalities that show similarities to human temporal lobe epilepsy. Long-term potentiation (LTP), an electrophysiological correlate of synaptic plasticity related to memory process, is compromised in epileptic rats due to hippocampal damage. Epileptic animals transplanted with bone marrow cells (BMC) showed LTP recover 10 and 120 days after SE. Associate with the brain insult, pro-inflammatory Interleukin-1β (IL-1β) was increased in the epileptic rats and was normal in rats transplanted with BMC. In order to better understand the role of the BMC in the inflammatory responses in acute and latent phase of the temporal lobe epilepsy, and its role in restoring the hippocampal synaptic activity, we investigate the level of IL-1β and correlated with the LTP obtained in rats submitted to SE. Male Wistar rats (45-50 days old) were initially treated with methylscopalamine (1 mg/kg; i.p.) in order to limit peripheral cholinergic effects. Twenty minutes later a single injection of pilocarpine (260 mg/kg; i.p.) was performed. SE describes an enduring epileptic state during which seizures are unremitting and are characterized by generalized tonic-clonic seizures. Diazepam (10 mg/kg, i.p.) was administered 1h after onset of SE, and these rats were randomly assigned to groups: the (1) rats submitted the SE (SE-saline), (2) SE transplanted with mononuclear bone marrow cells (SE-BMC). Control group (rats not submitted to induced SE). SE-BMC after Diazepam received BMCs (107 cells in 0.2 ml PBS) from GFP-donor and the SE and control group received saline solution, both through intravenous injections. At one, three and ten days after SE animals were anesthetized with thiopental (40 mg/kg i.p) and decapitated; one hippocampus was separated for in vitro electrophysiological study (LTP) and the other one for IL-1β ELISA assay. To further examine the impact of inflammatory changes on synaptic plasticity, besides the IL-1β evaluation we also investigated the potential effect of indomethacin (100uM) and celecoxib (10uM). We demonstrated that pilocarpine-induced SE increased IL-1β one day after SE and this effect was inhibited by BMC. Three and ten days after the onset of SE, no induction of LTP was obtained. Similarly occurred in animals treated with BMC one and three days after SE. We found that indomethacin and celecoxib did not significantly change the induction and maintenance of LTP in the control group but they were able to reverse the LTP induction in animals at 1-day post-SE. Our data suggest that LTP obtained in hippocampal slices of rats submitted to

SE by pilocarpine probably is in part related to inflammatory process modulated by BMC and reversed by an inhibitor of prostaglandin synthesis and COX-2.

W-2081
P14ARF IS A POTENT SUPPRESSOR OF SOLID TISSUE REGENERATION IN THE ZEBRAFISH CAUDAL FIN

Hesse, Robert G.[1], Kouklis, Gayle K.[1], Ahituv, Nadav[2], Pomerantz, Jason H.[1]
[1]Surgery, Division of Plastic Surgery, Orofacial Sciences, University of California, San Francisco, San Francisco, CA, USA, [2]Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, CA, USA

The *CDKN2A* locus encodes two distinct tumor suppressor proteins in humans and mice. One of the tumor suppressors, p16INK4a, is an inhibitor of cyclin-dependent kinases 4 and 6. The other, the alternate reading frame (ARF) protein, promotes TP53 pathway function by antagonizing MDM2, a negative regulator of TP53. ARF bridges the retinoblastoma (RB) and the TP53 pathways, by sensing aberrant RB pathway signaling. In addition to canonical TP53-dependent functions, ARF possesses TP53-independent functions such as regulating ribosome biogenesis and responding to oxidative stress. Unlike p16INK4a and its orthologs, which are highly conserved throughout evolution, ARF appeared more recently in vertebrate lineages and can inhibit dedifferentiation of skeletal muscle cells in culture. Therefore, we are investigating whether absence or presence of an ARF gene correlates with species' regenerative capacity and whether expression of ARF impacts regenerative capacity *in vivo*. We analyzed the Ensembl and UCSC Genome Browser databases and confirmed that ARF homologs exist in birds. Additionally, we found a homolog in alligator, but none in other reptiles including lizards and turtles, or *Xenopus*, or fish including fugu and zebrafish. We are using transgenesis to evaluate the impact of human ARF on regeneration of the zebrafish caudal fin. We found in zebrafish that, as in mammals, human ARF localizes to the nucleolus, interacts with endogenous Mdm2, and stabilizes Tp53. Induction of ARF in a transgenic zebrafish line (Tg(*hsp70l*:ARF)) inhibits caudal fin regeneration in a dose-dependent manner. In a transgenic zebrafish line that expresses human ARF under the control of its endogenous human promoter (Tg(*ARF*:ARF)) we tested whether the human ARF promoter responds to regenerative signals. Tg(*ARF*:ARF) fish are viable, grow to adulthood, and do not express ARF in the caudal fin. However, upon caudal fin amputation, ARF is expressed, and fin regeneration fails to occur. When expressed in the fin after amputation, ARF inhibits s-phase and induces apoptosis of blastema cells. Our findings provide experimental evidence that ARF is a potent suppressor of solid tissue regeneration *in vivo*, in addition to its known role as a tumor suppressor. We are investigating how the evolution of ARF may relate to distinct metabolic and cancer susceptibility variations among vertebrates.

W-2082
ROLE OF EXOSOMES AND THEIR MICRORNA CONSTITUENTS IN MEDIATING THE THERAPEUTIC BENEFITS OF HUMAN CARDIOSPHERE-DERIVED CELLS IN VITRO AND IN MICE WITH MYOCARDIAL INFARCTION

Ibrahim, Ahmed G.[1], Cheng, Ke[2], Marbán, Eduardo[1]
[1]Heart Institute, Cedars Sinai Medical Center, Los Angeles, CA, USA, [2]Cedars-Sinai Medcal Center, Los Angeles, CA, USA

Background: Exosomes are nano-sized bilayer vesicles that are secreted by most cell types. Exosomes are rich in microRNAs (mirs) which may function in a paracrine fashion. Cardiosphere-derived cells (CDCs) have been shown to regenerate heart after myocardial infarction (MI) in animal models and in the CADUCEUS clinical trial. However, most of the regenerated muscle is of innate origin, which suggests that CDCs function mainly through indirect pathways. Method and Results: In vitro and in vivo, we compared three treatment groups: vehicle only (control), CDC-derived exosomes and normal human dermal fibroblast (NHDF)-derived exosomes. Neonatal rat cardiomyocytes (NRCMs) incubated with CDC exosomes were more frequently positive for Ki67 (n=4, $p<0.001$), and less frequently tunel-positive, compared to NHDF exosomes or control (n=4, $p<0.01$). CDC exosomes also stimulated tube formation in HUVEC cells compared to NHDF exosomes or control ($p<0.05$). SCID mice injected with exosomes from CDCs during acute MI showed higher LVEF at two weeks (n=6, $p<0.05$) and four weeks (n=6, $p<0.05$) post MI, as well as increased viable mass. The therapeutic benefits of exosomes were equivalent to those of 105 injected human CDCs (p=ns). Inhibition of exosome secretion by ceramide synthesis inhibition abrogated the beneficial effects by CDCs in vivo and in vitro. Mir microarray analysis identified mirs146a as the most highly-up regulated mirs in CDC-exosomes compared to NHDF (262-fold, n=4). Mir-146a-treated NRCMs were more resistant to H2O2-induced stress compared to a mimic control. Array analysis of these NRCMs treated also showed suppression of IRAK1 and TRAF6 transcripts. SCID mice injected with 80 ng of mir-146a during acute MI showed higher LVEF at two weeks (n=6, $p<0.05$) and four weeks (n=6, $p<0.05$) post MI, as well as increased viable mass compared to mimic control. Conclusions: Mir-containing exosomes secreted by CDCs exhibit multiple beneficial effects on injured myocardium, suggesting that exosomes may mediate some of the therapeutic effects of CDCs. Most notably mir-146a provides cardioprotection in an acute model of MI.

W-2083
GENE EXPRESSION ANALYSIS OF HAIR CELL REGENERATION IN THE ZEBRAFISH LATERAL LINE

Jiang, Linjia, Romero-Carvajal, Andres, Haug, Jeff S., Seidel, Christopher W., Piotrowski, Tatjana
Stowers Institue for Medical Research, Kansas city, MO, USA

Deafness due to the loss of inner ear sensory hair cells is one of the most common sensory diseases. Such loss is permanent, as mammalian inner ear hair cells do not regenerate. However, non-mammalian animals (e.g., birds, amphibian and fish), regenerate damaged hair cells. In order to better understand the reasons underpinning such regeneration disparities in vertebrates, we set out to define at high resolution the changes in gene expression associated with the regeneration of hair cells in the zebrafish lateral line. We performed RNA-Seq analyses on regenerating support cells purified by fluorescence activated cell sorting (FACS). The resulting expression data were subjected to pathway enrichment analyses, and the differentially expressed genes validated in vivo via whole-mount in situ hybridizations. We discovered that cell cycle regulators respond hours before the activation of Wnt/β-catenin signaling, we propose that Wnt/β-catenin signaling is not involved in regulating the onset of proliferation but governs proliferation at later stages of regeneration. The pathways of Jak1/Stat3, nitric oxide and reactive oxygen species are quickly activated and might function to induce cell proliferation after hair cell death. In addition, and in marked contrast to mammals, our data clearly indicate that the Notch pathway is significantly downregulated shortly after injury, thus uncovering a key difference between the zebrafish and mammalian response to hair cell injury. Our efforts presented here have characterized the temporal activation pattern of signaling pathways during zebrafish sensory hair cell regeneration. These results will aid in the design and interpretation of functional studies aimed at elucidating the gene regulatory network underlying zebrafish hair cell regeneration, as well as guide experiments

to trigger hair cell regeneration in mammals.

W-2084
OLEIC ACID PROMOTES HUMAN UMBILICAL CORD BLOOD DERIVED MESENCHYMAL STEM CELLS MIGRATION AND THERAPEUTIC EFFICACY THROUGH THE EXPRESSION OF EPHB2 RECEPTOR

Jung, Young Hyun, Lee, Sei-Jung, Lee, Hyun Jik, Kim, Mi Ok, Jeon, Jihoon, Song, Eun Ju, Han, Ho Jae
Department of Veterinary Physiology, BK21 PLUS Creative Veterinary Research Center, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

Oleic acid (OA), a monounsaturated fatty acid (MUFA), plays a critical role in stem cell function. However, the effect of OA on clinical utility of cell-based therapy and its mode of actions in stem cells have not been reported. Therefore we investigated the role of OA on motility and skin wound healing effect of human umbilical cord blood derived mesenchymal stem cells (hUCB-MSCs). In in vitro, OA increased hUCB-MSCs migration and assembly of F-actin. The migration effect of OA was inhibited by GPR40 antagonist, a free fatty acid receptor-1. OA increased phosphorylation of protein kinase C (PKC), and then activated Src and VEGFR2. The VEGFR2 transactivation was attenuated by Src inhibitor. Activation of VEGFR-2 phosphorylated glycogen synthase kinase 3β (GSK3β), resulting nuclear translocation and activation of β-catenin. As a transcriptional target of β-catenin, OA significantly increased level of *EphB2* mRNA expression among *EphB1*, *EphB2*, *EphB3*, *EphB4* and *EphB6* mRNA. Transfection with *CTNNB1* (β-catenin) siRNA inhibited up-regulation of EphB2 induced by OA, suggesting that β-catenin is a transcription factor for EphB2. In addition, we found that EphB2 receptor was expressed in hUCB-MSCs membrane and the expression was increased by OA treatment. *EphB2* siRNA attenuated OA induced migration and actin filament reorganization. Pre-clustered ephrinB2 phosphorylated EphB2 and enhanced OA-induced hUCB-MSCs migration. Furthermore, OA pre-treatment enhanced ephrinB2 ligand mediated signaling involving AKT/FAK phosphorylation and RhoA activation, indicating that OA had an influence on Eph/ephrin signaling pathway of hUCB-MSCs. In mouse excisional wound splinting in vivo model, OA-induced hUCB-MSCs accelerated skin wound healing and angiogenesis surrounding wound sites. Interestingly, after treatment of OA-induced hUCB-MSCs, we found that EphB receptors expression as well as VEGFR receptors expression was increased in the process of wound healing. These findings demonstrate that increased expression level of EphB2 with microenvironmental changes by OA provides the evidence of improving therapeutic effect of hUCB-MSCs on wound healing.

W-2085
SYNERGISTIC EFFECT OF LOCAL CONTROLLED RELEASE STATIN AND ADIPOSE-DERIVED STEM CELLS IN A MOUSE HIND LIMB ISCHEMIA MODEL

Shintani, Takashi[1], Ii, Masaaki[1], Saito, Takashi[2], Tabata, Yasuhiko[2], Asahi, Michio[1]
[1]*Department of Pharmacology, Osaka Medical College, Takatsuki, Osaka, Japan,* [2]*Department of Biomaterials, Field of Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan*

Aim: Clinical trials of autologous adipose-derived stem cell (AdSC) therapy for peripheral artery disease (PAD) patients with critical limb ischemia are now on-going. We have investigated the hypothesis that statin, an agent for stem cell activation, could augment the therapeutic potential of AdSCs. Materials and methods: Human AdSCs were isolated from surgically resected subcutaneous adipose tissue by enzymatic digestion method. Nude mice underwent unilateral hind limb ischemia by femoral artery ligation and were assigned in the following 4 groups with different local intramuscular injection: 1) gelatin hydrogel (1mg, Control), 2) Simvastatin-conjugated gelatin hydrogel (18μg/1mg, ST), 3) AdSCs (5.0x104), and 4) ST+AdSCs. Laser Doppler Imaging analysis was performed weekly for blood flow assessment in ischemic area, and vascularity and transplanted AdSC retention/differentiation were examined by immunohistochemistry at 28 days after surgery. To determine whether statin improve survival rate and promote vascular differentiation of transplanted AdSCs with its anti-apoptotic effect, the retained AdSCs in ischemic tissue were examined by immunohistochemistry at 7 and 28 days after surgery. Next, to evaluate the direct effect of simvastatin on AdSC functions of proliferation/migration and differentiation/growth factor production, AdSCs were treated with different doses (0, 1, 10, 100, 1000nM) of simvastatin for MTT assay, Transwell assay, and real-time RT-PCR analysis. Results: Although all groups exhibited significant recovery of blood perfusion with increased vascularity, the fastest recovery was observed in ST+AdSCs group at 21 days after surgery. The survival rate of transplanted AdSCs was significantly increased in ST+AdSCs group compared with those in AdSCs group at 7 days after surgery. Moreover, a number of transplanted AdSCs were not only survived but also differentiated into endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) in ischemic tissue at 28 days after surgery in ST+AdSCs group. In vitro assay, simvastatin significantly promoted the migration activity of AdSCs without changing the proliferation activity, and also up-regulated mRNA expressions of various growth factors and EC or VSMC markers in a dose-dependent manner peaking at 100nM in AdSCs. Conclusions: Local/controlled-release statin augments both paracrine effect on angiogenesis and direct contribution to neovascularization of AdSCs by promoting cell survival and differentiation into ECs or VSMCs in ischemic tissue. Local combined application of statin and AdSCs might ameliorate therapeutic angiogenesis with their synergistic effect in severe PAD patients.

TISSUE ENGINEERING

W-2086
VASCULARIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELL ENGINEERED BONE GRAFTS

Sladkova, Martina, Lin, Charles, **de Peppo, Giuseppe Maria**
The New York Stem Cell Foundation Research Institute, New York, NY, USA

INTRODUCTION: Skeletal reconstructions are required to obviate bone deficiencies associated with trauma and diseases. Biomimetic approaches of bone engineering using human induced pluripotent stem cells (iPSCs) open the possibility to construct unlimited amount of patient-specific bone substitutes for personalized applications. Human bone is a highly vascularized tissue, and survival and functionality of engineered bone following transplantation strongly depends on angiogenesis and substitute vascularization. Engineering vascularized bone substitutes is therefore paramount for enhanced healing of large skeletal defects. MATERIALS AND METHODS: iPSC line 1013A (1013A-MSC) were co-cultured with human umbilical vein endothelial cells expressing the green fluorescent protein (GFP-HUVECs; Lonza) at different cell-to-cell ratios and under different concentrations of osteo- and vascular-inducing factors both in 3D clots of fibrin (20 mg fibrinogen/20U thrombin) and decellularized bone scaffolds (8 mm Ø x 2 mm height) for 4 and 6 weeks respectively. The effect of osteogenic pre-differentiation on bone vascularization was also evaluated. Human

bone marrow-derived mesenchymal stem cells (BMSC; Lonza) were used as control for all experiments. Functionality of GFP-HUVECs was compared with wild type HUVECs by assessing their proliferation rate, expression of specific markers, uptake of acetylated LDL, and tube formation assay for over 10 culture passages. Maturation and vascularization of engineered bone were assessed via confocal imaging, and by biochemical, histological and immunohistochemical analyses. RESULTS: Our studies demonstrate that co-culture of BMSC and 1013A-MSC with HUVEC resulted in long-lasting formation of vascular networks, either when cells were embedded in fibrin clots or seeded onto decellularized bone scaffolds. Formation of vascular structures in decellularized bone scaffold was concomitant with bone development as evidenced by increased expression of specific markers and positive staining for osteocalcin, osteopontin and bone sialoprotein. CONCLUSION: Development of proper vascularization protocols in combination with our biomimetic approach of bone engineering using patient-specific iPSC lines open the possibility to construct unlimited amount of vascularized bone grafts for enhanced treatments of the skeletal system.

W-2087
PRESERVATION OF VERY SMALL EMBRYONIC-LIKE STEM CELLS IN THE CORD BLOOD PROCESSING BY HESPAN SEDIMENTATION

Tsai, Ming-Song[1], Chang, Yu-Jen[2], Hwang, Shiaw-Min[3]
[1]Obstetrics and Gyn, Cathay General Hospital, Taipei, Taiwan, [2]Food Industry and Development Institute, Hsinchu, Taiwan, [3]Bioresource Collection and Res Center, Hsinchu, Taiwan

Very small embryonic-like (VSEL) stem cells are identified as the specific stem cells population present in bone marrow, cord blood and other tissues. The VSEL stem cells is a rare cell subset characterized by their small size (3-5 μm) and high nuclear/cytoplasmic ratio, and these cells have the ability to differentiate into cells of the 3 germ layers. These VSEL stem cells were reported to be lost with red blood cells by Ficoll-Paque density gradient centrifugation during bone marrow and cord blood specimens processing. However, most cord blood banks process specimens following the Cord Blood Transplantation Study: cord blood bank standard operating procedures (COBLT SOP). According to COBLT SOP, total nuclear cells are preserved during red blood cells removal using Hespan sedimentation but not Ficoll-Paque density gradient centrifugation. In this report, we showed that the rare sub-population of CD45-/Lin-/SSEA-4+ VSEL stem cells survived after Hespan sedimentation, and there was highly positive correlation between the recovery of total nucleated cells and the recovery of CD45-/Lin-/SSEA-4+ VSEL stem cells after Hespan processing. Furthermore, the results showed that the expression of pluripotent genes were slightly reduced after Hespan sedimentation, and the recovery of the gene expression was 92.0% in Oct-4, 81.2% in Sox2 and 80.0% in Nanog. However, a significant decrease in the level of these genes in mononuclear cells was observed after Ficoll-Paque processing. Immunocytochemical staining also revealed that the VSEL stem cells isolated with Hespan sedimentation had positive staining with Oct-4 and SSEA-4. In this study, we concluded that using Hespan sedimentation for cord blood processing and banking can retain the majority of VSEL cells in final products.

W-2088
DIRECT REPROGRAMMING OF FIRBROBLASTS TO FUNCTIONAL HEPATOCYTE-LIKE CELLS

Hui, Lijian
Shanghai Institute of Biochemistry and Cell Biology, Shanghai, China

Generating functional hepatocytes independent of donor liver organs is of great interests for regenerative medicine to cure liver diseases. Induced hepatic differentiation was achieved using embryonic stem cells or induced pluripotent stem (iPS) cells. However, induction of hepatocytes from iPS cells still composes complex steps, which can be replaced by improved technology. On the other hand, the generation of large numbers of functional human hepatocytes for cell-based approaches to liver disease is an important and unmet goal. We have previously induced mouse tail-tip fibroblasts (TTFs) into functional hepatocyte-like (iHep) cells by transduction of Gata4, Hnf1a and Foxa3 and inactivation of p19Arf (1). Lately, we generated human induced hepatocyte-like (hiHep) cells from fibroblasts by forced expression of human FOXA3, HNF1A, and HNF4A (2). HiHep cells express hepatic gene programs, can be expanded in vitro, and display functions characteristic of mature hepatocytes including cytochrome P450 enzyme activity and biliary drug clearance. Upon transplantation into mice with concanavalin A-induced acute liver failure and fatal metabolic liver disease due to fumarylacetoacetate dehydrolase (Fah) deficiency, hiHep cells restore the liver function and prolong survival. Collectively, our results demonstrate successful lineage conversion of non-hepatic human cells into mature hepatocyte-like cells with potential for biomedical and pharmaceutical applications.

W-2089
OSTEOCYTES FORM DUE TO CONCERTED EFFORTS OF POLARISED OSTEOBLASTS IN CONDENSATIONS

Kaul, Himanshu[1], Hall, Brian K.[2], Newby, Christopher[3], Ventikos, Yiannis[4]
[1]University of Oxford, Oxford, United Kingdom, [2]Department of Biology, Dalhousie University, Halifax, NS, Canada, [3]University of Leicester, Leicester, United Kingdom, [4]Mechanical Engineering, University College London, London, United Kingdom

Deposition of osteoid is essential to initiating the terminal differentiation of osteoblasts into osteocytes within or following osteogenic condensations. However, the dynamics regulating this process remain an open question. Multiple hypotheses exist, which, in proposing mechanisms of osteoid deposition, aim to clarify this executive event with immense clinical implications. The hypotheses either suggest self-entrapment of osteoblasts: resulting from random deposition of osteoid due either to (a) non-polar osteoblasts or (b) unidirectionally polarised osteoblasts; or entrapment due to neighbouring osteoblasts: resulting from (c) osteoblasts in the same generation acting in unison to deposit osteoid, thereby burying osteoblasts in the preceding generation or (d) certain osteoblasts switching-off their osteoid deposition capability and being buried by the surrounding osteoblasts. Visualising this executive event from the perspective of these hypotheses experimentally is near impossible due to inherent logistical and technical issues. Computational strategy being the only available investigative approach, we sought to test the veracity of these hypotheses by employing a computational method that can provide suitable ontologies for both the system (condensation) as well as the system constituents (cells and osteoid). Agent-based modelling was chosen to develop a 3D in vitro model of osteogenic condensation. Whereas the pattern-oriented method was adopted to construct the model, the strong-inference approach was used to test the various hypotheses. Agents, endowed with 'genes' as well as rules - pertaining

to proliferation, migration, differentiation, osteoid deposition, and apoptosis - were used to represent cells capable of detecting their local environment and acting at each discrete time step based on the rule-set assigned to them. Fibroblasts, pre-osteoblasts, osteoblasts, osteoid, and osteocytes were the only players modelled, with the condensation evolving from the heterogeneous, dynamic, bi-directional set of interactions between them. All hypotheses were simulated five times, with additional sensitivity analysis conducted on one of the hypotheses (amounting to 10 parametric changes). Furthermore, to ensure model robustness as well as insensitivity to random variables, the models were simulated on two additional workstations. In virtuo condensations were validated using in vitro data. Both were found to be quite similar in terms of structure, shape and composition. Two-way ANOVA conducted on the data indicated that transition from condensation to differentiation was a result of osteoid deposition and pre-osteoblast mitosis acting in synchrony with each other ($p<0.001$), which is in agreement with in vitro observations (and in vivo, at least, in terms of osteoid deposition). Kruskal-Wallis test for non-normally distributed data ($p=0.612$) suggested that models testing hypotheses a, b, and c produce largely similar condensations. However, hypothesis d failed to produce normal condensation. Further tests revealed that hypothesis d, similar to c but for the slowing down of osteoid deposition, produces normal condensations if the ability to slow-down matrix deposition is made redundant. Cluster analysis carried out on the data indicated that hypothesis c leads to most consistent condensation structures across generations. We conclude that polarised osteoblasts act in alignment to bury osteoblasts in preceding generations.

W-2090
CHONDROGENIC CELL SHEET FABRICATED FROM HUMAN DENTAL PULP STEM CELLS

Tatehara, Seiko, Tadokoro, Susumu, Imamura, Takahiro, Ide, Shinji, Tokuyama, Reiko, Satomura, Kazuhito

Department of Oral Medicine and Stolomatolgy, School of Dental Medicine, Tsurumi University, Yokohama, Japan

Articular hyaline cartilage is a connective tissue that covers articular surfaces and allows almost frictionless movement of synovial joints. This cartilage can be damaged due to a variety of causes such as traumatic accident, wear and tear over time, and other pathological conditions. Once this tissue is injured, however, it does not usually regenerate sufficiently because of its very less regenerating capacity. For the purpose of overcoming these situations, cell-based therapies have been recently performed for damaged articular cartilage. The cells necessary for this therapeutic modality have been usually obtained from healthy cartilage, bone marrow or adipose tissues. In these cases, however, there are some critical problems to be overcome, which are morbidity of the harvest site, quantitative and qualitative insufficiency of available cells, and difficulty of three dimensional structure, and so forth. Cell sheet technology has been proven to be useful for the regeneration of some tissues such as cornea and esophageal mucosa and myocardium. As the cell sheet is a complex consisting of functional cells and abundant extracellular matrices, it is considered to be available and useful even for cartilage regeneration. Accumulating evidence indicated that dental pulp tissues contain stem cells designated as dental pulp stem cells (DPSCs) and that these cells have very high proliferation capacity and multilineage differentiation potential. DPSCs are considered to be an advantageous cell source for tissue regeneration because they can be obtained from extracted teeth that are usually discarded as clinical waste. We propose, here in the present study, a new strategy for cartilage regeneration based on the combination of DPSCs and cell sheet technology.Dental pulp cells were isolated by enzymatic digestion from dental pulp tissues obtained from third molars in mandible of patients at 19 to 25 years of age. As the isolated cells were positive for STRO-1, a stem cell marker, these cells were considred to be DPSCs. To fabricate chondrogenic cell sheet, DPSCs were cultured in the presence of TGFBeta3 for 2 weeks. Thereafter, the cell sheets were analyzed by immunohistochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR) to confirm its chondrogenic characteristics. Moreover, these cell sheets were subcutaneously transplanted into the back of SCID mice and histologically observed at 6 weeks after transplamtation. The DPSCs formed mono- or two-cell layered sheet structure which was strongly positive for type II collagen (Col II) and intensely stained with alcian blue at 2 weeks of culture in chondrogenic induction medium. In addition, RT-PCR analysis revealed that thses chondrogenic cell sheets expressed mRNA encoding SOX 9, Col II and aggrecan. Histological analysis showed that the transplants contained alcian blue-positive cartilageous extracellular matrix and also immunohistologically positive for Col II. The cells surrounded by the extracellular matrices were positive for Col II and aggrecan. These results demonstrated that DPSCs were capable of forming a transplantable chondrogenic cell sheet. Taken together, the chondrogenic cell sheets fabricated by human DPSCs could be a new promising approach for effective cartilage regeneration.

W-2091
DEVELOPMENT OF AN IN VITRO SYSTEM TO STUDY THE INITIATION OF GASTRULATION DURING HUMAN DEVELOPMENT

Tewary, Mukul[1], Nazareth, Emanuel[2], Ostblom, Joel[1], Zandstra, Peter W.[3]

[1]IBBME, University of Toronto, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]University of Toronto Institute of Biomaterials and Biomedical Engineering, Toronto, ON, Canada

Somatic tissues of all species are formed by the initial formation of the three germ layers during development in an evolutionarily conserved principle called gastrulation. In amniotes, gastrulation initiates with the induction of the primitive streak (PS). During development, the epiblast is subjected to a gradient of morphogens. Specifically, a gradient of Wnt, and BMP4 that reduces anteriorly and ActivinA that reduces posteriorly. These gradients result in the differential primitive streak fates - anterior PS and posterior PS. Although a lot of work has been done in identifying the concentrations of the morphogens required to direct human pluripotent stem cells (hPSCs) toward either the anterior or the posterior primitive streak, dynamic regulation of the primitive streak induction and subsequent germ layer segregation in vitro remains elusive. The aim of this project is to use micro-engineering technologies to develop a platform that facilitates control of colony size, cell positioning, and morphogen gradients to mimic epiblast dynamics, and use this platform to study the germ layer segregation event that occurs during gastrulation. As a first step, we sought to develop a technology that permits high fidelity hPSC colony patterns, because it has been widely reported that micropatterning is able to elicit a homogenous response from hPSC colonies. We elected to develop a lithography based platform as it is known to produce high fidelity patterns. To avoid a laborious protocol that required the use of photoresists and access to a cleanroom, we chose to employ the use of substrates coated with organic polymers like polyethylene glycol (PEG) and phosphorylcholine (PC) presenting molecules as passivating agents, and utilize Deep UV light (<200nm) for patterning as it is able to photo-oxidize organic polymers. The surface chemistries of the two polymers, post photo-oxidization, were analyzed using X-Ray Photoelectron Spectroscopy (XPS) to detect functional groups that facilitated immobilization of extra-cellular matrix (ECM) proteins.

The PEG coated substrates presented carboxyl groups which were crosslinked with ECM proteins to allow for high fidelity patterns (60 colonies per well of a 96-well plate). The PC polymer did not contain bio-functionalizable groups, so a pretreatment with a synthetic peptide (Poly-L-Lysine) was used to crosslink ECM proteins. We next set out to validate that the hPSCs patterned colonies on these surfaces were able to differentiate robustly in response to inductive signals. The validation was done by comparing the response of the hPSC colonies to a variety of cytokine treatments of hPSC colonies patterned on these surfaces with micro-contact printing controls. The comparison yielded a poor correlation for the colonies patterned on the PC polymer (R2 = 0.69), but high correlation for PEG coated substrates (R2 = 0.89). We also investigated the differential induction of PS in varying shapes and sizes of colonies of a Mixl1 (PS marker) reporter hPSC line subjected to a saturating signal of BMP4 and ActivinA. We found that the Mixl1 expression localized in predictable regions depending on the size and shape of the hPSC colony. In conclusion, we have developed a novel high-throughput platform to study the effect of varying micro-environments on PS induction in hPSC colonies and report a differential spatial expression pattern of Mixl1 as a function of hPSC colony size and shape, indicating the possibility of in vitro control over PS patterning.

W-2092
HUMAN SKIN MESENCHYMAL STEM CELLS ASSOCIATED WITH DERMAL SUBSTITUTES AS POTENTIAL TOOL FOR SKIN REGENERATION

Trentin, Andrea[1], Jeremias, Talita Silva[1], Machado, Rafaela Grecco[1], Visoni, Silvia Beatriz Coutinho[1], Leonardi, Dilmar Francisco[2]
[1]Biologia Celular, Embriologia e Genética, Universidade Federal de Santa Catarina, Florianopólis, Brazil, [2]Cirurgia, Hospital Regional de São José Dr Homero de Miranda Gomes, São José, Brazil

New strategies for skin regeneration are needed in order to provide effective treatment for cutaneous wounds and disease. Among these possible strategies are the development of new biomaterials, cell therapy and the identification and application of factors involved in tissue repair. Mesenchymal stem cells (MSCs) have been suggested as an attractive source of cells for tissue engineering because of their multipotentiality and ability to release active molecules for tissue repair. Therefore, the present study established and evaluated a new method for treatment of skin lesions, based on the association of MSCs from human skin with commercial dermal substitutes (DS) currently used in clinical procedures. MSCs from human skin (dMSCs) were isolated and characterized according to their morphological, phenotypical and migratory features and potentiality. By these characteristics, dMSCs were similar to bone marrow MSCs. We also developed a new three-dimensional (3D) culture system that associate dMSCs with the DS, Pelnac® and Integra®. Both DS were equally efficient to support the adhesion, spread and growth of human dMSCs. In this 3D-culture system, dMSCs maintained the MSC phenotype and the expression of pluripotent, neural and mesenchymal markers. Next, the therapeutic potential of dMSCs associated with Integra® was evaluated in the repair of skin lesions. Therefore, we carried out an experimental *in vivo* analysis of full-thickness skin wounds, in a murine model. Histological and immunohistological sections performed at the 7rd, 14th and 21th days after surgery were analyzed with regard to vascularization, inflammatory response, deposition of extracellular matrix molecules and re-epithelialization. Animals treated with dCTMs associated with Integra® displayed increased granulation tissue, density of inflammatory cells (neutrophils and macrophages), vascularization, deposition of collagen fibrils and re-epithelization, and thus exhibited accelerated tissue repair. Furthermore, qPCR array analysis revealed that the dMSC-Integra® treatment also modulated the expression of genes related to tissue repair. In conclusion, the population of dermal MSCs isolated in the present study from human skin was efficiently associated with SDs in the described 3D-culture system and can be used for the treatment of cutaneous lesions in mice thus represents a new therapeutic tool for tissue engineering.

W-2093
INJECTABLE MYOCARDIAL MATRIX-GRAPHENE COMPOSITE HYDROGELS FOR FUNCTIONAL CARDIAC TISSUE ENGINEERING

Tsui, Jonathan[1], Jang, Jinah[2], Lefebvre, Austin E.[1], Neal, Nicholas[1], Cho, Dong-Woo[2], Laflamme, Michael[3], Kim, Deok-Ho[1]
[1]Bioengineering, University of Washington, Seattle, WA, USA, [2]Integrative Biosciences and Biotechnology, Pohang University of Science and Technology, Pohang, Republic of Korea, [3]Pathology, University of Washington, Seattle, WA, USA

Introduction: While direct stem cell injection therapies for treating cardiac infarcts hold much potential, it has now been demonstrated that the vast majority of grafted cells die shortly following introduction, thereby limiting their ability to contribute to the long-term restoration of cardiac function. Additionally, the key limitation of current synthetic matrices used for cardiac tissue engineering is their poor conductivity, which hampers signal propagation and cell to cell electrical communication between pores, leading to inadequate electrical coupling of implanted tissues with the host environment. This study examined the hypothesis that a composite biomaterial composed of reduced graphene oxide (rGO) integrated with decellularized extracellular matrix (dECM) can be used as an injectable scaffold that serves to properly mimic the native cell microenvironment and thereby create functional tissues that will contribute to the restoration of cardiac function in injured hearts due to enhanced electromechanical integration. Methods: Fabrication of rGO-dECM Composite Scaffolds: Porcine ventricular myocardium was sectioned into thin fragments and soaked for 12 h in 1% SDS and washed in 1% Triton-X100 before washing with PBS to remove residual detergent and cell debris. Single-layer graphene oxide (GO) stock solution was reduced by NaBH4 for 1 h before solid reduced graphene oxide (rGO) is filtered out. rGO and dECM were combined together in a acetic acid, pepsin, and riboflavin solution at various rGO concentrations and at 2% w/v dECM. In-vitro Characterization: Scaffold structure was examined using scanning electron microscopy (SEM). Conductivity of scaffolds was measured using a four-point probe method, and mechanical properties were measured using an Instron compressive tester. Scaffolds were seeded with human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes, and cell phenotype was assessed with immunohistological and western blot analysis of Cx43 and cTnI expression. Live-cell calcium dye imaging was used to examine the ability of the composite scaffolds to enhance cell signal propagation. In-vivo Characterization: Cell-seeded scaffolds were injected at the site of injury in a guinea pig model using a 27-gauge catheter, and restoration of cardiac function was assessed using intravital calcium imaging and echocardiography 4 weeks post-injection. Results: SEM imaging of composite scaffolds revealed a porous 3D structure with pores approximately 50-200μm in diameter. Higher magnification images of the pore walls showed a deposition of rGO flakes throughout. The collagen and GAG content of scaffolds was found to be comparable to that of native tissue, confirming that the ECM composition was unaffected by the decellularization process. Conductivity measurements showed an increasing trend in conductivity corresponding with both an increase in rGO content and an increase in the degree of reduction of GO. Scaffolds seeded with hiPSC-derived cardiomyocytes showed good cell viability, and

cardiomyocyte maturation was enhanced compared to those seeded in non-conductive scaffolds. Cardiac function was improved with scaffold treatment. Conclusions: In this study we have developed an electrically conductive 3D scaffold with tunable properties, and demonstrated the ability for these composite functional scaffolds to promote cardiomyocyte maturation and integration with host tissue.

W-2094
DO HGF-LOADED BIOMATERIALS RECRUIT MESENCHYMAL STEM CELLS

van de Kamp, Julia[1], Jahnen-Dechent, Willi[1], Woeltje, Michael[2], Boebel, Michael[2], Rheinnecker, Michael[2], Neuss, Sabine[3]
[1]Helmholtz Institue for Biomedical Engineering, Biointerface Laboratory, RWTH Aachen, Aachen, Germany, [2]Spintec Engineering GmbH, Aachen, Germany, [3]Institute of Pathology, RWTH Aachen University, Aachen, Germany

Wound healing - a topic that affects everyone. Usually the skin has the capacity to heal itself and restore its function. In the case of burns, (chronic) ulcers or large scale skin traumata, the skins' regenerative capacities might be disturbed, making external assistance, e.g. grafts, necessary. Auto- or allografts are well-established in the clinics, although both methods show several deficits. In tissue engineering, an alternative is stem cell application. Adult mesenchymal stem cells (MSC) are ideal, owing to their self-renewal capacity and differentiation potential. They can be harvested from several tissues and take part in tissue regeneration, repair and wound healing. MSC secrete, among others, hepatocyte growth factor (HGF). HGF concentration is enhanced in wounded areas, and it is shown to act as a chemoattractant for MSC and other cell types related to wound healing processes. Our objective is the identification of a biomaterial suitable to carry and release growth factors in an appropriate manner. This combinatory wound dressing should recruit endogenous MSC from their tissue-specific niche towards the wounded area. Additionally, we aim to circumvent the costly multi-step procedures (cell isolation, expansion, differentiation, transplantation) generally used in tissue engineering today. HGF was incorporated into collagen and fibrin gels during polymerization, silk was purchased from Spintec Engineering, Aachen. MSC motility and migration in the presence and absence of HGF was analyzed with scratch assays. Quantification of chemotactic behaviour of MSC towards HGF-loaded biomaterials was assessed with Boyden Chamber Assays. MSC were transfected with c-met siRNA to identify the HGF receptor c-met as an important factor for MSC migration. HGF release from all biomaterials was evaluated with ELISA. We implanted HGF-loaded and HGF-free biomaterials subcutaneously into mice; animals were sacrificed at 1, 3, and 12 weeks after operation. Residual biomaterials and adjacent tissue were resected and processed for histology (HE, Elastica van Gieson) and immunohistochemistry (CD20, CD68, CD117). Cells were quantified by means of their marker positivity. All results were analyzed with One-way ANOVA (*$p<0.05$). Incorporation of HGF into all biomaterials led to enhanced and directed migration of MSC *in vitro*. Transfection of MSC showed the importance of the receptor c-met for directed migration of MSC. HGF release was controlled and detectable over a period of 7 days. Animals tolerated biomaterials well and showed low to moderate inflammatory response. Collagen gels and silk were macroscopically still visible after 12 weeks while fibrin was dissolved even before 1 week. Vascularization was more prominent proximal to HGF-loaded biomaterials than proximal to HGF-free biomaterials. Cells infiltrated collagen and fibrin gels while silk was covered with a dense cell layer at all time points. We identified HGF as effective for MSC migration *in vitro* and cell recruitment *in vivo*. Collagen and fibrin gels as well as silk are applicable carriers for the delivery of drugs, growth factors and/or cytokines. This novel approach of growth factor-loaded biomaterials is an excellent solution to sidestep time-consuming *ex vivo* cell expansion and differentiation processes. Patients requiring immediate and/or intensive wound care, such as burn victims or chronic ulcer patients, can profit from an off the shelf, easy to handle product.

W-2095
COCULTURE OF STEM CELLS FROM THE APICAL PAPILLA AND HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS ENHANCES THE ANGIOGENIC POTENTIAL OF DENTAL PULP TISSUE

Wang, Penglai[1], Yuan, Changyong[2], Zhang, Chengfei[2]
[1]Xuzhou Stomatological Hospital, Xuzhou, China, [2]Faculty of Dentistry The University of Hong Kong, Hong Kong, Hong Kong

PURPOSE**:** To observe the effect of coculture of stem cells from the apical papilla (SCAPs) and human umbilical vein endothelial cells (HUVECs) on the angiogenic potential of dental pulp tissue. METHODS: SCAPs were incubated in osteo/odontogenic, adipogenic, neurogenic induction medium and α-MEM medium, whose multilineage differentiation capacities were confirmed using alizarin red staining, Oil red O staining and βIII-Tubulin immunofluorescent staining. The tubular length, branching points number and junctional areas were detected after 3 h, 6 h and 9 h since cells were seeded onto Matrigel, and the data were calculated using SPSS 16.0. RESULTS: SCAPs of experimental groups were detected having more lipid droplets, mineralization nodules and neuron-like cells. Coculture of SCAPs and HUVECs formed more vessel-like structures in tubular formation assay. CONCLUSIONS**:** SCAPs are capable of differentiating into fat, bone, and nerve-like cells in vitro. Coculture of SCAPs and HUVECs is able to enhancethe angiogenic potential of dental pulp tissue.

W-2096
INVESTIGATION OF THE OPTIMAL SOURCE OF ISOLATING HUMAN SOFT TISSUE PROGENITOR CELLS FOR CLINICAL APPLICATION

Yang, Tsunglin[1], Chen, You-Tzung[2], Hsiao, Ya-Chuan[3]
[1]National Taiwan University Hospital and College of Medicine, Taipei, Taiwan, [2]National Taiwan University College of Medicine, Taipei, Taiwan, [3]Department of Ophthalmology, Zhongxing Branch, Taipei City Hospital, Taipei, Taiwan

The area of head and neck is essential for many physiological functions and also relevant to personal identity and self-esteem. The craniofacial damage is particularly devastating to personal life. The current method to restore normal structures of head and neck is always restricted by a shortage of donor tissue. Therefore, the tissue engineering approach based on autologous progenitor cells of soft tissue is another choice. Since craniofacial area is composed of complex and exquisite musculature, to harvest cells by taking biopsy in situ may be inappropriate. Soft tissue progenitor cells now plays an important role in many bioengineering approaches for soft tissue regeneration such as skeletal muscle. However, the influence of clinical parameters and patient characteristics on the quantity and quality of harvested soft tissue progenitor cells remains elusive. Therefore, the aim of current proposal is to explore the method of harvesting soft tissue progenitor cells, and to define the optimal cell sources. Based on clinical information and patient variables, the methodology serves as a routine procedure that can be widely applied to facilitate clinical translation. In the present study, soft tissue progenitor cells are isolated from muscle samples and expanded. This methodology can be employed in patients with different demographic backgrounds, and adequate numbers

of soft tissue cells can be efficiently obtained and expanded. By our procedure, a small piece of soft tissue is sufficient to generate adequate cell numbers for tissue engineering applications within a reasonable time frame, which indicates that minimally invasive procedure of biopsies is sufficient.

W-2097
TISSUE STRANDS AS BIOMATERIAL FOR SCALE-UP ORGAN PRINTING

Yu, Yin[1], Ozbolat, Ibrahim[2]
[1]*Biomedical Engineering, University of Iowa, Iowa City, IA, USA,* [2]*Mechanical and Industrial Engineering, University of Iowa, Iowa City, IA, USA*

Objective: Organ printing has taken tissue engineering to a new era with the interdisciplinary effort spanning biology, medicine, and engineering. As the foundation of organ printing, cell aggregates, or tissue spheroids have recently been attracted enormous attention, where spheroid-shaped cell aggregates are used as building blocks for tissue fabrication. Although a wide array of cell aggregate techniques has been investigated, there has been no reported research in fabrication of bioprintable cell aggregate strands for scale-up tissue fabrication. In this paper, we introduced a new micro-fabrication technique to create cellular tissue strands as a feeding material for robotic organ printing. Methods: Sodium alginate hydrogel was used to directly print micro-tubular conduits with pneumatic pressure-assisted bioprinter. Later, these conduits were used as semi-permeable capsules for mouse insulin producing cells (Beta-TC-3) encapsulation to facilitate cell aggregation and maturation followed by dissolving the capsules leaving cellular tissue strands. Mechanical property and cell viability were recorded at different time point to evaluate biocompatibility of fabircation process as well as the process of strands maturation. Finally tissue strands were bioprinted together with human umbilical vein smooth muscle cell (HUVEC) loaded microvasculature tissue to demonstrate the capability of scale-up vascularized organ printing with customized multi-arm bioprinter (MABP). Results: Tissue strands were succesffully formed upon 4 days post fabrication with reasonable mechanical strength, structural integrity. The diameter of tissue strands decreased from 672 ± 21μm to 512 ± 11μm till day 10 without further shrinkage. Cell viability increased from 75 ± 5% to 85 ± 4% over 10 days in vitro culture. Fusion were readily observed between strands as soon 24 hours after placing them together, and furthered during prolonged culture, and completed at day 7 without visible gap. Also, tissue strands were printed layer by layer with vascular conduits together to fabricate miniature tissue analog, which successfully fused in culture. Immunofluorescent staining examination showed overall tissue specific c-peptide, insulin, glucagon expression on matured tissue strands as well as hybrid tissue constructs, which demonstrated the potential function of fabricated tissue analog with its nature counterpart. Conclusion: Our study provided a novel approach for cellular tissue strands fabrication, which have high viability and quick fusion capability, that might expedite tissue maturation thus facilitate scale-up organ printing process.

W-2098
HYPOXIA ENHANCES THE ANGIOGENIC POTENTIAL OF DENTAL STEM CELLS FROM APICAL PAPILLA VIA UPREGULATION OF VEGF AND EPHRINB2 EXPRESSION

Yuan, Changyong[1], Wang, Penglai[2], Zhu, Lifang[1], Dissanayaka, Waruna L.[1], Green, David William[1], Tong, Edith H.Y.[1], Jin, Lijian[1], Zhang, Chengfei[1]
[1]*Faculty of Dentistry The University of Hong Kong, Hong Kong,* [2]*Dental Implant Center, Xuzhou Stomatological Hospital, Xuzhou, China*

The ultimate success of bioengineereddental pulp depends on two principal elements, (1) whether the transplanted tissue can develop its own vascular endothelial tubule network and (2) whether host vasculature can be induced to penetrate the bioengineered pulp replacement and conjoin. Principal inductive molecules that participate in laying down blood vessels include: vascular endothelial growth factor (VEGF), ephrinB2 and hypoxia-inducible factor 1α (HIF-1α). Being able to modulate the genes that control these angiogenic molecules is a target for therapy in pulp regeneration for endogenous blood vessel formation, prevention of graft rejection and infection exclusion. Once implanted inside the root canal, a bioengineered pulp is subject to severe hypoxia and is the cause of tissue degeneration. However, short-term hypoxia is known to stimulate angiogenesis. Thus, it may be feasible to potentiate dental cells for angiogenic activity prior to implantation. Stem cells from apical papilla (SCAP) are arguably one of the most potent and versatile dental stem cell populations available for bioengineering pulp in the petri dish. Coculture of SCAP with endothelial cells (EC) promotes endothelial tubules, the formative blood vessel network. The aim of this study was to investigate whether coculture of these two sorts of cells under hypoxia was able to enhance the angiogenic potential of EC. In addition, we aim to clarify the interplay between the genes that orchestrate these important angiogenic molecules in hypoxic conditions in SCAP. We showed that SCAP cocultured with human umbilical vein endothelial cells (HUVEC) promoted VEGF and HIF-1α protein expression combined with increases in: the number of endothelial tubules, the tubule lengths, and branching points. Transcripts of ephrinB2 mRNA were significantly increased in this artificially induced hypoxic environment. We show evidence that this is directly mediated by HIF-1α signaling pathway. SCAP cultured with HUVEC in a 1:5 ratio promoted endothelial tubules and the overexpression of key angiogenic gene transcripts for VEGF, HIF-1α and ephrinB2. The significance of this study is to highlight hypoxia as a targeted way of stimulating angiogenic responses in SCAP committed to the engineering of dental pulp replacement. The results from this study will help us focus on the best therapeutic target in promoting angiogenesis so that future bioengineered pulp replacements integrate faster and permanently within the host.

W-2099
THREE-DIMENSIONAL MICROTISSUE SPHEROIDS OF DENTAL PULP STEM CELLS AND ENDOTHELIAL CELLS IN VASCULARISED PULP REGENERATION

Zhang, Chengfei
Faculty of Dentistry The University of Hong Kong, Hong Kong

Objectives: To fabricate the microtissue spheroids of dental pulp stem cells (DPSCs) and human umbilical vein endothelial cells (HUVECs); and to examine the potential of these three-dimensional spheroids in vascularised pulp regeneration. Methods**:** Three-dimensional microtissue-spheroids of DPSC-alone and DPSC-HUVEC co-cultures were fabricated using 12-series micro-molds (MicroTissues Inc.). Cellular organization within the spheroids (CellTracker dyes) and cell viability (live/dead assay) were assessed at day-1, 7 and 14.

Microtissue-spheroids were induced for odontogenic differentiation (21-days), examined for expression levels of osteo/odontogenic markers: alkaline phosphatase (ALP), bone sialoprotein (BSP) and RUNX2 (Real-time PCR), mineralization (von-Kossa) and for prevascularisation (Immunohistochemistry for CD31). Microtissues were inserted into the canal space of tooth-root slices and implanted into the subcutaneous space on the back of 6-8-week-old female severe combined immunodeficient (SCID) mice. Four weeks after the transplantation, the mice were euthanized and the tooth fragments were removed for histological (Haematoxylin and eosin) and immunohistochemical (human mitochondria, human CD31, Human Nestin antibodies) analysis. Experiments were conducted in triplicate using DPSCs from three different donors and statistically analysed (ANOVA). Results: DPSCs and HUVECs were self-aggregated into spheroids with no evidence of cell death at the centre. HUVECs were organized into a dense-network of tubular-like structures throughout the DPSC:HUVEC co-cultured microtissues. Elevated levels of ALP, BSP and RUNX2 ($p < 0.05$) in DPSC:HUVEC microtissues compared to DPSC-alone microtissues confirmed that HUVECs enhanced osteo/odontogenic differentiation. Both DPSC-alone and DPSC: HUVEC groups showed vascularized pulp-like tissue with an odontoblast-like cell layer adjacent to the dentin after transplantation in SCID mice. DPSC-HUVEC microtissue groups showed a significantly higher amount of extracellular matrix, vascularisation and mineralization compared to DPSC-alone microtissues both in-vitro and in-vivo. Positive staining for antibodies against human mitochondria confirmed the contribution of transplanted microtissues in regenerated pulp-like tissue and vasculature. DPSCs and HUVECs in three-dimensional microtissue spheroids synergistically enhance osteo/odontogenic differentiation and angiogenesis in-vitro. These prevascularized microtissue spheroids can successfully regenerate vascularised pulp-like tissue in-vivo.

W-2100
REOVIRUS PURGING OF UNDIFFERENTIATED PLURIPOTENT STEM CELLS FROM DIFFERENTIATION CULTURES IS NOT MEDIATED THROUGH ERAS

Zhang, Ben Tianzong[1], Hsu, Charlie Yu-Ming[2], Ito, Ken[2], Rancourt, Derrick[2]
[1]Biomedical Engineering, University of Calgary, Calgary, AB, Canada, [2]Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada

Pluripotent stem cells (PSC) provide a viable source of cells for regenerative medicine owing to their ability to proliferate indefinitely and develop into any cell type in the body. However, one of the prevailing issues currently facing the clinical translation of PSC is the resurgence of undifferentiated cells in PSC-derived tissue culture, which poses the risk of tumorigenesis following in vivo transplantation. One solution to overcome this safety hurdle is to apply a negative selection strategy to preferentially remove the contaminating cells from the culture. In this study we examined the possibility of using Respiratory Enteric Orphan virus (reovirus), currently in clinical trials as an oncolytic virus, as a purging agent to selectively remove undifferentiated mESC from differentiated tissue culture. We demonstrate that reovirus is able to significantly reduce the viability of mESC in a viral-titre dependent manner at an optimal time frame of 72hrs after viral infection. As a proof-of-concept, we infected a mixed population of mouse embryonic fibroblasts (MEF) and mESC with reovirus and found that reovirus was able to preferentially reduce the viability of the mESC by up to 80%, without affecting the viability of MEFs. Further, the reduction in overall cell viability is dependent on the proportion of mESC present in the heterogeneous cell population. Since reovirus was known to infect cells that constitutively express the signal transducer Ras, we found that ERas, a homolog of Ras, is differentially expressed in mESC but absent in MEF. However, when reovirus was applied to infect a hemizygous ERas exon knockout mESC strain, the viability of the mutant mESC did not increase, suggesting ERas may not be directly involved in mESC susceptibility to reovirus infection. Taken together, these results showed that reovirus can be used to selectively remove undifferentiated PSC from differentiated cell culture to reduce the risk of tumor formation prior to in vivo transplantation. However, the mechanism behind reovirus selectivity of mESC remains to be clarified.

TECHNOLOGIES FOR STEM CELL RESEARCH

W-2101
REPEATED COCULTURE WITH ADIPOSE-DERIVED STEM CELLS ENHANCED CHONDROGENIC PROPERTIES OF DEGENERATIVE NUCLEUS PULPOSUS CELLS

Kim, Jin-Su[1], Ryang, Ah-Yeon[2], Park, Kwang-Sook[1], Arai, Yoshie[1], Moon, Bo Kyung[1], Han, Inbo[1], Park, Hansoo[2], Lee, Soo-Hong[1]
[1]CHA University, Seoul, Republic of Korea, [2]Chung-ang University, Seoul, Republic of Korea

Co-culture has been suggested to be an effective method for stem cell differentiation. In this work, we used porous membranes for co-culture of adipose stem cells (ASCs) and nucleus pulposus cells (NPCs), and investigated whether repeated co-cultures enhanced the chondrogenic properties of degenerative NPCs (dNPCs), which in turn stimulated the dNPCs in a consecutive repeated co-culture. The dNPCs with low level of chondrogenic gene (such as collagen type II, aggrecan and sox-9) and alcian blue were collected, classified as dNPCs, and co-cultured with ASCs for 1 week (first co-culture). The dNPCs co-cultured with ASCs for 1 week showed slight increase of expressions of chondrogenic genes and alcian blue compared with dNPCs cultured alone. The dNPCs were then collected and co-cultured again with ASCs for 1 week (second co-culture). The second co-cultured dNPCs exhibited higher expression of chondrogenic genes than first co-cultured dNPCs and much more than dNPC cultured alone. Furthermore, Six weeks after in vivo transplantation, second co-cultured dNPCs also showed a significantly higher cartilage tissue formation compared to first co-cultured dNPCs. These data demonstrate that repeated co-culture with ASCs would be a useful technique to recover chondrogenic characteristics of degenerative NPCs.

W-2102
3D CULTURE OF RAT EMBRYONIC STEM CELLS AND RAT INDUCED PLURIPOTENT STEM CELLS IN A NOVEL PGMATRIX HYDROGEL

Rajanahalli, Pavan[1], Huang, Hongzhou[2], Sun, Xiuzhi[2], Weiss, Mark[1]
[1]Anatomy and Physiology, Kansas State University, Manhattan, KS, USA, [2]Grain Science and Industry, Kansas State University, Manhattan, KS, USA

Expanding embryonic stem cells (ESCs) in a synthetic 3D microenvironment in vitro while maintaining the undifferentiated state has been challenging. It would be advantageous to identify a substrate which could be modified such as by changing ligands that interact with cell surface receptors or by changing the substrate's stiffness. We developed a synthetic PGmatrix hydrogel to include these design specifications and determined whether it could support the expansion of undifferentiated ESCs in a 3D microenvironment. Rat embryonic stem cells (rESCs) and rat induced pluripotent stem cells

(riPSCs) were used for evaluation. The cells were grown both in 2D and 3D in PGmatrix hydrogel containing 2 inhibitors (2i), e.g., GSK3β and MEK inhibitors. Dynamic rheological tests of PGmatrix hydrogel in 2i medium showed a rapid increase of storage moduli (G' = 600 Pa for 3mM in 1 hr) indicating gelation after mixing. The mechanical strength of hydrogel was full recovered after shear-thinning into liquid phase. Also, the hydrogel reached a self-supporting strength within 1000sec which is a reasonable rate to ensure cells were suspended in the hydrogel matrix before they reached the bottom of the plate. A range of hydrogel peptide concentration was tested (0.5 mM to 12 mM) for ESC culture and cells grown in 3mM showed the best results. Both cell lines were cultured for more than 30 passages using regular trypsinization method to produce single cells at passage prior to plating. We harvested cells from the hydrogel for passaging by shearing the gel to liberate the cells. Phase contrast images of pluripotent stem cells grown in 3D showed similar morphology which had rounded phase-bright borders compared to cells grown on murine embryonic fibroblast (MEF) feeder layer. They retained pluripotent stem cell properties and showed positive staining for alkaline phosphatase, Oct-4, Nanog, Sox-2 and SSEA-1. Karyotypes of pluripotent stem cells cultured in 3D were normal after 20 passages. Embryoid bodies (EBs) could be formed by hanging drop after the cells were trypsinized from 3D cultures. We plan to compare the gene expression profiles of rESCs and riPSCs grown in 2D (MEFs) vs 3D (PGmatrix hydrogel) and determine if the 3D cultured cells can undergo differentiation into all the 3 germ layers and form teratomas. Growing ESCs in a fully synthetic 3D culture can significant advantages for directing differentiation and examining the interactions with surface receptors. Such knowledge will enable 3D culture for their use in drug development or for encapsulation of cells for clinical use.

W-2103
VALIDATION OF CLINICAL GRADE HUMAN EMBRYONIC STEM CELLS PRODUCED UNDER GMP

Hirai, Masako[1], Takada, Kei[1], Hamao, Mari[1], Kashigi, Fumi[1], Kawase, Eihachiro[1], Suemori, Hirofumi[1], Nakatsuji, Norio[2], Takahashi, Tsuneo A[1]

[1]Department of Embryonic Stem Cell Research, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan, [2]Institute for Intergrated Cell-Material Sciences, Kyoto University, Kyoto, Japan

In our laboratory five human embryonic stem cell lines were established for research use and these human embryonic stem cells (hESCs) have been distributed worldwide. The hESCs have been maintained using mouse feeder cells and culture medium containing animal-derived components. We report here a new method for production of clinical grade hESCs using a defined culture medium and synthetic substrate. The cell processing was performed in the manufacturing GMP-based cell processing facility built in our institute. The clean up procedures to ensure cell purity and safety were performed by culturing of hESCs for multiple passages under xeno-free conditions, and stocking expanded cells as a master cell bank (MCB). A working cell bank was made by the culture of the MCB cells for few more passages. For the process of clean up of hESCs, we cultured the cells for several passages for stocking the working cell bank (WCB). Characterization of the cells include assaying for viability, morphology, growth rate, undifferentiation state and pluripotency. Quality and safety testing comprised karyotype (G-banding), animal and human derived viruses, sterility, mycoplasma and endotoxin screening. The amount of residual animal serum was analysed by an ELISA, and the presence of residual mouse feeder cells was measured by an immune fluorescence staining method and by a new real-time PCR method. Viral screening of the MCB determined that animal derived viruses were not present. For cryopreservation, we developed a slow cooling method using a non-toxic cryoprotectant. All the test results met the requirement of domestic guidelines and that of ISCBI. This system developed for the production of clinical grade hESCs will be applicable for production of other types of stem cells for advanced cell therapy.

W-2104
A STEM CELL COMMONS FOR THE HARVARD STEM CELL INSTITUTE: BUILDING DATA INTEGRATION AND SHARING, ANALYTICAL CAPABILITIES AND KNOWLEDGE

Ho Sui, Shannan J.[1], Das, Sudeshna[2], Gehlenborg, Nils[3], Merrill, M. Emily[2], Sytchev, Ilya[4], Park, Richard W.[3], Haseley, Psalm[3], Hutchinson, John[4], Corlosquet, Stephane[2], Hansen, Terah A.[4], Hofmann, Oliver[1], Park, Peter J.[3], Hide, Winston[1]

[1]Harvard Stem Cell Institute, Harvard School of Public Health, Boston, MA, USA, [2]MIND Informatics, Massachusetts General Hospital, Cambridge, MA, USA, [3]Center for Biomedical Informatics, Harvard Medical School, Boston, MA, USA, [4]Biostatistics, Harvard School of Public Health, Boston, MA, USA

Biology has become a data intensive field: large-scale biological screens generate large volumes of data that require bioinformatics expertise and specialized infrastructure to synthesize. Omics data stored in most databases are not consistently associated with experimental and molecular phenotypic information, resulting in added expenses and lost opportunities to perform biological analysis, integration and comparison at the systems level. Manually keeping track of software and data used in analyses is tedious and error prone, and visual exploration to study the results of such analyses is currently not well supported. To address these challenges, we have developed the Stem Cell Commons (SCC) for the Harvard Stem Cell Institute (HSCI), a system that facilitates collaboration by enabling curation, storage, retrieval and sharing of data and analyses, as well as large-scale integration, analysis, and comparison. The SCC repository, built on the open source eXframe platform, has rich metadata annotations and uses the widely accepted ISA-Tab data standard to curate and structure data with standard ontologies/vocabularies, making it possible to share and compute upon this data in consistent and innovative ways. Coupled with the new Refinery Platform, the SCC provides a flexible environment for managing data, running diverse analysis workflows using the popular Galaxy analysis framework, and saving data provenance information to enable reproducible analyses. The SCC currently contains data from 17 HSCI laboratories and 89 public datasets, covering more than 100 cell types. Data in the SCC can be clustered by its shared molecular characteristics and experimental descriptions, making it comparable and searchable in more powerful ways. Refinery also allows for powerful searching and retrieval of experiments that can be submitted for analysis or visualization. The SCC is available at http://stemcellcommons.org. All SCC data and tools are open source and freely available. Through the SCC, we hope to build a community of stem cell researchers centered on its data and tools, with the ultimate goal of providing a platform for new knowledge and discoveries in stem cell biology.

W-2105
FUNCTIONAL STUDIES OF EXOSOMES FROM WHARTON'S JELLY MESENCHYMAL STEM CELLS

Hsu, Li-Wen, Hwang, Shiaw-Min

Bioresource Collection and Research Center, Food Industry Research and Development Institute, Hsinchu, Taiwan

The transplantation of mesenchymal stem cells (MSCs) has been proved as a potent therapeutic tool for a diversity of aging-related and

degenerative diseases. Most of these pathologies are linked to increased levels of reactive oxygen species. Besides restoration of tissue function by MSCs differentiation, the paracrine effects of MSCs participate in anti-oxidative stress are also demonstrated in many studies. Exosomes, one of paracrine mediators released from MSCs, affect tissue repair after MSCs application. Exosomes are microvesicles secreted from cells via exocytosis pathway. These small extracellular vesicles can function as carriers of proteins and genetic materials to neighboring or distant target cells. Therefore, exosomes play an essential role in the regulation of cell behaviors. However, the role of exosomes in the management of oxidative stress remains to be established. In this study, we isolated exosomes from Wharton's Jelly MSCs (WJ-MSCs) and analyzed their characteristics and anti-oxidants potentials. The immunophenotypic profile showed that WJ-MSCs were positive for CD73, CD90 and CD105, and negative for CD14, CD19, CD34, CD45 and HLA-DR for the surface markers of mesenchymal stem cells. Exosomes derived from WJ-MSCs were also expression exosomal protein markers, including CD9, CD63, CD81 and Hsp70. In addition, we found that WJ-MSCs-derived exosomes contributed to anti-oxidative activities by limiting oxidative stress-induced DNA damage in vitro. Through this study, we can elucidate the mechanism and therapeutic potential regarding how exosomes act on oxidative stress-mediated cell injury, in hopes of bringing this exciting technology toward an effective clinical reality.

W-2106
INHIBITION OF NF-KAPPA-B ACTIVATION ATTENUATES CARDIAC FAILURE BY SECRETION OF BONE MARROW MESENCHYMAL STEM CELL

Il-Kwon, Kim[1], Wei, Chuanyu[2], Kim, Sung-Hwan[3], Lee, Jeong-Sang[3], Song, Byeong-Wook[3], Kim, Han-Soo[3], Gupta, Sudhiranjan[2]
[1]International St. Mary's Hospital, Innovative Cell and Gene Therapy Center, Incheon, Republic of Korea, [2]Texas A and M Health Science Center, Temple, TX, USA, [3]Innovative Cell and Gene Therapy Center, Incheon, Republic of Korea

Cardiovascular disease remains the leading cause of mortality irrespective of type and etiology. Stem cell therapy (SCT) is emerging as a potential novel therapy to repair the damaged heart from myocardial infarction (MI). Various adult stem cells, including bone marrow-derived cells (BMC), endothelial progenitor cells, and bone marrow-derived mesenchymal stem cells (BM-MSC) have been shown to be able to differentiate into cardiomyocytes, support survival of residential cells; ultimately promote the repair mechanism of injured myocardium. Among donor cell types used for SCT, MSC have been the focus of much research because of their plasticity and availability. Accumulating evidence further indicates that MSC may exert their beneficial effects by releasing cytoprotective paracrine factors e.g. SDF1, which contribute to the recovery of cardiac contractile function. The paracrine factors secreted from BMC have been shown to prevent adverse cardiac remodeling, but the effect of secreting factors released from MSC in cardiac cells are not well known. Recently few studies indicated a salutary effect of genetically modification of stem cells which enhances the survival of engraftment of MSC after myocardial injury. However, the mechanism mediating this process is currently unknown. NF-κB, a pleiotropic transcription factor has become the major focus of attention over the past decade in the patho-physiology of ischemia-reperfusion injury, cardiac hypertrophy (CH) and congestive heart failure. NF-κB activation is necessary for the development of cardiac hypertrophy and inhibition of NF-κB attenuated cardiac hypertrophy indicated a potential candidate for therapeutic intervention. Although, NF-κB has been studied extensively in various cardiac pathologies, but there is no evidence to delineate the role of this transcription factor in MSCs as a better cell based therapy. We carried out the experiment using conditioned media (CM) from MSC isolating from IκBα triple mutant transgenic mice (3M, resistant to NF-κB activation) and, compared the efficacy with wild type (WT) CM. In this study, we induced rat neonatal cardiac myocyte and fibroblast with angiotensin II (Ang II) in the presence and absence of WT and 3M CM, to investigate the hypertrophic, pro-inflammatory, apoptotic and pro-fibrotic responses, respectively. Our results suggest that the CM from 3M-MSC provide better efficacy compared to the WT-MSC in cardiac cells. We propose that anti-inflammatory function of genetically modified BM-MSC has advantage in overcoming the limitation and risk associated STC for cardiovascular disease. This study was supported by a grant of the Korean Health Technology R and D Project, Ministry of Health and Welfare, Republic of Korea. (HI13C1270)

W-2107
STEM CELL SCREENING FOR THE DISCOVERY OF CHONDROGENIC DRUGS

Jeyakumar, Jey[1], Mennecozzi, Milena[1], Coote, Victoria[1], Cerqueira, Antonio[2], Choo, Yen[2]
[1]Plasticell Ltd, Stevenage, United Kingdom, [2]Progenitor Labs Ltd, Stevenage, United Kingdom

Drugs that promote chondrogenesis could be used to induce self-repair and regeneration of damaged articular cartilage after traumatic joint injury or osteoarthritis (OA). This could be achieved by targeting the resident chondrogenic progenitor cell populations that exist within or adjacent to the articular cartilage. Using Plasticell's bead-based combinatorial cell culture system CombiCult, we have discovered a number of highly efficient, serum-free chondrogenic media capable of generating chondrogenic progenitor cells and chondrocytes from MSC cultures in monolayer with high reproducibility. This was achieved via screening of a large number of chemicals and growth factors in 3,375 of serum-free media, where the optimal combinations of factors and time windows were identified. Chondroprogenitor cells derived in this way were further characterized by phenotype, stability and suitability for compound screening. We then carried out phenotypic screening using these cells on chemical libraries including FDA approved drugs, and identified a number of "hits" capable of promoting chondrogenesis in vitro. We anticipate intra-articular administration of drugs developed from these hits will target the resident chondrogenic progenitor cell populations in the joint to promote chondrogenesis or stimulate resident chondrocytes to produce new cartilage matrix within the tissue, thus preventing additional joint damage and improve joint pain and function.

W-2108
LN521 STEM CELL MATRIX ENABLES EASY SINGLE-CELL PASSAGE OF HPSCS AND CLONAL SURVIVAL WITHOUT ARTIFICIAL INHIBITORS

Kallur, Therese, Sun, Yi, Tryggvason, Kristian
BioLamina, Stockholm, Sweden

There has been a lack of defined, xeno-free, easy and robust methods for the labor- and cost-effective expansion of human pluripotent stem cells (hPSC) and the possibility to culture these cells under clonal conditions without using inhibitors of anoikis. This has hindered both basic research experiments due to high experimental variation and introduction of genetic changes as well as the development of human cell therapy requiring absolute safe methods and large numbers of low passage cells. By using a human recombinant protein naturally expressed by hPSCs, LN-521™, we can culture hPSCs for over 130 single cell passages without any abnormal genetic aberrations and

with maintained expression of pluripotency markers. Cells cultured on LN-521™ grow twice as fast compared to other matrices and can be split 1:20 or up to 1:30 as single cells without the addition of artificial ROCK inhibitor (Rodin et al, 2014). The simplicity and reliability of the procedure, speed of cell amplification and the rational of biorelevance make LN-521™ a cost-effective and scientific sound alternative of hPSC matrix. Furthermore, LN-521™ based CloneStem-To-Go pre-coated plates support the clonal survival and expansion of hPSCs. Not only does this allow the defined and xeno-free derivation of new human embryonic stem cells lines, even without destroying the embryo (Rodin et al, 2014), but is important for e.g. small molecule, siRNA and high-throughput screening experiments. In conclusion, we show that LN-521™ is an optimal and cost-effective matrix for hPSC culture due to its biological relevance allowing derivation, clonal cultivation and robust long-term pluripotent cell growth, making LN-521™ a suitable choice for the advancement of human cell therapy.

W-2109
HIGH-THROUGHPUT MICROFLUIDIC SCREENING PLATFORM FOR CRATION OF AN ARTIFICIAL THREE NICHE OF HUMAN PLURIPOTENT STEM CELLS

Kamei, Kenichiro, Koyama, Yoshie, Mashimo, Yasumasa, Fockenberg, Chris, Yoshioka, Momoko, Nakajima, Minako, Nakashima, Miyuki, Chen, Yong
Kyoto University, Kyoto, Japan

Human pluripotent stem cells (hPSCs) can be used in drug development, cell-based therapies, tissue engineering as well as to elucidate underlying mechanisms of organ development in humans. Developing these applications requires a predictive understanding of the factors that control cell fates and functions. Therefore, establishing artificial in vitro cellular microenvironments (or niche) is necessary because they play critical roles in the precise regulation of cell fate decision and functions in the body; they also segregate the appropriate cells into a heterogeneous cell population for developing rigorously arranged functional tissues. However, the current artificial niches established in most in vitro tissue culture systems are vastly different from the in vivo conditions due to the lack of controllability of the topological features of cellular scaffolds at subcellular levels, precise configuration of three-dimensional (3D) extracellular space, and diffusion of soluble factors. Therefore, there is a clear need to establish artificial regulatory niche to precisely control stem cell functions. Here, we describe a high-throughput microfluidic device in combination with biocompatible and thermo-responsive hydrogel to create artificial 3D niche for the systematic investigation of how a niche regulates cellular functions and fate decisions. This platform allows the manipulation of chemical (e.g., growth factors) and physical (e.g., mechanical stress) cues within a microfluidic cell-culture channel; NSP also helps in identifying the optimal niche conditions for inducing desired hPSC functions. In this study, we identified a completely defined 3D niche condition for maintaining the self-renewal of hPSCs.

W-2110
APPLICATION OF A CAPILLARY BASED VACUUM ASSISTED MICRODISSECTION INSTRUMENT FOR RAPID AND EFFICIENT COLLECTION OF INDIVIDUAL CELLS FROM ADHERENT CULTURES FOR SINGLE CELL AND CLONAL ANALYSIS

Karsten, Stanislav L., Ma, Zhongcai, Kudo, Lili C.
NeuroInDx, Inc., Signal Hill, CA, USA

Our understanding of normal stem cell function is greatly challenged by the diversity of stem cells and progenitor populations often present concurrently during organism development. Therefore traditional analysis at the population level often fails, and demand for single cell research becomes very high. Numerous technologies and protocols tailored for single cell analyses have been developed, which include whole genome sequencing and expression analyses. Although current flow sorting technology permits collection of single cells for their further analysis, rapid acquisition of cells directly from cell culture dishes based on their morphology, location or label remained challenging. Recently we introduced a novel cell and tissue acquisition system (KuiqpicK) and demonstrated its application for brain tissue microdissection (Kudo eta l, 2012; PLoS One). Here, we examine KuiqpicK's ability to efficiently collect single and multiple cells from adherent cell cultures. Collection of individual live cells was performed from various types of adherent cultures, including primary neural progenitor cells established from embryonic and adult mouse and rat brains, neuroblastoma SH-SY5Y, Chinese hamster ovary (CHO) and human melanoma MDA-MB-435 cell cultures. Single and multiple cell acquisitions were performed based on their morphology, location in the plate and fluorescent label (e.g. CellTracker probes). To test the viability of collected cells, Trypan blue exclusion test and single cell recultivation experiments were performed. Trypan blue assay demonstrated survival rates ranging from 80 to 95% (n>300) for all cells tested. The clonal expansion of collected single SH-SY5Y and CHO cells was demonstrated within 6 and 25 days, respectively. In addition, applicability of the instrument for the collection of single cells grown in three-dimensional (3D) culture system was shown using MDA-MB-435 cells. Moreover, isolation of RNA from collected cells exhibited high integrity and low degradation rates. Subsequent T7 based labeling procedure consistently produced high incorporation yields making it suitable for downstream sequencing and gene expression analyses. To summarize, our experimental data convincingly demonstrated that using the capillary based vacuum assisted instrument is a convenient approach to collect single and multiple cells (e.g. colonies) from both adherent and 3D cell cultures, capable of providing cells with high survival rate and suitable for an array of downstream functional studies.
This work was supported by NIH/NIMH 2R44MH091909-03.

W-2111
LARGE CRISPR/CAS9-MEDIATED DELETIONS ALLOW FOR CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF GENOMIC REGULATORY ELEMENTS IN AN ALLELE SPECIFIC MANNER

Katsman, Yulia, Mitchell, Jennifer
Cell and Systems Biology, University of Toronto, Toronto, ON, Canada

The study of transcriptional regulatory elements is critical for understanding cellular processes and responses, as well as for elucidation of disease mechanisms. Traditionally, gain-of-function luciferase reporter assays have been used for enhancer validation. Large scale loss-of-function studies have not been feasible as the traditional homologous recombination approach for knocking out genomic elements is extremely inefficient. The TALENs approach, although more efficient, is difficult to conduct at several locations as new proteins must be engineered to target different genomic regions. The recent advancement of the CRISPR/Cas9 system, which uses a guide RNA to target the Cas9 nuclease to the desired genomic location, has opened the door for large scale exploration of genomic elements. This system presents a powerful tool for validation of regulatory elements, due to its specificity, efficiency, and accessibility. In our studies, we used the CRISPR/Cas9 system to delete proximal and distal enhancers surrounding the *Sox2* gene in mouse embryonic stem cells. To this end, a Cas9-GFP expression vector was transfected into mouse F1 (*M. musculus*^{129} x *M. castaneus*) ES cells along with pairs of guide

RNAs flanking the targeted enhancer region. GFP expressing cells were sorted 48 hours post transfection, and individual clones were picked for propagation, screening and analysis. Incorporation of SNPs in the screening primers allowed for allele specific colony screening by real-time PCR. Deletion breakpoints in heterozygous and homozygous deleted clones were confirmed by sequencing. Allele specific gene expression was measured by real-time PCR using SNP-containing primers. Using this approach we deleted the two gene proximal enhancers, *Sox2* regulatory region 1 (SRR1) and SRR2, as well as two distal downstream enhancer regions (*Sox2* control region 104-112 ($SCR^{104-112}$) and SCR^{18}). The CRISPR/Cas9 system allowed for efficient and precise deletion of both large (>10kb) and small (<2kb) enhancer regions. We found that deletion efficiencies, although remarkably high compared to those expected to be obtained by traditional methods, were quite variable. Single allele deletion efficiencies ranged from 1% (for SRR1) to 38.5% (for $SCR^{104-112}$), while double deletion efficiencies ranged from 0 to 10%. This variability could result from a range of factors, such as individual guide efficiencies, the specific region being targeted, or possibly even break point homology, but appeared to be independent of the deletion size. Despite this variability in deletion efficiency, we were able to obtain multiple clones from each screen to perform gene expression analysis which revealed the necessity of downstream distal regulatory elements for *Sox2* transcription in ES cells. In summary, we have demonstrated the utility of large Cas9-mediated deletions in F1 ES cells for the study of genomic regulatory elements in an allele-specific manner. The efficiency and specificity of targeted genome modifications conducted via the CRISPR/Cas9 system provide an opportunity for high throughput validation of distal regulatory elements in mammalian genomes, allowing for a mechanistic understanding of the 80% of disease-linked loci which are found in uncharacterised intergenic regions of the human genome.

W-2112
INJECTABLE MULTIFUNCTIONAL MICROGEL ENCAPSULATING OUTGROWTH ENDOTHELIAL CELLS AND GROWTH FACTORS FOR ENHANCED NEOVASCULARIZATION

Kim, Pyung-Hwan[1], Yim, Hyun-Gu[2], Choi, Young-Jin[1], Kang, Byung-Jae[1], Kim, Joohyun[1], Kwon, Sang-Mo[3], Kim, Byung-Soo[2], Hwang, Nathaniel S.[2], Cho, Je-Yoel[1]
[1]College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea, [2]School of Chemical and Biological Engineering, Seoul National University, Seoul, Republic of Korea, [3]Department of Physiology, Pusan National University, Yangsan, Republic of Korea

Recent cell-based therapy approaches have employed both nanotechnologies and other biomedical technologies to enhance their therapeutic potential. A combined strategy using therapeutic stem/progenitor cells and angiogenic proteins is attractive for the treatment of vascular disease. In this study, we developed an injectable multifunctional micro-sized gel system (microgel), composed of arginine-glycine-aspartic acid (RGD)-conjugated alginate, for the delivery of both cells and growth factors *in vivo*. The microgels were formed via electrosparying, and outgrowth endothelial cells (OECs) and growth factors (vascular endothelial growth factor, VEGF, and hepatocyte growth factor, HGF) were encapsulated for multifunctionality. Incorporation of RGD into the microgel resulted in enhanced cell viability. Cells encapsulated within the microgel exhibited a time-dependent proliferation, and the size-controlled microgels resulted in sustained release of growth factors for enhanced new vessel formation, as demonstrated by tube formation and rat aorta sprouting in the *in vitro* assay. Angiogenesis was also estimated *in vivo*, in which strong, thick blood vessel formation was observed at the site injected with the RGD-microgel containing OECs and growth factors in comparison with that of single treatment groups. Furthermore, injection of the multifunctional microgel into a hindlimb ischemia model improved blood flow perfusion and increased the capillary density, which was assessed by histological analysis. Compared with hydrogel system, our injectable microgel system was shown to be superior, demonstrating no toxicity, for increased new vessel formation. Overall, our injectable multifunctional microgel system has been shown to be an effective method to deliver potential therapeutic agents/cells for the treatment of vascular diseases.

W-2113
SPECIFIC DETECTION OF STEM CELL BIOMARKERS WITH RNA PROBES IN LIVE CELL POPULATIONS

Koong, Victor, Weldon, Don, Su, Kevin, Hsu, Matthew, Wells, Erika
EMD Millipore, Temecula, CA, USA

Although cell surface protein markers for embryonic stem cells have been widely employed for detecting and sorting pluripotent and differentiated stem cell populations researchers are currently limited to surface markers for live cell sorting. Gold nanoparticle RNA probes can be used to detect specific RNA expression within living cells without harming or effecting cell health. The use of RNA markers in live cells would allow for greater flexibility in enriching for cells with high and low expression profiles through Fluorescent Activated Cell Sorting (FACS). Live cell detection of RNA markers would leave cells unchanged and unharmed allowing the use of the enriched population for downstream assays. Here we show the ability to detect specific RNA expression of single cells within live stem cell populations using RNA probes attached to gold nanoparticles. We generated probes to detect mRNA levels of OCT4, UTF1, SOX2, REX1, DPPA2, TERT, KLF4 and TLE1 in human embryonic stem cells (hESC) and hESC-derived neural progenitor cells (NPC). We employed this technique to detect changes in Oct4 mRNA in hESC and NPC and thus were able to demonstrate the downregulation of OCT4 mRNA in the differentiated cells by flow cytometry. Fluorescent imaging also revealed the heterogeneity of OCT4 mRNA expression within individual hESC. Finally, we also demonstrated the differential mRNA expression of seven additional stem cell biomarkers in hESC and NPC. This technology enables researchers to analyze and sort live stem cell populations for additional downstream applications, based on intracellular RNA expression, allowing for new advancements in the fields of regenerative and personal medicine.

W-2114
IMPROVED CRYOPRESERVATION AND RECOVERY SOLUTIONS FOR PLURIPOTENT STEM CELLS AND DIFFICULT-TO-PRESERVE PRIMARY CELLS

Newman, Rhonda, Sangenario, Lauren, **Kuninger, David**
Cell Biology, Life Sciences Solutions Group, Thermo Fisher Scientific, Frederick, MD, USA

Pluripotent stem cells (PSCs) and primary cells are foundational tools for basic research and applied applications including regenerative therapy, drug discovery, and toxicological assessment. While stem cells have a tremendous proliferative capacity, long term culture of these cells has been shown to cause an accumulation of mutations that result in genetic instability, increasing tumorigenicity and thus limiting their usefulness in research and clinical applications. Improved solutions for cryopreservation of early passage cells that minimize loss of viability, maximize post-thaw recovery, and minimize unwanted differentiation are essential components to PSC, as well as primary cell, workflows. While many cryopreservation reagents afford high viability immediately post-thaw, significant apoptosis and necrosis is

often observed following the first 24 hours post-thaw, decreasing the effective viability, reducing cell numbers and adding additional stress and selective pressure to cultures. Further, this extends the time post-thaw cells must be cultured prior to use in downstream experiments. Using a series of Design of Experiments (DOE) and mathematical modeling methods, we describe the development of a xeno-free cryomedium for use in cryopreservation of PSCs and ESCs, and a chemically defined post-thaw recovery supplement for use in recovery of PSCs, ESCs, as well as difficult-to-preserve primary cells. When used together, we show this system provides >80% direct post-thaw viability of PSCs with >70% cell survival following 24 hours post-plating. As a result of increased post-thaw survival rate, cells recover faster and are ready to passage sooner than with current solutions, while maintaining pluripotency and normal karyotype over 10 passages. Additionally, the post-thaw recovery supplement was tested in combination with other cryopreservation reagents which lead to markedly improved 24 hour post-thaw viability of difficult-to-preserve primary cells, including primary cortical neurons and human corneal epithelial cells.

W-2115
GENE TARGETING AT SILENT GENES IN HUMAN PLURIPOTENT STEM CELLS

Li, Li B.[1], Gornalusse, German[1], Deyle, David R.[2], Pilat, Kristina[1], Russell, David W.[1]
[1]Medicine, University of Washington, Seattle, WA, USA, [2]Medical Genetics, Mayo Clinic, Rochester, MN, USA

Gene targeting is a genetic engineering technique that is used to make precise modifications to the genome. Many genes of interest are silent in pluripotent stem cells, and it can be difficult to obtain targeted cells for these genes. This could be due to reduction in homologous recombination at silent loci, and/or suppression of the exogenous promoter that drives the selection marker. Here we designed an assay to study gene targeting frequencies and to compare exogenous promoter activity at a silent locus in human iPSCs. The iPSCs were reprogrammed from mesenchymal stem cells, in which an IRES-*NEO* cassette had been inserted into exon 1 of the robustly expressed *COL1A1* gene. The iPSCs became G418-sensitive due to silencing of the *COL1A1* promoter driving the *NEO* gene. This silent *COL1A1*-IRES-*NEO* locus was then targeted with a series of AAV vectors designed to insert different promoters between IRES and *NEO* sequences, such that only gene-targeted cells with an active exogenous promoter would become G418-resistant. The results showed that UCOE was the best promoter, achieving a G418-resistance frequency of 0.4%, followed by PGK and EF1α promoters, and distantly followed by SOX2, REX1 and EPC promoters. We next quantified the homologous recombination frequencies by Taqman qPCR, which measured the copy number of targeted alleles in all infected cells without G418 selection. The results showed that AAV vectors with EPC, REX1 and UCOE promoters recombined with the target locus in 0.16%, 0.42% and 0.52% of total cells respectively, which were all similar frequencies. Therefore the differences between G418-resistance and recombination frequencies reflect exogenous promoter silencing, which was not observed with the UCOE promoter. Our study proves that AAV vectors target silent genes efficiently and that the UCOE promoter can be used to ensure stable transgene expression. We next performed epigenetic analysis to examine the chromatin status of the targeted *COL1A1* locus. The chromatin-immunoprecipitation results showed that introduction of the UCOE promoter resulted in the removal of histone markers of transcriptional silencing and introduction of active chromatin markers. Bisulfite sequencing analysis showed that the UCOE promoter insertion also caused demethylation of the CpG island in the *COL1A1* locus. Thus the UCOE promoter is able to activate repressed chromatin and maintain an active chromatin status. Mutations in the *Interleukin-2 Receptor Gamma* (*IL2RG*) gene cause X-linked severe combined immunodeficiency (X-SCID), featured by a lack of T, NK and activated B cells, and this gene is silent in pluripotent stem cells. As a proof-of-principle, we targeted *IL2RG* in human ESCs and set up an in vitro model for X-SCID. In the targeting vector, the UCOE-*NEO* expression cassette is flanked by two *IL2RG* homology arms. Human ESCs were infected with this AAV vector and selected with G418. The results showed that 0.15% of all infected cells and 19% of G418-resistant cells underwent gene targeting at *IL2RG*, similar to what can be obtained at expressed loci. The *IL2RG*-targeted clones were then differentiated to CD45+ hematopoietic cells, which only expressed non-functional *IL2RG*. The *IL2RG* mutation does not affect the production of CD45+ cells from ESCs. Additional experiments are being done to generate T and NK cells for comparison. A similar targeting strategy could be employed to fix *IL2RG* mutations in X-SCID patients for cell therapy and modify other silent genes in pluripotent stem cells.

W-2116
HUMAN GENOME EDITING USING LAMBDA INTEGRASE-MEDIATED SITE-SPECIFIC GENE INSERTION

Makhija, Harshyaa[1], Vijaya Chandra, Shree Harsha[1], Peter, Sabrina[1], Siau, Jia Wei[2], Ghadessy, Farid John[2], Droge, Peter[1]
[1]School of Biological Sciences, Nanyang Technological University, Singapore, Singapore, [2]p53 Laboratory, Immunos, A-Star, Singapore, Singapore

The manipulation of the human genome is an important tool for applications in basic biological, biomedical and biotechnological research, and forms the basis of gene therapy. Based on the desired outcome, various tools exist to alter the human genome. Our tool employs λ integrase-mediated site-specific recombination, and our strategy is to select single, specific docking sites in the human genome which can be targeted and allow for homogenous stable long term expression of a transgene. We identified sequences in the human genome which can be targeted by a circular vector containing a corresponding recombination partner sequence in the presence of a novel factor-independent λ integrase. The majority of these target nucleotides are conserved and found in many locations in the human genome. Conservative single site-specific targeting events, i.e. without changes in nucleotide compositions as a consequence of recombination, were confirmed by PCR, sequencing and Southern blot analysis. Site-specific gene adding has been achieved and validated in human HT1080, HeLa, NEB-1 and hESC cell lines. Targeting is possible with mutant integrase expressed from co-transfected mRNAs. It is, therefore, a simple, safe and efficient novel tool with many applications in human stem cell-related research and gene therapy.

W-2117
DEVELOPMENT OF A GMP CRYOGENIC COLD CHAIN FOR CLINICAL DELIVERY OF REGENERATIVE MEDICINE THERAPEUTICS

Man, Jennifer[1], Hunt, Charles J.[2], Healy, Lyn E.[1], Stacey, Glyn[3], Morris, G J.[4]
[1]UK Stem Cell Bank, National Institute for Biological Standards and Control, Hertfordshire, United Kingdom, [2]UK Stem Cell Bank, National Institute for Biological Standards and Control, Hertfordshire, United Kingdom, [3]UK Stem Cell Bank, National Institute of Biological Standards and Control, Hertfordshire, United Kingdom, [4]Asymptote Ltd, Cambridge, United Kingdom

The potential use of human embryonic stem cells (hESCs) for

therapeutic applications has been met with increasing interest within the regenerative medicine community. One of the key challenges in using cells for therapy is the ability to cryopreserve and store these cells for eventual clinical application without compromising loss of functionality, viability and efficacy. The advantage of successful banking of clinical grade cell products is the development of "off-the-shelf" therapies with significantly increased shelf-life. The use of stem cells for clinical applications requires that all the steps within the production processes conform to Good Manufacturing Standards (GMP), defined by both the European Medical Agency and the US Food and Drug Administration. This project focuses on key elements within the cold chain, including controlled rate freezing of large banks of cells, short and long-term frozen storage and controlled thawing, with the intention of developing the first GMP compliant cryogenic cold chain for the delivery of regenerative medicine therapeutics. The use of liquid nitrogen-free, Stirling-cycle cooling technology will enable the development of equipment that can be used within GMP environments where control of particulates is an important consideration. One key element in this project is the qualification of a novel Stirling cycle engine controlled rate freezer (the CBCRF) for the cryopreservation of large banks of hESCs and induced pluripotent stem cells. Short and long-term culture of cells post-thaw has been investigated for markers of self-renewal and a panel of biological tests including membrane integrity and markers of early and late apoptosis, applied to investigate cell quality and function following cryopreservation. Expression of extracellular stem cell markers; SSEA-1, SSEA-3, SSEA-4 and TRA-1-60 were comparable to those using standard protocols and no change in relative expression of key signalling molecules regulating adhesion, cell cycle, apoptosis and cell renewal were detected in any sample following cryopreservation when compared to pre-freeze control. The results show that the CBCRF can be as effective at maintaining cell viability and functionality as current alternatives when used with standard freezing protocols thereby providing a novel approach for cryopreservation.

W-2118
MESENCHYMAL STEM CELLS ORGANISE ENDOTHELIAL NETWORKS THROUGH PLATELET-DERIVED GROWTH FACTOR AND INTEGRIN-LINKED KINASE SIGNALLING

Marshall, Julia[1], Yang, Xuebin[2], Genever, Paul[1]
[1]The University of York, York, United Kingdom, [2]The University of Leeds, Leeds, United Kingdom

In addition to their capacity for skeletogenic differentiation, there are suggestions that mesenchymal stem/stromal cells (MSCs) have additional roles in organising tissue vasculature through interactions with endothelial cells (ECs). However, suitable experimental models to test these unique MSC activities are lacking and the mechanisms are unclear. Here, we have developed a novel 3D in vitro co-culture spheroid model of MSCs and ECs to track endothelial restructuring and identify the signalling processes involved. Human bone marrow derived MSCs and ECs (human umbilical vein endothelial cells) were labelled with CellTracker™ green and red respectively. The cells were counted and mixed suspensions totalling 30,000 cells were added to non-adherent, U-bottomed 96-well plates in optimised MSC/EC media containing 0.25% (w/v) methylcellulose. By trialling different MSC:EC cell ratios, we identified a 50:50 composition as being most suitable for cell tracking and reorganisation events. The 3D MSC:EC spheroids were grown in the presence and absence of a range of specific inhibitors of different signalling pathways, ultimately focusing on platelet-derived growth factor receptor (PDGFR) and integrin-linked kinase (ILK), which can act downstream of PDGFR. MSC-EC self-organisation was tracked by multiphoton confocal microscope for up to 7 days. Spheroid volume changes were determined using Volocity image analysis software and internal cellular organisation were examined following snap-freezing and cryosectioning. Control, dual-labelled (green and red) MSC:MSC spheroids were also generated to test EC-specific self-organisation events. Between days 1 and 3 of culture, dramatic self-organisation of the MSC:EC spheroids was observed. ECs formed inter-connected vascular-like lattices surrounded by MSCs, which extended from peripheral EC assemblies to internal networks throughout the spheroid. MSC:MSC spheroids remained randomly mixed, with no evidence of self-organisation. Exposure to inhibitors of PDGFR, and to a lesser extent ILK, induced significant increases in MSC:EC spheroid volume within 24 hours (59% ± 14% and 31% ± 20% respectively (n=9) compared to controls). The PDGFR inhibitor-treated MSC:EC spheroids were also 157% ± 15.9% larger at day 2, however beyond this time point, no significant differences in size were observed. Inhibition of PDGFR and ILK also disrupted spheroid self-organisation by altering the peripheral distribution of ECs at the spheroid surface and causing them to form enlarged cell aggregates without the connected lattices observed in untreated MSC:EC controls. Again, these observations were most pronounced following PDGFR inhibition. This study showed that MSCs and ECs have an intrinsic capacity to self-organise when co-cultured under defined 3D conditions to form elaborate vascular-like networks in a mechanism that is dependent on PDGF and ILK-mediated signalling. ILK inhibition is known to suppress VEGF function in ECs resulting in disrupted angiogenesis, whilst PDGF signalling plays an important role in vascular stabilisation. Our novel 3D co-culture model represents a simplified system to decipher the mechanisms guiding MSC-dependent remodelling of host vasculature, which may be exploited to augment MSC-based tissue repair.

W-2119
INTEGRATED MICROFLUIDIC PLATFORM WITH NANOFIBERS TO CREATE ARTIFICIAL NICHE FOR CONTROLLING HUMAN PLURIPOTENT STEM CELLS

Mashimo, Yasumasa[1], Kamei, Kenichiro[1], Li, Liu[1], Chen, Yong[2]
[1]Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Kyoto, Japan, [2]Ecole Normale Supérieure, Paris, France

Human pluripotent stem cells (hPSCs), which they can self-renew indefinitely and differentiate into most of the cell types, hold a great potential for drug development, cell-based therapies, tissue engineering as well as biological investigation. These applications require precise regulation of cellular microenvironments (or niche) as cell fates and functions are finely controlled in our body. Thus, establishment of artificial in vitro niche is necessary to understand what factors are critical in obtaining cells or tissues which we desired. In this study, we created artificial in vitro niche controlling cell-cell contact and consisting of engineered cellular scaffolds by utilizing micro/nanofabrication technologies. Our designed niches consisted of (i) a microfluidic device for miniaturization of experiments and high-throughput analysis and (ii) an engineered cellular scaffold for mimicking in vivo extracellular matrix and realizing the fully defined culture condition. Furthermore, the niches were arranged in the pattern of standard 96-well plate format to perform semi-automatic experiment, and screened based on expression of cell-fate factors with defined media, a single-cell imaging and data analysis pipeline. Utilizing the screening platform, we optimized cell density, and material and density of the scaffold to allow robust maintenance of pluripotency of hPSCs. Here, we used a 3D printer for fabricating the mold of a microfluidic device within a few hours, while a conventional lithography technique needs at least one week. It allowed us to easily investigate the designs of an optimal microfluidic device for the experiment, and to fabricate culture chambers of a different height at

resolution of tens of micrometer. For proof-of-concept, we investigated how engineered niches influenced cell fate decision by monitoring their statuses of pluripotency (OCT3/4), cell proliferation (EdU) as well as apoptosis (Annexin V) in individual cells. The investigation was performed by microfluidic image cytometry, which is capable of quantitative, single-cell proteomic analysis of multiple molecules using only thousands of cells. Moreover, adapting bioinformatics analysis (i.e., self-organizing maps and unsupervised hierarchical clustering), we obtained a visual summary of the similarities, dissimilarities, and the degree of cellular heterogeneity in each niche, and identified the optimal niche for stem cell self-renewal from multi-parameteric analysis.

W-2120
DMSO-FREE FORMULATIONS FOR CRYOPRESERVATION: A STUDY ON FIBROBLASTS, MESENCHYMAL STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS

Matosevic, Sandro, Zylberberg, Claudia
Akron Biotech, Boca Raton, FL, USA

While traditional cryoprotective agents (CPAs) such as dimethylsulfoxide (DMSO) have been successfully used for the cryopreservation of stem cells for decades, side effects such as elevated toxicity call for new strategies for cryopreservation that maintain cell viability while eliminating damage to the cells. We have been developing non-toxic CPA formulations based on polyampholytes as well as solutes such as ectoine. One such formulation utilizes polyampholytes with cryoprotective properties. The active form of such CPAs is obtained after carboxylation of reactive amino groups. The exact mechanism of cryoprotection by polyampholytes is postulated to occur by adsorption to the cell membrane which reduces ionic permeability. Ectoine, on the other hand, penetrates the cell interior and creates ectoine-water complexes that stabilize the cell membrane. Here we present validation data following assessment of cryopreservation efficiency for a number of cell types, including human lung fibroblasts, mesenchymal stem cells, hematopoietic stem cells and induced pluripotent stem cells. We show that cryoprotectant performance is strongly dependent on cell type. In vitro data on the effect of cryoprotectant toxicity on cell proliferation is also reported by expanding cells in the presence of these DMSO-free CPAs. Cell toxicity of DMSO-free CPAs is cell type-dependent. Moreover, we validate these findings by reporting cell viability post-thaw following a number of freeze-thaw cycles, and compare this to traditional CPAs containing DMSO as well as sugar-rich DMSO CPAs. As cryoprotectants, we demonstrate that both polyampholyte and ectoine-based CPAs are promising alternatives to DMSO with important implications in the fields of tissue regeneration and regenerative medicine.

W-2121
INTEGRATED MULTI-STAGE TISSUE ON A CHIP GENERATION FROM HUMAN PLURIPOTENT STEM CELLS

Giobbe, Giovanni Giuseppe[1], **Michielin, Federica**[1], Martewicz, Sebastian Lukasz[1], Giulitti, Stefano[1], Giulitti, Stefano[1], Luni, Camilla[1], Floreani, Annarosa[1], Elvassore, Nicola[2]
[1]University of Padova, Padova, Italy, [2]University of Padova, Padova, Italy

The development of human organs-on-chips, in which the microscale engineering technologies are combined with cultured human cells, offers a unique opportunity to study human physiology and pathophysiology in an organ-specific context. The possibility of developing direct organogenesis-on-chip from human pluripotent stem cells (hPSCs) could overcome the limited availability of human primary cells, such as hepatocytes and cardiomyocytes. Human embryonic (hESCs)and induced pluripotent stem cells (hiPSCs) open a wide perspective for multi-organ generation on a chip. Integrated functional hPSCs differentiation on a chip has not yet been developed. Here we demonstrate how to control stem cell expansion, selective germ layer commitment and derive functional tissue-specific cells on a chip from both hESCs and hiPSCs through a multi-stage microfluidic-based technology. Experimentally, we showed that a discontinuous periodic medium delivery with stage-dependent frequency, f, (number of cycles of medium change per day) is an effective strategy for modulating stem cell niche specification in vitro, ensuring optimal delivery of exogenous factors and removal of endogenous cell-secreted factors. HPSCs homogeneously express pluripotency markers Oct4, Sox2 and Tra1-60, along the microfluidic channel. QRT-PCR analysis of Oct4 and Nanog, showed an optimal frequency of f=2d-1 and a significant three-fold higher expression compared to lower and higher frequencies was observed. HPSCs were induced to spontaneously differentiate within the microfluidic platform and a frequency-dependent germ layer enrichment was observed. Compared to standard culture in Petri dish, we obtained a significant increase in ectoderm markers OTX2 and TUBB3 expression by adopting f=1d-1 and a dramatic reduction with f=8d-1, confirming that ectoderm commitment is mainly driven by endogenous factors accumulation. Conversely, by adopting f=8d-1, we observed a significant increase of meso-endoderm T and GATA4 markers and early endoderm FOXA2, EOMES and AFP markers expression. More selective germ layer commitment was successfully obtained following specific differentiation protocols and an optimal f for each germ layer commitment was established. Cardiac cells on a chip were derived in 15 days of frequency-dependent multi-stage differentiation protocol. 60% Troponin-T positive cells showed defined sarcomeric organization, spontaneous contractile activity and proper calcium dynamics. Cells were also responsive for caffeine and verapamil stimulation. Similarly, hepatic-like cells on a chip were obtained starting from endoderm commitment up to hepatic maturation. Cells express cytokeratin 18, CYP-3A and albumin. Cells are also responsive to indocyanine green digestion, glycogen storage (75% of total cells) and 40% increase albumin secretion, compared to static conditions was observed. Drug-induced cytotoxicity assays have been performed, in order to verify effective responsivness of hepatocyte-like cells. Microfluidic-differentiated cells showed higher sensitivity to acetaminophen stimulation in a dose- and time-dependent manner, compared to Petri dish cells. Functionally differentiated cells derived in the microfluidic channels can be directly used for dynamic multi-parametric and large-scale drug screening or for developing micro-engineered human organ models, overcoming issues related to the limited availability of human primary cell sources.

W-2122
A VERSATILE ADENOVIRAL VECTOR SYSTEM FOR BOTH TRANSIENT AND STABLE VISUALIZATION OF BONE-FORMING OSTEOBLASTS

Mitani, Ko, Sone, Takefumi, Katagiri, Takenobu
Research Center for Genomic Medicine, Saitama Medical University, Saitama, Japan

Osteoblasts are specialized cells for bone formation and are derived from undifferentiated mesenchymal progenitor cells. The expression of osteocalcin (OC) is widely used as a specific marker for bone-forming osteoblasts. While some studies have reported isolation of the osteoblasts using an anti-OC antibody by FACS, this technique appears to be less efficient since OC is released from the cell surface as a secretory protein. To generate a human OC-Venus helper-dependent adenoviral vector (HDAd-hOC-Venus), a BAC clone containing the human OC locus was modified so that the ATG start codon of Venus

was fused in frame with the ATG of the OC gene. Subsequently, a total of 21.4 kb of OC genomic sequences including the marker cassette was subcloned into the HDAdV plasmid, and the vector was propagated by serial passages. The MG-63 human osteosarcoma cell line was infected with the HDAdVs at an MOI of 10000. With this MOI, 99% of the cells were transduced by the control HDAd-CAG-Venus vector. The specific expression from hOC-Venus was examined by FACS analyses and qRT-PCR, and the mean fluorescence intensity of the total cells was observed to increase after treatment with 1α,25(OH)2D3 in a dose-dependent manner in parallel with the level of mRNA. The mature osteoblast-specific expression of hOC-Venus was further examined in cultures of murine primary osteoblasts, which were infected with the HDAdVs at an MOI of 1000. With this MOI, 60% of the cells were transduced with the HDAd-CAG-Venus vector. With the HDAd-hOC-Venus virus, Venus was specifically expressed in the cells forming bone-like nodules. The merged images of the HDAd-hOC-Venus expression and the anti-OC staining indicated that the expression of hOC-Venus correlates with that of endogenous OC. The Venus (+) fraction in the HDAd-hOC-Venus infected cultures was 5.0% of the total cells, and the purity after FACS sorting was 87%. Five endogenous genes, OC, Bsp, Pth1, Alp and Osx, showed higher mRNA levels in the Venus (+) cells than in the Venus (-) cells. A transcription factor, Runx2, was also slightly higher (1.1-2.4 fold) in the hOC-Venus (+) fraction. The results are expected for Bsp because it has been reported to be highly expressed in mature mineralizing osteoblasts, while they are unexpected for Alp and Pth1r because these genes have been regarded as an early marker of osteogenesis. Although Runx2 has been identified as the critical transcription factor for osteoblast-specific expression of OC mRNA, there was little difference in the Runx2 mRNA levels between the hOC-Venus (+) and (-) fractions. The same vector was also used to make a reporter human iPS cell (hiPSC) lines, with the Venus integrated in the ATG-coding exon of OC locus. Human iPS cells were infected with the vector and subject to neomycin selection to isolate hiPSC clones with chromosomal vector integration. From 2.4 million infected cells, we obtained 29 colonies, among which 7% were gene-targeted confirmed by PCR and Southern analyses. These iPSC clones would be useful to optimize conditions for inducing osteoblast differentiation from hiPSCs. In summary, we established a novel HDAdV-based system to transiently detect living bone-forming osteoblasts and to stably establish osteoblast-specific reporter hiPSCs by using the long regulatory sequences of the human OC gene. Our HDAdV-based hOC-Venus reporter will thus be useful to visualize, isolate and characterize bone-forming osteoblasts in various systems.

W-2123
PROLIFERATION OF MOUSE NEURAL STEM/PROGENITOR CELLS ON POLYVINYL ALCOHOL HYDROGELS

Mori, Hideki, Hara, Masayuki
Graduate School of Science, Osaka Prefecture University, Sakai, Osaka, Japan

Aqueous solution of polyvinyl alcohol (PVA) can be modified into soft hydrogels by radiation cross-linking and can be used in several pharmaceutical and biomedical applications. We investigated the mechanical and biological properties of PVA hydrogels for use as culture substrates to maintain neural stem/progenitor cells (NSPCs). PVA hydrogels were synthesized from 3.75, 7.5, and 15% PVA solution by γ-irradiation with the dose of 10, 20, and 40 kGy. The mechanical properties of these hydrogels were characterized by calculating the swelling ratio based on the data of weight measurement, the Young's modulus based on the data of compression test. PVA hydrogels close to a swelling ratio of 100%, which are synthesized from 3.75% PVA solution by 10 kGy radiation (3%-10kGy PVA), 7.5% PVA solution by 20 kGy radiation (7%-20kGy PVA), and 15% PVA solution by 40 kGy (15%-40kGy PVA), were selected to test the ability of the culture substrate for NSPCs. The Young's modulus of PVA hydrogels were increased with increasing the concentration and the irradiation dose (3.3±0.5 kPa for 3%-10kGy PVA, 10.7±1.0 kPa for 7%-20kGy PVA, and 26.3±8.1 kPa for 15%-40kGy PVA). The PVA hydrogels were well dried, and then the structure was investigated by Fourier Transform Infrared Spectroscopy (FTIR). The FTIR spectra of PVA hydrogels showed a little difference of the relative intensity of the band related to hydroxyl groups (ν = 3,550-3,200 cm^{-1}). The NSPCs that were isolated from embryonic forebrain tissue of E14 ICR mouse and were cultured in DMEM/F-12 medium supplemented with B-27 solution and growth factors (EGF, b-FGF) applied to test the ability of PVA hydrogels as the cell culture substrate. NSPCs were incubated on each PVA hydrogel at 37°C, 5%CO_2 for 7days, and then were counted their cell numbers. Additionally, the cell populations in the NSPC culture were examined using immunocytochemistry and quantitative RT-PCR analysis. The NSPCs attached and proliferated on respective PVA hydrogels. On the 15%-40kGy PVA, the cells showed obviously spreading and extension. However, the neural stem cell markers, nestin- and sox-2-positive cells were detected abundantly in NSPCs cultured on every PVA hydrogels in immunocytochemical analysis using fluorescence microscopy. The RNA expressions of *Nestin* and *Sox2* showed similar values among these PVA hydrogels. PVA hydrogels prepared by γ-irradiation cross-linking will enable the new application as the adhesion culture of NSPCs.

W-2124
PLURITEST - PROVIDING A PLURIPOTENCY TEST FOR THE GLOBAL STEM CELL COMMUNITY

Mueller, Franz-Josef[1], Brändl, Björn[1], Lenz, Insa[2], Williams, Roy M.[3], Mason, Dylan[4], Schuldt, Bernhard[5], Loring, Jeanne F.[6]
[1]Department for Psychiatry, Zentrum Für Integrative Psychiatrie, Kiel, Germany, [2]Zentrum Für Integrative Psychiatrie, Kiel, Germany, [3]The Moores Cancer Center, University of California, San Diego, La Jolla, CA, USA, [4]Independent Consultant, Encinitas, CA, USA, [5]Department for Data-Driven Modeling, Aachen Institute for Advanced Study in Computational Engineering Sciences, Aachen, Germany, [6]The Scripps Research Institute, La Jolla, CA, USA

We developed PluriTest as an alternative to the teratoma assay, which requires the use of laboratory animals, for determining whether human stem cells are pluripotent. PluriTest is a bioinformatics-based assay based on gene expression profiles of cells of interest. An important feature of PluriTest was the establishment of a website (www.pluritest.org), which enables the non-expert in bioinformatics to process and retrieve the assay results from data that has been uploaded to the website. Our goal is to make assessment of pluripotency in human cells both reliable and user-friendly. PluriTest is increasingly being used for testing large collections of human iPSCs, where the teratoma assay is impractical. As of the time of this submission nearly 8,000 gene expression datasets have been uploaded by 524 unique registered users from 29 countries, and the test has been used in publications in Nature, Nature Methods, PNAS, Cell Stem Cell, Science Translational Medicine, Genome Research, and others. Here we will present and discuss extensions to the current PluriTest report, anonymized usage data and data trends, our ongoing improvements in the PluriTest data model for pluripotency, and new applications using the bioinformatics-based model.

W-2125

INVESTIGATING THE IMPACT OF FLUIDIC AGITATION ON HUMAN PLURIPOTENT STEM CELLS IN DYNAMIC SUSPENSION

Nampe, Daniel[1], Joshi, Ronak[2], Beaudette, Chad[3], Liew, Chee Gee[2], Tsutsui, Hideaki[3]

[1]Bioengineering, University of California, Riverside, Riverside, CA, USA, [2]Cell Biology and Neuroscience, University of California, Riverside, Riverside, CA, USA, [3]Mechanical Engineering, University of California, Riverside, Riverside, CA, USA

Due to the unique ability to self-renew indefinitely and differentiate into any cell type, human pluripotent stem cells (hPSCs) are an ideal cell source for future cell therapy applications. Despite its potential, a robust scalable culture system that can produce a sufficient number of clinical stem cell products is currently lacking. Dynamic suspension culture is a promising platform because it is easily scalable and automated, thereby reducing cost of stem cell production. However, propagation of undifferentiated hPSCs in dynamic suspension has not been explored until recently, and the microenvironmental factors that regulate the fate decision of hPSCs are poorly understood. In particular, fluidic agitation is unique to dynamic suspension culture and can play an important role in survival, self-renewal, and differentiation of hPSCs. We assessed impacts of different agitation rates (0-120 rpm) on cell output using a conventional spinner flask. After 7 days of dynamic suspension culture in mTeSR medium, moderate agitation at 60 rpm achieved the highest cell yield (55 fold increase) while maintaining high expression of pluripotent markers. This condition also produced the most uniformly sized cell aggregates with 200-300 μm in diameter, whereas the other agitation rates resulted in broader size distribution. Specifically, agitation conditions below 60 rpm resulted in large aggregates, mostly above 400 μm in diameter, and a low cell yield (5-15 fold increase), and conditions above 60 rpm resulted in smaller sized aggregates below 400 μm and a high cell yield (30-40 fold increase). This result presented a strong relationship between size of the cell aggregates and the cell yield, indicating there is an optimal aggregate size for survival and growth of hPSCs. To confirm this observation, we cultured aggregates of hPSC of prescribed sizes (100-500 μm) in mTeSR under static condition for 7 days. Overall, aggregate size at 300 μm had the highest cell yield (40 fold increase) and viability (90%) as well as high expression of pluripotency makers. Sizes below or above 300 μm displayed a decrease in both cell yield (10-25 fold increase) and viability (78-82%). Moreover, as confirmed by qPCR, large sizes (>400 μm) resulted in early germ layer differentiation, likely due to diffusion limitation of soluble factors. Collectively, this set of studies suggest that cell aggregate size is a critical parameter which can affect survival, growth and differentiation of hPSCs and that fluidic agitation can control their fate decisions at least partially by modulating aggregation and agglomeration kinetics. Another potential means of stem cell fate control in dynamic suspension culture is shear-induced mechanotransduction, although it is to be explored in our future study. Understanding cellular and molecular mechanisms that are induced by fluidic motion in suspension will help the development of scalable stem cell culture systems to meet the anticipated demands of stem cells and their derivatives.

W-2126

A FULLY AUTOMATED SYSTEM FOR LARGE-SCALE INDUCED PLURIPOTENT STEM CELL PRODUCTION AND DIFFERENTIATION

Paull, Daniel[1], Sevilla-Hernandez, Ana[1], Zhou, Monica[1], Hahn, Aana[1], Kim, Hesed[1], Napolitano, Chris[1], Sun, Bruce[1], Woodard, Chris[1], Shang, Linshan[2], Zimmer, Matthew[1], Moroziewicz, Dorota[1], Jagadeesan, Premlatha[1], Krumholz, Katherine[3], Tsankov, Alexander[4], Kahler, David[3], Forero, Eliana[1], Sy, Reese[1], Martina, Hector[1], Dusenberry, Carmen[3], Vensand, Lauren[3], Solomon, Susan[1], Chang, Stephen[3], Meissner, Alexander[5], Eggan, Kevin Carl[5], **Noggle, Scott**[1]

[1]New York Stem Cell Foundation, New York, NY, USA, [2]The New York Stem Cell Foundation, New York City, NY, USA, [3]The New York Stem Cell Foundation, New York, NY, USA, [4]Harvard, Cambridge, MA, USA, [5]Harvard University, Cambridge, MA, USA

Induced pluripotent stem (iPS) cells represent a source of patient-specific cells with the potential to differentiate into all the cell types in the human body. Given its significant utility in basic and clinical research, as well as in clinical applications, the demand for iPS is growing. However, the protocols, procedures, and reagents for iPS cell generation vary significantly between laboratories, leading to major concerns about the product's safety, quality, and consistency. Moreover, the current methods of iPS cell generation are mostly carried out manually, resulting in derivation that is time-consuming with variable efficiency, accuracy, and consistency. Therefore, a combined approach of iPS cell production, characterization, and differentiation using a standardized protocol in an automated cell production platform is desired. The NYSCF Global Stem Cell Array is a new technology platform for the derivation and manipulation of stem cell lines as well as the differentiation of adult cell types in a high-throughput, parallel process using automation. Standardization and scale-up capabilities achieved through this automated process are critical in efforts to reduce methodological variability to uncover true biology by improving signal to noise ratios in phenotype studies. The automated system has the capacity to generate hundreds of stem cell lines per month allowing large-scale investigation into disease pathologies and drug toxicity and efficacy studies. The platform will generate panels of stem cell lines from thousands of genetically diverse individuals representing both diseased patients and controls. In this fully automated system, the robotic methods for fibroblast cell thawing and seeding, reprogramming induction, iPS cell enrichment and purification, as well as iPSC colony selection and consolidation are optimized and tested. The entire production process is recorded with the cells imaged using an automated image-processing method and analyzed simultaneously, allowing for tracking every step and enabling the measurement of cell properties and consistent quality control. iPSC cultures have been generated, characterized, and maintained in feeder free conditions. The Array has the capacity for automated tri-lineage differentiation in embryo bodies as well as lineage specific differentiation into adult cell types such as dopaminergic neurons and pancreatic beta cells.

W-2127

VERAVEC ENDOTHELIAL CELLS RECREATE THE VASCULAR STEM CELL NICHE FOR MODELING AND EXPANSION OF STEM AND PROGENITOR CELLS OF HEALTHY AND MALIGNANT ORIGINS

Nolan, Daniel Joseph
Angiocrine Bioscience, New York, NY, USA

The scarcity of stem cells from various tissues has created a bottle neck for basic research, transplantation, and regenerative medicine at large. Current methodologies for the expansion of stem cells in vitro invariably reduce the capacity for engraftment and multi lineage commitment of the expanded stem cells. The ability to recapitulate the stem cell niche in vitro has eluded scientists as the creation of such a niche often requires irreducibly complex media additives, such as serum. The recent appreciation of the association of stem cells with the endothelial cells forming the vascular stem cell niche has only recently been understood. Angiocrine factors, the growth factors supplied by endothelial cells, are indispensable for the maintenance and expansion of stem cells in vivo. Historically, this observation has not been transferable to in vitro cultures due to the numerous additives needed to cultivate endothelial cells. VeraVec endothelial cells are capable of adapting endothelial cells from various species to tissue culture conditions without the need for such toxic and deleterious media additives. VeraVec endothelial cells are phenotypically stable for numerous passaging until their eventual senescence and are capable of recreating the vascular stem cell niche in vitro with defined media conditions. The co-culture of stem cells, with hematopoietic stem cells used as an example, with VeraVec endothelial cells resulted in a profound 1200 expansion within 12 days. Importantly, the expanded stem cell population maintained multi lineage engraftment capacity while simultaneously having enhanced engraftment. Transplantation studies revealed that the long term engrafting stem cells were both serially transplantable and at a higher frequency when compared to primary stem cell populations. This is in stark contrast to the loss of long term engraftment and lineage bias normally observed with HSC expansion protocols which rely on reductionist conditions. In addition to other adult stem cell lineages, recent studies have also demonstrated the effect on VeraVecs on cancer stem cells in a similar fashion. Primary malignant cells were demonstrated to retain their in vivo phenotype only when co-cultured on VeraVec ECs. Traditional mechanisms of culture cause a rapid departure from the original phenotype. Collectively, these observations underscore the capacity to use the the vascular stem cell niche in vitro for the expansion of stem cells for both research and clinical applications.

W-2128

REPLACEMENT OF BASIC FGF BY A STABLE-TYPE CHIMERIC FGF IN HUMAN PLURIPOTENT STEM CELL CULTURE

Onuma, Yasuko[1], Higuchi, Kumiko[1], Aiki, Yasuhiko[1], Shu, Yujing[1], Asada, Masahiro[2], Asashima, Makoto[1], Suzuki, Masashi[2], Imamura, Toru[2], Ito, Yuzuru[1]
[1]Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan, [2]Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan

FGF signal is essential for self-renewal of human embryonic stem cell and induced pluripotent stem cells. Recombinant basic FGF (bFGF or FGF2) is conventionally used for culture of the pluripotent stem cells, however because of its instability, consecutive addition of fresh basic FGF protein into the culture medium is required. In this study, we demonstrated that a recombinant stable-type FGF, FGFC, was useful for maintaining of human pluripotent stem cells. FGFC is a chimeric protein composed of human FGF1 and FGF2 domains, and is excellent at thermal stability and protease resistance than FGF1 and FGF2. Both human embryonic stem cells and induced pluripotent stem cells were maintained in ordinary culture medium containing FGFC instead of FGF2, without a significant change either in pluripotent markers expression, global gene expression, karyotype or differentiation potential into three germ lineages. Therefore, FGFC is an effective tool for stable maintenance of human pluripotent stem cells.

W-2129

GENOME ENGINEERED HUMAN EMBRYONIC STEM CELLS EFFICIENTLY REPORT GENE EXPRESSION DURING THE TRANSITION FROM PLURIPOTENCY TO THE DIFFERENTIATED CARDIOMYOCYTE STATE

Ortiz, Mariaestela[1], Ortmann, Daniel[1], Mascetti, Victoria[1], Mendjan, Sasha[1], Bernardo, Andreia Sofia[1], Janeway, Weslie[1], Mujica, Alejandro[2], Bekele, Yalem[2], Skarnes, William C.[2], Rosen, Barry[2], Pedersen, Roger A.[1]
[1]The Anne McLaren Laboratory for Regenerative Medicine, WTMRC, Cambridge Stem Cell Institute, The University of Cambridge, Surgery Department, Cambridge, United Kingdom, [2]T87 Wellcome Trust Genome Campus, The Wellcome Trust Sanger Institute, Hinxton, Cambridge, United Kingdom

Genome engineering of human embryonic stem cells (hESCs) enables visualization of gene expression during the transition from a pluripotent to a differentiated state. BAC recombineering methodology was used to generate vectors for gene targeting of hESCs. A gene targeting strategy was employed in hESC H9 cells to study cardiac progenitors previously described in mouse to arise from the mesoderm in the primitive streak, which expresses the T-gene (BRACHYURY/T), and the subsequent cardiac lineage committed progenitors that express NKX2.5 and TBX5. Knockin targeting vectors were engineered to encode H2B_Venus YFP or H2B_Cherry RFP reporter gene, which faithfully reported activity of the endogenous promoter of each gene expressing bright, nuclear localized fluorescence. While mesoderm formation and cardiomyocyte specification are typically studied in hESCs, gene dosage effects during these processes has been more difficult to model. To assess the precise gene dosage activity levels of those genes that are not expressed by pluripotent cells two design strategies were used: First, knockin reporter insertions which perturbed one copy of the endogenous locus resulting in heterozygousity (+/-), which should potentially model human haploinsufficient disease states. An additional targeting event of a (+/-) reporter line with the same vector (after marker removal) rendered the locus homozygous null (-/-). Second, the insertion of T2A_H2B_Venus YFP at the 3' end of the locus was used to render a non-perturbed wild type state (+/+). To enable functional interrogation of genes that might be necessary for pluripotent hESC growth a CRE-conditional targeting approach was developed. Targeting efficiencies of 1% to 3% were observed for T_T2A_H2B_Venus YFP and TBX5_H2B_Cherry RFP knockins. Similar gene targeting frequencies were observed for double targeted lines, which reported stably TBX5 with H2B_Cherry RFP and NKX2.5 with H2B_Venus YFP upon induction of differentiation of targeted clones to beating cardiomyocytes. The modular reporter cassettes and strategy for regulating gene dosages described here can equally be applied to engineer hESCs and hiPSs using CRISPR based approaches applicable to human cardiovascular development and disease.

W-2130

3D SPHERE CULTURE WITH FUNCTIONAL POLYMERS FOR LARGE-SCALE HUMAN PLURIPOTENT STEM CELL PRODUCTION

Otsuji, Tomomi G.[1], Bin, Jiang[1], Yoshimura, Azumi[1], Tomura, Misayo[2], Tateyama, Daiki[3], Minami, Itsunari[1], Yoshikawa, Yoshihiro[3], Aiba, Kazuhiro[1], Heuser, John E.[1], Nishino, Taito[2], Hasegawa, Kouichi[1], Nakatsuji, Norio[1]

[1]Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Kyoto, Japan, [2]Nissan Chemical Industries, Ltd., Tokyo, Japan, [3]Nipro Corporation, Kusatsu, Japan

Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), are expected to contribute greatly to regenerative medicine and drug discovery. These practical applications require defined and quality-controlled large-scale cell production. However, scaling up conventional adherent cultures presents challenges of maintaining a uniform high quality at low cost. In addition, current three-dimensional (3D) culture systems for large-scale hPSC production have disadvantages such as low survival rates, control of aggregate size and cell damage due to physical forces. Here, we have solved such problems in the suspension culture system through addition of functional polymers. Spontaneous fusion between spheres made variation in sphere sizes including very large spheres, which may cause cell death and autonomous differentiation. Addition of polymer 1 was able to produce uniform hPSC spheres through decrease of spontaneous fusion. Moreover we found polymer 2 that inhibits sedimentation and maintains the sphere in suspension without high viscosity or gel formation. Combination of polymer 1 and 2 improved our sphere culture system toward 3D culture without mechanical or dynamic stirring. In our 3D culture, hPSC spheres can be passaged with around 6-8 times split rate every 5 days, at least more than 50 passages, and the cells maintained a normal karyotype and pluripotency. Finally, to demonstrate the capability of our novel 3D sphere culture system, we tried to use 200 mL volume capacity culture bag made of gas permeable membrane. The morphologies of spheres and growth rate were similar to those of sphere in the small-scale culture, and average cell number yield was 1.4E+8 per bag. Thus, this simple and robust 3D sphere culture method would present new possibility of suspension culture for the large-scale hPSC production.

W-2131

DEVELOPMENT OF COMPARTMENTALIZED CULTURE PLATFORMS TO STUDY THE CELLULAR AND MOLECULAR PHENOTYPES IN SCHIZOPHRENIA PATIENT-DERIVED NEURONS

Pankonin, Aimee Ree, Kim, Hyung Joon, Kim, Yongsung, Wright, Rebecca, Gage, Fred H.

Salk Institute for Biological Studies, La Jolla, CA, USA

Schizophrenia (SZ) is prevalent in approximately one percent of the world's population over the age of 18. This disorder is ranked amongst the top ten causes of disability in developed countries worldwide. Postmortem studies have concluded that a patient inflicted with SZ shows a decreased cerebral volume, particularly in the frontal cortex and temporal lobes. This has been thought to be due to a decreased neuronal size in conjunction with a loss of neurites, as well as an increased neuronal density. However, these studies can only give us a snapshot of the inner workings of the brain without neuronal activity. With the scientific advancement of induced pluripotent stem cells (iPSC), we can now observe the activity of live neurons and create a more authentic model of neuronal activity. In order to reproduce the cellular and molecular defects of SZ, we used reprogramed SZ patient-derived iPSCs, which were then differentiated into neurons representing the diseased state. Due to the complex integrity of the brain, it is a challenging task to establish in vitro assays for quantitative studies of the synaptic connectivity in SZ. By interconnecting two 100μm-high cell culture chambers with 3μm-high microgrooves, we successfully compartmentalized neuronal cell bodies and neurites (axons and dendrites). This approach establishes a valuable in vitro assay for identifying cellular and molecular phenotypes in patient-derived neurons from iPSCs along with better quantification strategies. Using microfluidics-based compartmentalized culture, we are able to reproduce some key cellular and molecular events in the nervous system, such as cellular migration and neuronal differentiation, mitochondrial dynamics, and synaptic connectivity in both healthy control and disease states. Here, we report that the SZ neurons have shorter neurite length, less mobile mitochondria and decreased synaptic connectivity than control neurons. This technological ingenuity will impact research in neurological disorders by providing for the possibility of improved drug screening assays and the quantification of other phenotypes within the nervous system.

W-2132

AN IMPROVED WHOLE GENOME BISULFITE SEQUENCING (METHYL-SEQ) LIBRARY CONSTRUCTION METHOD FROM LOW $_{G}$DNA INPUTS FOR EPIGENETIC STUDIES

Pease, Jim, Czyz, Agata, Khanna, Anu, Gabel, Dixie, Ruotti, Victor, Syed, Fraz, Vaidyanathan, Ramesh

Illumina, Madison, WI, USA

Changes in DNA methylation patterns play a significant role in a variety of epigenetic processes including cell differentiation and disease on-set. Genome-wide analysis of methylated CpG nucleotides is possible by whole-genome bisulfite sequencing (WGBS; methyl-seq) which provides DNA methylation status at single nucleotide resolution. Conventional WGBS sample prep involves DNA shearing, ligation of methylated sequencing adaptors, bisulfite conversion of unmethylated cytidine nucleotides and sequencing on an Illumina sequencer. However, a major challenge with conventional WGBS sample prep is that large amounts of gDNA must be used because ~90% of the adaptor-tagged DNA is degraded during the bisulfite conversion step rendering them incapable of being sequenced. We describe an improved WGBS library construction method that features bisulfite conversion of cytosines *prior* to addition of the Illumina sequencing adaptors. As a consequence, our method enables use of as little as 50 ng of gDNA because virtually 100% of the DNA fragments, independent of position of bisulfite-mediated strand breaks, remain as viable templates for library construction and sequencing. Our WGBS library construction method is faster and produces highly diverse libraries with uniform CpG coverage.

REPROGRAMMING

W-2133

REPROGRAMMING OF MOUSE SOMATIC CELLS BY MICRORNA 302.367 CLUSTER

Kang, Kyung-Ku, Kim, Ah-Young, Lee, Eun-Mi, Lee, Eun-Joo, Min, Chang-Woo, Lee, Myeong-Mi, Kim, Sang-Hyeob, Sung, Soo-Eun, Hwang, Meeyul, Ghim, Soong-Koo, Jeong, Kyu-Shik

Department of Veterinary Pathology, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea

Induced pluripotent stem cells (iPSC) have been directly generated

from fibroblast cultures though retrovirus-mediated ectopic gene overexpression of only a few defined transcriptional factors. This remarkable achievement has greatly enhanced the promise of regenerative medicine through overcome immunological and ethical problem of embryonic stem cells. Recently, many kinds of somatic cells from different tissues have exhibited a capacity for reprogramming toward an embryonic stem cell-like state, but limitations in iPSC derivation and therapeutic use remain, including low reprogramming efficiency and safety of the generated iPSC. In this study, to overcome these limitations of iPSC, we're trying to reprogramming by using microRNA 302/367 cluster without exogeneous transcriptional factors. There was overexpressed exogeneous microRNAs into mouse embryonic fibroblasts via lentivirus transduction of microRNA 302/367 cluster expression vector. After lentiviral transduction, transducted cells showed immature colony characteristic such as assembled cells and blunt boundary. But RT-PCR results show upregulation of pluripotentcy gene expression such as Nanog and Klf4 compared to mouse embryonic fibroblasts. These results suggested that microRNA 302/367 cluster transducted cells were progressed partially reprogramming but not fully that. Thus, this study showed possibility of mouse somatic cells reprogramming by microRNA 302/367 cluster as alternative method.

W-2134
HETEROTYPIC CELLULAR REPROGRAMMING TO CORTICOFUGAL SUBTYPES USING A NOVEL GENETIC SYSTEM FOR INDUCIBLE EXPRESSION OF FEZF2

Akhtar, Aslam, Kim, Gi Bum, Molina, Jessica, Breunig, Joshua
Regenerative Medicine Institute, Cedars Sinai Medical Center, Los Angeles, CA, USA

The neurons of the cerebral cortex arise from neural stem cells during embryogenesis to generate a six layer structure. These cells terminally differentiate into precise subtypes of neurons. The intrinsic and extrinsic determinants of cerebral cortex development and the establishment of laminar neuronal subtype identities are increasingly understood. In particular, the transcription factor Fezf2 has been identified as a critical determinant of layer 5 corticofugal projection neurons. This class of neurons includes corticospinal motor neurons that are lost in degenerative motor neuron disorders such as amyotrophic lateral sclerosis (ALS). Previous work has explored the ability of Fezf2 to reprogram cells to a corticofugal phenotype within a very spatial and temporal window of early development. We have developed an electroporation based method for stably misexpressing Fezf2 in neural stem cells lining the lateral ventricle. Misexpression of Fezf2 in these cells reduces astrogliogenesis and olfactory bulb (OB) neurogenesis. To avoid acute OB neurogenesis alterations, we developed an inducible and reversible, 3rd generation, doxycycline(Dox)-regulated genetic system for expressing Fezf2. When our expression vector is induced by Dox in cultures of mouse neural stem cells or astrocytes, Fezf2 causes morphological conversion into neuron-like cells despite the presence of growth factors and serum. We have verified that this system is very robust upon in vivo electroporation, displaying little or no leakage and excellent inducibility. When Fezf2 expression is induced postnatally in the olfactory bulb, we see changes in nuclear size, increased ER81 expression, and evidence of ectopic axonal growth from the olfactory bulb. Using this new technology, we are exploring the ability of Fezf2 to reprogram heterogeneous populations of stem, progenitor and terminally differentiated cells to corticofugal subtypes in postnatal and adult mice, including in cortical lesion and neurodegenerative disease models. In the future, we will explore the suitability of this tool for generating enriched populations of motor neurons for studying disease processes in patient derived induced pluripotent stem (iPS) cells.

W-2135
VALIDATION OF AUTOMATIC, LABEL-FREE AND REAL-TIME SELECTION OF FULLY REPROGRAMMED IPSC COLONIES USING TIME-LAPSE MICROSCOPY AND KINETIC IMAGE PATTERN RECOGNITION

Alworth, Samuel V.[1], Hendriks, William T.[2], Kenyon, Zakary A.[1], Collins, Nicholas J.[3], Nakada, Chieko[4], Kiyota, Yasujiro[4], Daheron, Laurence[5], Rubin, Lee[2], Cowan, Chad A.[2], Lee, James[6]
[1]DRVision Technologies LLC, Bellevue, WA, USA, [2]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA, [3]Department of Stem Cell and Regenerative Biology, Havard University, Cambridge, MA, USA, [4]Nikon Corporation, Tokyo, Japan, [5]Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA, [6]DRVision Technologies LCC, Bellevue, WA, USA

The ability to reprogram somatic cells to an embryonic stem cell-like state has had a landmark impact on basic biological research, drug screening, and drug discovery. However, picking fully reprogrammed induced pluripotent stem cell (iPSC) colonies can be unreliable, costly and time consuming. In particular, currently there are no methods to consistently and automatically pick fully reprogrammed iPSC colonies without fluorescent surface markers. Label free and automated selection of iPSC colonies would greatly reduce the complexity of automated reprogramming and expansion systems, save time and resources in iPSC production, and facilitate high-throughput application of iPSC technology. In addition, for researchers less familiar with iPSC technology, computer assisted iPSC colony selection would ease implementation of reprogramming in their laboratories. We have discovered that the way iPSC colonies form contains very useful information that can be used to predict whether they will become fully reprogrammed. We have developed software methods to quantify and score these colony formation patterns using phase contrast video microscopy, and predict which colonies will become iPSCs. Since, in general, only a few fully reprogrammed iPSC colonies per patient sample are needed, we designed our approach to achieve very high specificity while allowing lower sensitivity. We developed the method using a database of seven patient samples, comprised of time-lapse recordings showing thousands of colonies forming, rigorous annotated ground truth (TRA 1-60 staining and manual review) on over 1800 colonies, and pluripotency characterization of ten cell lines. In the current study, we have validated the method in a double blinded study using healthy and disease-specific fibroblasts and Sendai virus-mediated reprogramming with Klf4, Oct3/4, Sox2 and c-Myc, imaged continuously for three weeks on the Nikon BioStation CT. The kinetic image pattern recognition software automatically identifies and ranks up to 10 fully reprogrammed colonies for selection per patient using only time-lapse phase contrast microscopy image sequences without fluorescence. Concurrently, skilled technicians in the HSCI iPSC core select fully reprogrammed colonies based on morphology and TRA-1-60 staining. Here we present the kinetic image pattern recognition method and validation results that show fully blinded and automated selection of iPSC colonies with high specificity. Pluripotency validation was performed and comparison between human and computer selected colonies shows no significant difference in colony quality using standard and rigorous pluripotency assessment methods. This research is supported in part by grant number 4R44HL106863 from the NHLBI and the Harvard Stem Cell Institute.

W-2136

IDENTIFICATION OF A TIME WINDOW FOR SPONTANEOUS ESTABLISHMENT OF PLURIPOTENCY IN MOUSE SPERMATOGONIAL STEM CELLS IN VITRO

Azizi, Hossein[1], Conrad, Sabine[2], Hinz, Ursula[1], Asgari, Behrouz[3], Nanus, Daniel[1], Peterziel, Heike[1], Moghadam, Akbar Hajizadeh[4], Baharvand, Hossein[3], Skutella, Thomas[1]

[1]University of Heidelberg, Heidelberg, Germany, [2]University of Tuebingen, Tuebingen, Germany, [3]Royan Institute, Tehran, Iran, [4]University of Mazandaran, Babolsar, Iran

Although testis-derived embryonic stem cell-like (ES-like) cells have been obtained in several studies, the time window for the shift to pluripotency is not clear yet. Here we describe, that only during a special time window (41 until 125 days) after initiation of germ line stem cell (GSCs) cultures from neonate and adult promoter-reporter Oct4-GFP transgenic mouse the spontaneous appearance of germline-derived pluripotent stem (gPS) cells from both neonate and adult GSCs occurred. The isolated and long-term cultured (more than one year) GSCs which were isolated by a morphology based selection procedure expressed germ cells markers and exhibited a similar morphology with a high nucleus/cytoplasm ratio in comparison to undifferentiated SSCs (spermatogonial stem cells) in vivo. The generated gPS cells expressed pluripotency marker, in-vitro differentiated into all three germ lineages, formed complex teratoma after transplantation in SCID mice and produced chimeric mice. Although the exact mechanism of the development of gPS cells from GSCs is still unclear, this new information could provide an ideal strategy for scheduling natural conversion mechanisms of ES-like cells from mouse testis.

W-2138

HIGHLY EFFICIENT REPROGRAMMING OF A SMALL NUMBER OF HUMAN SOMATIC CELLS INTO STABLE INDUCED PLURIPOTENT STEM CELL LINES USING A COMBINATORIAL RNA-BASED APPROACH

Bilousova, Ganna[1], Kogut, Igor[1], Ortega, Sandra M.[1], Pavlova, Maryna V.[1], Astling, David P.[2], Jones, Kenneth L.[2], Cambier, John C.[3], Roop, Dennis R.[1]

[1]Department of Dermatology, Charles C. Gates Center for Regenerative Medicine and Stem Cell Biology, University of Colorado, Aurora, CO, USA, [2]Department of Biochemistry and Molecular Genetics, University of Colorado, Aurora, CO, USA, [3]Department of Immunology, University of Colorado and National Jewish Health, Denver, CO, USA

Reprogramming somatic cells into induced Pluripotent Stem Cells (iPSCs) has greatly advanced the field of regenerative medicine and stem cell biology. However, there are still many obstacles that limit the broad application of iPSCs in the clinic and research, such as: (1) the relatively low efficiency and high cost of reprogramming protocols, including clinically relevant integration-free approaches; (2) substantial time required to establish a cell line from a patient's biopsy before iPSC generation can be initiated; (3) difficulties in reprogramming a low number of somatic cells with integration-free approaches. To address these obstacles, we optimized a synthetic mRNA-based approach described by Warren et al. through incorporating previously reported reprogramming mimic miRNAs, employing a feeder-free system, enhancing synthetic mRNA transfections, as well as improving the reprogramming regiment, medium composition, mRNA/miRNA concentrations, and cell plating conditions. As a result of these optimizations, we were able to attain an unprecedented efficiency of human cell reprogramming starting from as little as a single human cell. Using human primary neonatal fibroblasts, we generated ~1100 Tra-1-60 positive iPSC colonies from 200 starting cells within 2 weeks of reprogramming with only 6 transfections. A minimum of 3 transfections was required to obtain a few iPSC colonies, and 6 - 7 transfections to achieve the maximum reprogramming efficiency. The first colonies started to emerge as early as day 8, and mature iPSC colonies could be picked on day 12 of this reprogramming regiment. We were also able to reprogram ~75% of individually plated human neonatal fibroblasts that survived through the remainder of the reprogramming protocol; with each reprogrammed cell producing on average 22 Tra-1-60 positive iPSC colonies. When our protocol was employed for the reprogramming of adult fibroblasts derived from 41 and 50 year old individuals, the resulting efficiency of iPSC generation was ~14% and ~36% respectively. To address the applicability of our protocol in aging research, the same adult fibroblast lines were serially passaged until more than 91% of cells exhibited a senescent phenotype and then reprogrammed into iPSCs. The reprogramming of these senescent lines took only 2 weeks and resulted in an efficiency of ~0.22% and ~0.33% respectively. The established iPSC lines generated from neonatal, adult and senescent human fibroblasts exhibited normal karyotypes and have been successfully maintained for at least 15 passages. The pluripotency of the generated iPSCs was confirmed by gene expression analysis and the differentiation into cell types of all three germ layers both *in vitro* and *in vivo*. Thus, our protocol allows for the integration-free reprogramming of a variety of somatic human cells with kinetics and the efficiency which surpass all previously published reports. The approach is cost effective, provides an opportunity to shorten the time between the biopsy and the generation of stable high-quality iPSC lines, and allows for the production of iPSCs from individually plated cells in a feeder-free system. We anticipate that the reported method can be used to reprogram fibroblasts derived from both young and elderly patients into clinical grade iPSCs and, due to its ultra-high efficiency, opens up new horizons to studying the biology of reprogramming at a single cell level using primary human cells without any prior genomic modifications.

W-2140

RAPID AND EFFICIENT REPROGRAMMING OF HUMAN SOMATIC CELLS INTO INDUCED NEURAL STEM CELLS BY MIR-A/NTF-1

Yu, Kyung-Rok, **Shin, Ji-Hee,** Kim, Jae Jun, Lee, Jin Young, Koog, Myung Guen, Choi, Soon Won, Tae-Wook, Kang, Kim, Hyung-Sik, Seo, YooJin, Lee, Byung-Chul, Kang, InSung, Sung, Eun-Ah, Kang, Kyung-Sun

Seoul National University, Seoul, Republic of Korea

Recent advances have suggested that somatic cells can be generated to human induced neural stem cells (hiNSCs) by expressing defined sets of transcription factors. From miRNA expression profile analysis and validation, we identified miR-A acts as an inhibitory influence on the hiNSC generation. Furthermore, NTF-1, one of miR-A targeting gene, significantly promotes hiNSC derivation efficiency. Coexpressing NTF-1 with NTF-2, previously reported hiNSC reprogramming factor, greatly accelerated reprogramming so that reprogramming of human dermal fibroblast to hiNSCs requires only 7 day with 10-fold higher efficiency compared to NTF-2 alone. These induced hiNSCs display morphology, molecular features and tri-lineage differentiation potential with in vitro functionality similar to that of H9-derived NSCs. With NTF-1/NTF-2 combination, we successfully generated hiNSCs from human mesenchymal stem cells and human cord blood CD34+ cells. Finally, we demonstrated that only NTF-1 expression is sufficient to induce hiNSCs with combination of a small molecule, SB431542. Together our findings suggest miR-A/NTF-1 pathway plays critical roles in direct reprogramming into hiNSCs.

W-2141
DEFINED AND XENO-FREE MEDIUM FOR REPROGRAMMING BLOOD-DERIVED CD34+ CELLS OR ERYTHROID CELLS

Chang, Wing Yean, Hunter, Arwen L., Ng, Alvin, Wong, Matthew, De Jong, Susan, Yu, Irene, Wognum, Bert, Peters, Carrie, McQueen, Karina, Fairhurst, Maureen, Hadley, Erik, Antonchuk, Jennifer, Thomas, Terry E., Eaves, Allen C., Louis, Sharon A.
STEMCELL Technologies Inc., Vancouver, BC, Canada

Peripheral blood (PB) is the second most commonly used primary cell source, after skin, for generating human induced pluripotent stem cells (hiPSCs), due to the minimally invasive procedure for sample collection. However, challenges with reprogramming PB mononuclear cells (MNCs) include low frequencies of CD34+ hematopoietic stem and progenitor cells (0.01-0.1%), and high frequencies of T- and B-cells, which are less ideal as target cells for reprograming as they have VDJ rearrangements that may affect the downstream applicability of generated hiPSCs. We aimed to develop a defined feeder-free, xeno-free reprogramming medium for use with culture-expanded CD34+ and erythroid cells as primary cell sources. MNCs were isolated from umbilical cord blood (CB) or PB by fractionation over a Ficoll™ density gradient in SepMate™-50 tubes. CD34+ cells were then enriched (80-90% purity) by immunomagnetic separation using EasySep™ CD34+ positive selection, and expanded for 7 days in StemSpan™ SFEM II Medium with CD34+ Expansion Supplement (up to 18-fold expansion with 50-70% CD34+ cell purity). Alternatively, erythroid cells were generated by expansion of PB MNCs or CD34+-enriched CB cells for 10-14 days in StemSpan™ SFEM II with Erythroid Expansion Supplement (up to 20- or 3000-fold expansion for PB or CB, respectively, with 75-90% CD71+GlyA+ cell purity). Vectors containing reprogramming factors were then transfected into each of these starting cell populations, and the cells immediately transferred to Matrigel®-coated plates at 20,000 cells/cm2 in either TeSR™-E7™, KnockOut Serum Replacement-containing medium (KOSR medium), or a new defined xeno-free blood reprogramming medium (BRM). Cells were cultured with complete media changes every other day until day 7; at each media change all non-adherent cells were recovered by centrifugation and returned to the culture. After day 7, the media were changed daily up to day 28 without the return of non-adherent cells. Putative hiPSC clones were identified by microscopic examination of colony morphology and counted after 21-28 days. Reprogramming efficiencies for CB-derived culture-expanded CD34+ and erythroid cells in BRM were 4- and 5-fold higher (n=1) than in KOSR medium, and 1.5- and 2-fold higher (n=1) than TeSR™-E7™, respectively. Reprogramming efficiency for PB-derived culture-expanded CD34+ and erythroid cells in BRM were 2.25- and 2.5-fold higher (n=1) than TeSR™-E7™, respectively. In addition, by day 28, the hiPSC colonies generated in BRM from PB- or CB-derived CD34+ were more easily identified due to their compact morphology, well-defined borders, and larger size than colonies generated in TeSR™-E7™ or KOSR medium. In summary, our results demonstrate the application of a novel, xeno-free and defined blood reprogramming medium for efficient reprogramming in feeder-free conditions that produces hiPSCs exhibiting excellent colony morphology. We have established an integrated workflow for the isolation, expansion, and reprogramming of blood-derived cells using EasySep™ CD34+ cell selection, StemSpan™ media and supplements for CD34+ and erythroid cell expansion, and reprogramming in blood reprogramming medium (BRM).

W-2142
A POLYMERIC TAG FOR EFFICIENT REPROGRAMMING VIA PROTEIN HATHFUN

Christiansen-Weber, Trudy[1], Chu, Tiffany[1], Abramhina, Tatiana[1], Noskov, Alexander[1], Dillberger, Alexa[1], Garitaonandia, Ibon[2], Ostrowska, Alina[1], Semechkin, Ruslan[1]
[1]International Stem Cell Corporation, Carlsbad, CA, USA, [2]International Stem Cell Corporation, San Marcos, CA, USA

Transcription factors can be used as programming modules to generate specific cell types. The most famous is the creation of induced pluripotent stem (iPS) cells from fibroblasts. The methods to create these cell types, however, are good for research grade material, but not as a therapeutic because of safety concerns. To answer such concerns, HATHFUN, a fusion protein tag, was created as a tool to specifically target proteins to the nucleus utilizing protein transduction technology. The use of proteins carries no risk genomic DNA alteration and will not reactivate at inappropriate times. In terms of reproducibility, all cells are transduced with extremely consistent results. This system can be shut off without further manipulation. Importantly, there are no host-specificity issues and cells need not be proliferating in order to take up protein. Finally, in order to reach a target tissue, multiple transcription factors are required at different times, with different levels of expression which must be shut off as differentiation progresses. Viral systems emphatically cannot meet this need. This tag will allow the finest control possible for reprogramming cells and limit the footprint of *in vitro* manipulation. Our HATHFUN protein library can use these transcriptional master switches to direct differentiation of cells for definitive endoderm, skeletal muscle, brown fat and hepatocytes. We are pursuing two different reprogramming strategies: one set of HATHFUN-tagged proteins are being used to mature hepatocytes beyond the fetal stage of development. The second set of HATHFUN-tagged proteins generates iPSCs by increasing both the efficiency and speed of reprogramming. Morphology changes are observed by Day 4 and expression of the three pluripotency markers, NANOG, SOX2 and POUF51 are detectible by Day 5 with as much as a 12-fold increase of POUF51 by Day 8.

W-2143
SIMPLIFICATION OF INDUCTION TO PLURIPOTENCY USING A SINGLE POLYCISTRONIC EPISOMAL PLASMID

Patel, Zankesh[1], Woloszyn, Derek[2], Krugman, Jessica L.[3], Morgenstern, Ari[4], Lee, Alicia[5], Wong, Wilson[6], **Cohen, Rick I.**[5]
[1]Cell Biology and Neuroscience, Rutgers University, Piscataway, NJ, USA, [2]Biology and Psychology, Program in Behavioral Neuroscience, Northeastern University, Boston, MA, USA, [3]Biology, Emory, Atlanta, GA, USA, [4]Biology, University of Rochester, Rochester, NY, USA, [5]Biomedical Engineering, Rutgers University, Piscataway, NJ, USA, [6]Chemistry and Chemical Engineering, Rutgers University, Piscataway, NJ, USA

Reprogramming of somatic cells into Induced Pluripotent Stem Cells (IPSCs) can be accomplished by a variety of methods utilizing recombinant proteins, modified RNAs, DNA plasmids, or viral vectors hosting a popular set of four transcription factors: Oct4, Sox2, KLF4, and C-Myc (or L-Myc). More recently non-genetically modifying methods have gained popularity as they lend themselves to translational and eventually clinically related research paradigms. One such method involves insertion of 4 or 5 plasmids harboring EBNA-1 and OriP, two motifs that allows for episomal duplication of these plasmids in human cells. Therefore the transferred DNA not only expresses the desired set of genes, but also maintains their presence over the course of several cell divisions. While this simple method allows for robust reprogramming, preparation

of 5 plasmids is costly and time consuming. Here we developed a single episomal plasmid, pERC-V1, which contains Oct4, Sox2, KLF4, L-Myc and a fusion of RFP and Blasticidin-S-Deaminase. pERC-V1is capable of reprogramming human foreskin fibroblasts when transferred to cells via electroporation using the NEON system in conjunction with an optimized culturing methodology. Together the system produces proto-colonies within 7 days, and mature expandable colonies within 21 days. In the long term, we aim to improve the efficiency of the plasmid, and to remove the few remaining animal derived products and still achieve robust, and rapid reprogramming in a simple single step manner.

W-2144
COMPARISON AND EVALUATION OF NON-INTEGRATING REPROGRAMMING METHODS

Daheron, Laurence M.[1], Schlaeger, Thorsten[2], Lynes, Maureen[3], Brickler, Thomas R.[4], Chan, Karrie[2], Cianci, Amelia[2], DeVine, Alexander[2], Entwisle, Samuel[5], Andrew, Ettenger[2], Fitzgerald, Kelly[2], Godfrey, Michelle[2], Dipti, Gupta[2], McPherson, Jade[1,2], Malwadkar, Prerana[2], Doi, Akiko[6], Feinberg, Andrew P.[6], Meissner, Alexander[7], Cowan, Chad[3], Rubin, Lee[3], Daley, George[2]

[1]Harvard Stem Cell Institute, Harvard University, Cambridge, MA, USA, [2] Boston Children's Hospital, Boston, MA, USA, [3]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA, [4]Virginia Tech Regenerative Medicine, Blacksburg, VA, USA, [5]HSCI iPS Core, Cambridge, MA, USA, [6]Hopkins University, Baltimore, MD, USA, [7]Harvard University/Broad Institute, Cambridge, MA, USA,

The HSCI iPS core facility was launched in 2008 to facilitate stem cell research by providing services to the community. These services include: 1/ iPS derivation, 2/ iPS distribution and 3/genome editing. In the initial phase of the core facility, we generated iPS cell lines from fibroblast samples only and with the retrovirus system, an integrating technology. In the past 3 years, we focused on implementing new reprogramming technologies and diversifying the sources of somatic cells. We currently offer three non-integrative reprogramming technologies: Sendai virus, episomal vector and mRNA/microRNA. Moreover, we have derived iPS lines from a number of cell types including fibroblasts, mesenchymal stem cells (from Bone marrow and umbilical cord), T cells, erythroblasts, CD34+ (from adult peripheral blood) and keratinocytes. Together with the hESC Core Facility at Boston Children's Hospital, we have performed a comprehensive comparison of the three predominant non-integrating reprogramming methods based on the following criteria: efficiency, reliability, speed of colony emergence, kinetics of loss of exogenous factors, cost, ease of use, scalability, and the quality of the resulting lines. The experiments were performed in parallel in two separate laboratories to account for inter-laboratory variability. Here, we present our results and discuss the advantages and shortcomings of these reprogramming methods. In addition, we present and review the results of a survey of a large number of human reprogramming laboratories on their independent experiences and preferences.

W-2145
MIRNAS ARE REQUIRED FOR INDUCED NEURONAL CONVERSION

Davila, Jonathan[1], Ang, Cheen E E.[1], Kareta, Michael[2], Parchem, Ron[3], Shenoy, Archana[3], Blelloch, Robert[4], Wernig, Marius[5]

[1]Stanford University, Palo Alto, CA, USA, [2]Stanford University, Stanford, CA, USA, [3] University of California, San Francisco, San Francisco, CA, USA, [4]University of California, San Francisco, San Francisco, CA, USA, [5]Stanford University, Palo Alto, CA, USA

It is well established that exogenous expression of specific transcription factors can reprogram fibroblast to an induced neuronal cell (iN). microRNAs (miRNAs) have also been shown to enhance iN conversion. Because miRNAs serve as key regulators of biological functions, we wanted to test whether changes in the miRNA population are not only beneficial but also required for the reprogramming process. miRNA sequencing shows highly dynamic changes in miRNA expression profiles during iN conversion. To test if these changes are required for iN conversion, we have taken advantage of a miRNA deficient mouse embryonic stem cell (mESC) line that lacks DGCR8, a critical component for miRNA processing. Wild type (WT) mESCs rapidly convert to Map2+ neurons within 5days upon induction of the Ngn2 transcription factor. Meanwhile, DGCR8 -/- mESCs do not show any change in their state after Ngn2 induction and ultimately die during the reprogramming process. In addition, DGCR8 conditional KO MEFs infected with lentiviruses containing Tet inducible Brn2, Ascl1, and Myt1L (BAM) transcription factors show a decrease in iN formation after treatment with CRE-recombinase when compared to controls. These observations indicate that mESC directed differentiation and MEF transdifferentiation towards iNs requires a change in the population of expressed miRNAs. We are currently screening miRNAs to test whether we can rescue iN conversion deficiencies.

W-2146
DIRECT CONVERSION OF HUMAN FIBROBLASTS TO FUNCTIONAL CORTICAL NEURONS

Devaraju, Karthikeyan[1], Miskinyte, Giedre[1], Monni, Emanuela[1], Madsen, Marita G.[1], Prieto, Daniel T.[1], Woods, Niels-Bjarne[2], Lindvall, Olle[3], Kokaia, Zaal[4]

[1]Lund University, Lund, Sweden, [2]Lund University, Molecular Medicine and Gene Therapy, Lund, Sweden, [3]University of Lund, Lund, Sweden, [4]Stem Cell Center, Lund University, Lund, Sweden

Several neurological disorders such as hypoxia-ischemia of infant brain, traumatic brain injury, Alzheimer's disease and stroke, lead to neuronal death in cerebral cortex. Intracortical transplantation of cortical neurons could provide a potential novel therapeutic strategy for these disorders. Somatic cells like fibroblasts can be directly converted to functional neurons by forced expression of transcription factors, and differentiated to subtype-specific neurons, such as spinal motor and dopaminergic neurons. However, subtype-specific cortical neurons have so far not been derived by direct conversion of somatic cells. Here we have derived cortical neurons (iCtx cells) from human fetal lung fibroblasts using combinations of cortical transcription factors. The iCtx cells were pyramidal in shape with long neurites and most of the cells were bipolar or multipolar. The iCtx cells expressed transcripts that are developmental as well as layer- and functional area-specific cortical markers, namely, Pax6, Emx2, Tbr2, Bhlhb5, Satb2 and Ctip2. The cells could fire multiple action potentials and respond to glutamate or GABA application. We demonstrate, for the first time, by combined immunohistochemical, morphological, transcript and electrophysiological analysis, that human fibroblasts can be directly converted to functional cortical neurons, which may be useful in regenerative medicine and for disease modeling.

W-2147

THE ROLE OF THE NURD COMPLEX IN THE PROCESS OF INDUCED PLURIPOTENCY

dos Santos, Rodrigo Luiz[1], Tosti, Luca[2], Radzisheuskaya, Aliaksandra[1], M. Caballero, Isabel[3], Kaji, Keisuke[2], Hendrich, Brian D.[1], Silva, Jose[1]
[1]WT-MRC Stem Cell Instute, Cambridge, United Kingdom, [2]MRC Centre for Regenerative Medicine, Edinburgh, United Kingdom, [3]Laboratory of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Sweden

Reprogramming of somatic cells to naïve pluripotency can be robustly driven by the combined action of transcription factors and culture cues. Among the reprogramming transcription factors, Oct4 plays a central role, as it is sufficient and essential for the induction of pluripotent cells. Oct4 interactome studies in embryonic stem cells (ESCs) revealed members of the Nucleosome Remodelling and Deacetylase (NuRD) complex as its highest confidence interactors. Mbd3 is an essential subunit of the NuRD complex, in the absence of which the complex is not assembled. Embryos lacking Mbd3 die shortly after implantation and Mbd3-null ESCs are viable but show severely impaired lineage commitment and exhibit limited differentiation capacity. Since the NuRD complex is a high confidence interactor of Oct4 and a key regulator of developmental cell state transitions, we investigated if NuRD is also involved in the reverse biological process of induction of pluripotency. have addressed this question by means of genetics or siRNA to manipulate Mbd3 lelvels. We made use of different reprogramming cells systems, such as neural stem cells (NSCs), embryonic fibroblasts (MEFs), epiblast Stem Cells (EpiSCs) and pre-induced pluripotent Stem Cells (preiPSCs). In summary, our results identify a key role of the Mbd3/NuRD complex in the induction of pluripotency and show that a chromatin complex required for cell differentiation also promotes the reversion of these cells back to a pluripotent cell state. I will present my findings and discuss these in light of recent publications.

W-2148

GENERATING SOMATOSENSORY NEURONAL LINEAGES THROUGH DIRECT REPROGRAMMING OF HUMAN AND MOUSE FIBROBLASTS

Eade, Kevin[1], Blanchard, Joel[2], Lo Sardo, Valentina[3], Tsunemoto, Rachel[4], Baldwin, Kristin K.[5]
[1]The Scripps Research Institute, San Diego, CA, USA, [2]The Scripps Research Institute, Clairemont, CA, USA, [3]The Scripps Research Institute, La Jolla, CA, USA, [4]University of California, San Diego, TSRI, La Jolla, CA, USA, [5]Scripps Res Inst, La Jolla, CA, USA

Sensory neurons of the dorsal root ganglion and trigeminal nerve comprise a diverse lineage responsible for detecting pain, itch, temperature, pressure and stretch. The study of sensory disorders is confounded by individualized response to sensory inputs and discordance between phenotype and underlying pathology. The primary methods used to investigate these disorders have largely relied on animal models and heterologous expression systems due to the difficulty of obtaining and manipulating human neurons. A major consequence, due to the limitations inherent in these models, is a failure of many promising therapeutic candidates during clinical trials. Direct reprogramming offers the promise of modeling individualized phenotypes and genotypes in disease relevant cells through in vitro manipulation and screening. Here we show that transiently co-expressing two transcription factors selectively reprograms mouse and human fibroblasts into neurons that display hallmark morphological, gene expression, synaptic, and electrophysiological signatures of the primary somatosensory neuronal lineage. These induced sensory lineage neurons (iSLNs) acquire key defining morphologic features of somatic sensory neurons including pseudounipolar neurite structure, and match the characteristic gene expression patterns of endogenous sensory neurons including selective expression of TrkA, TrkB or TrkC receptors. Furthermore we show that functionally, different iSLNs respond selectively to diverse ligands known to activate nociceptors and itch sensitive neurons. This direct reprogramming technique provides a rapid and efficient method for the generation of sensory neurons from each of the three major modalities of perception (nociception, mechanoreception, and proprioception), delivering new technology for therapy and investigating the fundamentals of pain itch and other pathologies affecting peripheral sensory neurons.

W-2149

INDUCTION AND ISOLATION OF A NOVEL EARLY NEUROEPITHELIAL CELL POPULATION CAPABLE OF DIFFERENTIATION INTO CENTRAL AND PERIPHERAL NEURAL LINEAGES

Edenhofer, Frank[1], Thier, Marc Christian[2], Wörsdörfer, Philipp[3]
[1]Anatomy and Cell Biology, University of Wuerzburg, Wuerzburg, Germany, [2]German Cancer Research Center, Heidelberg, Germany, [3]University of Wuerzburg, Wuerzburg, Germany

Reprogramming differentiated cells into multipotent somatic stem cell-like cells developed into a promising alternative to the induction of pluripotency followed by subsequent differentiation. Recently, we demonstrated the successful derivation of induced neural stem (iNS) cells (Thier et al, Cell Stem Cell 2012). iNS cells represent both, a virtually unlimited source of Patient-specific neural cells for disease modeling and a robustly accessible model for early neural development. However, iNS are of radial glia (RG)-type and thus exhibit a limited developmental capacity and restricted regional identity. Here, we describe a novel stably expandable neuroepithelial cell population with greatly expanded multi-lineage differentiation capacity that was identified by modifying the iNS reprogramming protocol by pharmacological intervention. Using defined differentiation paradigms we derived from early neuroepithelial cells common pan-neural precursor populations, such as rosette- and RG-type neural stem cells as well neural crest progenitors together with the respective differentiated post-mitotic progeny. Intriguingly, this novel cell population does not only represent an induced artificial reprogramming outcome but we show that it has a physiological correlate. Thus far, RG-type NS cells represent the only murine neural stem cell population, which can be isolated from embryonic neural tissue and maintained in culture. Now, based on our new reprogramming findings we show that one can isolate similar early neuroepithelial cells from E9.5 forebrain vesicles by the same inductive cues as applied in the reprogramming protocol. We anticipate that these cells provide a robust and versatile cellular platform for the analysis of early neurodevelopment and biomedical applications.

W-2150

HUMAN OOCYTES REPROGRAM ADULT SOMATIC NUCLEI TO DIPLOID PLURIPOTENT STEM CELLS

Egli, Dieter[1], Yamada, Mitsutoshi[2], Johannesson, Bjarki[3], Sagi, Ido[4], Benvenisty, Nissim[4], Sauer, Mark[5]
[1]The New York Stem Cell Foundation Research Institute, New York, NY, USA, [2]Keio University School of Medicine, Tokyo, Japan, [3]NYSCF, New York, NY, USA, [4]Hebrew University, Jerusalem, Israel, [5]CWRC, New York, NY, USA

The transfer of somatic cell nuclei into oocytes can give rise to pluripotent stem cells, holding promise for autologous cell replacement

therapy. Though reprogramming of somatic cells by nuclear transfer was first demonstrated more than 60 years ago, only recently have human diploid embryonic stem cells been derived after nuclear transfer of fetal and neonatal fibroblasts. Because of the therapeutic potential of developing diploid embryonic stem cell lines from adult cells of normal and diseased human subjects, we have systematically investigated the parameters affecting efficiency and developmental potential in their derivation. We found that improvements to the oocyte activation protocol, including the use of both a kinase and a translation inhibitor, and cell culture in the presence of histone deacetylase inhibitors enable development of diploid cells to the blastocyst stage. Developmental efficiency varied significantly between oocyte donors, and was inversely related to the number of days of hormonal stimulation required to reach mature oocytes, while the daily dose of gonadotropin or the total number of MII oocytes retrieved did not affect developmental outcome. Using these modifications to the nuclear transfer protocol, we successfully derived diploid pluripotent stem cell lines from both postnatal and adult somatic cells of a type 1 diabetic subject.

W-2151
DIRECT REPROGRAMMING OF MOUSE MACROPHAGES INTO CARDIOMYOCYTE-LIKE CELLS

Tsuchida, Hiroshi[1], Martinson, Amy[1], Futakuchi-Tsuchida, Akiko[1], Pabon, Lil[1], Reinecke, Hans[1], Murry, Charles[2]
[1]University of Washington, Seattle, WA, USA, [2]University of Washington - Center for Cardiovascular Biology, Seattle, WA, USA

Direct reprogramming of fibroblasts into cardiomyocytes was reported to improve cardiac function in a mouse myocardial infarction model. Myeloid cells, monocytes/macrophages, are known to migrate into inflammatory sites. Here we tried to utilize the homing ability of macrophages and convert them into cardiomyocytes at mouse infarcted heart. We screened a retroviral library of transcription factors recently identified as regulators of cardiogenesis in human embryonic stem cells. Three 3 transcription factors, Gata4/Mef2c /Tbx5 (GMT), induced GFP expression in bone marrow cell-derived macrophages (BMM) isolated from Myh6-GFP transgenic mouse. GFP-positive cells appeared onward from 2 days after retrovirus infection, and the number increased gradually to reach the peak on day 8. Cardiac gene expression as measured by cardiac troponin T and ryanodine receptor 2 expression was also detected in reprogrammed BMM with quantitative PCR. Individual and combination of GMT expression revealed that Gata4/Tbx5 expression (without Mef2c) induced the same number of GFP-positive cells as GMT expression, and Gata4 expression itself strongly induced cardiac troponin T expression. FACS analysis indicated that GFP-positive cell population induced by GMT expression was less than 1% of total cells on day 8. However, treatment with JAK inhibitor 1 showed a 4-fold enhancement of reprogramming efficiency induced by GMT. Retinoic acid receptor agonist AM580 also doubled the number of GFP-positive cells induced by GMT. C/EBP alpha, C/EBP beta and PU.1 are the transcription factors important for myeloid differentiation that help establish macrophage cell identity. siRNA knockdown experiments of these 3 transcription factors indicated that repressing the expression of C/EBP beta doubled the reprogramming induced by GMT. miRNAs are known to induce direct reprogramming of fibroblasts into cardiomyocytes, and transfection of miR-208 into BMM prior to GMT expression showed a 2-fold enhancement of reprogramming efficiency into cardiomyocyte-like cells. Thus, reprogramming of mouse macrophages into cardiomyocyte-like cells can be accomplished with a combination of overexpressing cardiac transcription factors, repressing macrophage transcription factors, controlling JAK and retinoic acid signaling and overexpressing miR-208. Further improvements in reprogramming efficiency should enable more detailed assessment of phenotype and therapeutic potential.

W-2152
GUIDED CONVERSION OF HUMAN FIBROBLASTS TO STRIATAL NEURONS

Victor, Matheus B., Richner, Michelle, Yoo, Andrew S.
Developmental Biology, Washington University in St. Louis, St. Louis, MO, USA

MicroRNAs (miRNAs) regulate fundamental cellular processes such as proliferation and cellular differentiation through post-transcriptional regulation of gene expression. We have previously shown that brain-enriched miRNAs promoted conversion of non-neuronal cell types into functional neurons, demonstrating the potential of miRNAs for somatic cell reprogramming. Ectopic expression of miR-9/9* and miR-124 directly converted human fibroblasts into a heterogeneous population of excitatory and inhibitory neurons. However, the capability of this miRNA-based reprogramming approach to derive a discrete neuronal subpopulation remains unexplored. We now show that microRNA-mediated neuronal reprogramming can be refined by the addition of lineage-specific transcription factors to guide neuronal conversion towards specific subtypes. In this study, we report the direct conversion of human neonatal and adult fibroblasts into a nearly pure population of striatal medium spiny neurons (MSNs) by co-expression of miRNAs and striatum-enriched transcription factors. MSNs are a class of inhibitory projection neurons which comprise 90-95% of the striatum, an area of the brain involved in voluntary motor movements. Importantly, MSNs are the primary cell type affected in Huntington's disease (HD) and therefore are clinically relevant for transplantation studies and in vitro modeling of HD. In our study, we demonstrate by immunocytochemistry and single-cell gene expression profiling that our reprogrammed cells express key markers associated with MSNs but not of striatal interneurons or other neuronal subpopulations. In addition, we show that reprogrammed MSNs share similar gene expression profile to single cells microdissected from postmortem human striatum sections. We further characterized the reprogrammed MSNs by electrophysiological analysis and show that these cells are functional and synaptically competent. Furthermore, transplantation studies into the murine striatum demonstrate that the reprogrammed MSNs are able to survive and engraft into the local striatal circuitry in vivo. Our findings demonstrate a novel approach for converting human fibroblasts directly into striatal medium spiny neurons and the potential of miRNAs to act synergistically with terminal fate determinants to guide reprogramming into specific neuronal subtypes. We believe that neuronal subtype-specific reprogramming may prove to be a useful approach for modeling inherited neurological diseases affecting specific neuronal subtypes and brain regions.

W-2153
FUNCTIONAL SIGNIFICANCE OF PROTEIN SIALYLATION IN THE REGULATION OF PLURIPOTENCY

Wang, Yu-Chieh, Stein, Jason, Lynch, Candace, Tran, Ha T., Peterson, Suzanne, Loring, Jeanne F.
The Scripps Research Institute, La Jolla, CA, USA

Human pluripotent stem cells (hPSCs), including human induced pluripotent stem cells (hiPSCs) and embryonic stem cells (hESCs), hold great potential for expanding the frontiers of regenerative medicine. Recent studies show that post-translational modification of glycoproteins may be a significant factor involved in the regulation of pluripotency. Further understanding of how these mechanisms operate in hESCs and hiPSCs may facilitate the use of hPSCs for

research and clinical applications. Our group previously observed that genes encoding sialyltransferases that catalyze the terminal addition of sialic acid to β-galactosides on glycoproteins were preferentially expressed in undifferentiated hPSCs compared to non-pluripotent cells. We therefore investigated the potential function of sialyltransferases in the regulation of cellular pluripotency. We have confirmed that undifferentiated hESCs and hiPSCs had enhanced expression of sialyltransferases, compared to their differentiated derivatives or parental somatic cells used for reprogramming. In addition, lectins which preferentially bind to sialylated galactosides showed stronger binding reactivity with glycoproteins extracted from hESCs and hiPSCs in the pluripotent state, suggesting that a group of glycoproteins was specifically sialylated in undifferentiated hPSCs due to the high expression of sialyltransferases. To test the functional significance of sialyltransferases in the maintenance and establishment of cellular pluripotency, we used shRNA-mediated gene knockdown to downregulate the expression of sialyltransferases in undifferentiated hPSCs and somatic cells undergoing cellular reprogramming. We found that the downregulation of a specific sialyltransferase led to a decrease in POU5F1/OCT4 protein in hESCs and hiPSCs. In addition, the efficiency of retrovirus-mediated reprogramming of human dermal fibroblasts with POU5F1/OCT4, SOX2, KLF4 and MYC was also reduced when this sialyltransferase was knocked down as determined by the decrease of alkaline phosphatase and NANOG expression in the reprogrammed cells. Global gene expression analysis revealed that the expression of a panel of genes known for their roles in cell cycle and embryogenesis modulation differed in hPSCs with the down-regulated expression of this sialyltransferase, further attesting to the involvement of sialylation in the regulatory circuitry of cellular pluripotency and differentiation. In summary, our data indicate that sialyltransferases and their enzymatic products are highly expressed in undifferentiated hPSCs, and suggest that the protein glycosylation conducted by certain sialyltransferases may be critically involved in the regulation of pluripotency in human cells.

W-2154
DIRECT CONVERSION OF HUMAN GASTRIC EPITHELIAL CELLS INTO INDUCED ENDODERMAL MULTIPOTENT STEM CELLS BY SMALL MOLECULE COMPOUNDS

Wang, Yunfang, Qin, Jinhua, Wang, Shuyong, Zhang, Wencheng, Yan, Fang, Chang, Mingyang, Pei, Xuetao
Stem Cell and Regenerative Medicine Lab, Beijing Institute of Transfusion Medicine, Beijing, China

Internal organ-associated diseases such as end stage liver diseases and diabetes afflict millions of people each year. Cell-based therapy, emerging as a revolutionary strategy for the treatment of those lethal diseases has drawn great interest of clinicians all over the world. However, the severe shortage of cell source has hampered the widespread application of this strategy. Human pluripotent stem cells supply a new source for cell-based therapies due to their unlimited proliferation and potential to differentiate into almost all cell types. However, the pluripotent stem cells derivatives are tumorigenic. Recent studies have demonstrated that transcriptional factors can not only reprogram mature somatic cells to pluripotent stem cells, but also convert one kind of somatic cell to another differentiated or stem cell types. Although these strategy share advantages of iPSCs such as patient-specific and have some superiority like less tumorigenity and more purity, those methods are tedious and the integration of virus constructs into resulting cells raises safety concerns. Small molecules have some distinct advantages such as, permeable, controllable, non-immunogenic, non-integrative, cost-effective, and tunable, etc. However, no study so far has directly converted cell type of one lineage into another only by small molecules in all three germ layers. In this study, we generated endodermal multipotent stem cells (iEMSCs) from human gastric epithelial progenitor cells, derived from gastric biopsy or gastrectomy, by defined small molecule(s) under the supporting of stomach stromal cells in vitro. iEMSCs expressed endodermal transcription factors (e.g., FOXA2, Sox9, PDX1, HES1, NGN3, PROX1) and stem/progenitor surface markers (EpCAM, CD133, CXCR4). They clonally expanded on plastic remaining morphologically, phenotypically stable as undifferentiated cells for months with doubling time at around 36 hours. They could give rise to hepatocytes, pancreatic endocrine cells and intestinal epithelial cells under certain induction conditions. The hepatocytes derived from iEMSCs lost stem cell markers and gained markers of mature, functional parenchymal cells, such as albumin, transferrin, CK8 and 18. The cells could be infected by HCV, had the metabolism activities of CYP450s and secreted albumin and urea. Transplantation into the livers of Fah -/- immunocompromised mice resulted in functional human hepatocytes and cholangiocytes integration. Moreover, we identified that wnt/β-catenin and TGFβ signaling pathways were involved in the regulation of the reprogramming process. The non-tumorigenicity, expansion and differentiation potential and availability from all age donors suggest that iEMSCs have considerable potential for regenerative therapies of liver, intestinal and pancreatic diseases including liver failure and diabetes.

W-2155
A LYMPHOCYTE BASED CELL-TO-CELL THERAPEUTIC DELIVERY SYSTEM

Woodsworth, Daniel, Sharma, Govinda, Holt, Robert
University of British Columbia, Vancouver, BC, Canada

With their ability to sense and integrate a wide range of signals, home to specific tissue compartments, and actuate context-dependent responses, engineered cell-based systems are promising next-generation therapeutics, particularly for cancer. Cytotoxic lymphocytes (CLs) are an ideal chassis for developing cell-based therapeutic systems for two reasons: (i) CLs possess a unique cell-to-cell molecular transfer system in the granzyme-perforin pathway, minimizing off-target effects; and (ii) T-cell receptors (TCRs), or the related chimeric antigen receptors (CARs), can endow CLs with an exquisite level of specificity in targeting a cell population defined by its antigen profile. One of the main mechanisms by which CLs kill target cells is via secretion of granzyme B (GzB) and perforin, resulting in GzB induced target cell apoptosis. Importantly, malignant cells are often apoptosis resistant. To address this issue, we are engineering CLs to transfer a GzB-toxin fusion protein to targeted cells, where the granzyme motif of the fusion protein acts as a chaperone to ensure appropriate CL intracellular packaging, and then trafficking and delivery of the toxin payload to the target cell. We have designed and constructed a modular mammalian expression vector that codes for a GzB-payload fusion protein, where the gene encoding the payload may be cloned into a dedicated site at the C-terminus of granzyme B. We have constructed plasmids with payloads of Diphtheria toxin A (DTA), Pseudomonas exotoxin A (PE), and Saporin (SAP), as well as beta-lactamase (BL) as a negative control and the enhanced cytotoxicity of these fusions has been functionally verified in Hela cells. To test GzB fusion protein trafficking we constructed a plasmid encoding a GzB beta-lactamase (BL) fusion protein, and functionally verified BL activity in K562 cells. This plasmid was then transfected into the natural killer cell line NK-92MI, which were then co-cultured with target K562 cells. GzB mediated transfer of beta lactamase activity to K562 cells was assessed by staining this co-culture with a FRET based beta-lactamase substrate and then flow cytometry analysis. Our experiments to date have been hampered by

low transfection efficiency and we are in the process of selecting stable, BL-expressing NK92-MI cell lines to continue these experiments. These proof of principle studies will pave the way for similar experiments using the GzB-toxin fusion proteins, where we will aim to show that NK92 cells expressing GzB-toxin fusions are capable of killing K562 cells that have been rendered apoptosis resistant by overexpression of the inhibitor of apoptosis protein XIAP, a common finding in various tumour types.

IPS CELLS: DIRECTED DIFFERENTIATION

W-2157
LEVERAGING THE INNATE IMMUNITY PATHWAY FOR TRANSDIFFERENTIATION OF FIBROBLASTS TO ENDOTHELIAL CELLS

Sayed, Nazish[1], Cooke, John P.[2]
[1]Cardiovascular Sciences, Houston Methodist Research Institute, Houston, TX, USA, [2]Center for Cardiovascular Regeneration, Houston Methodist Research Institute, Houston, TX, USA

Cell fate is fluid, and may be altered experimentally by the forced expression of master regulators mediating cell lineage. Such nuclear reprogramming has been achieved using viral vectors encoding transcription factors. We recently discovered that the viral vectors are more than passive vehicles for transcription factors, as they participate actively in the process of nuclear reprogramming to pluripotency (Lee and Sayed et al, Cell 2012). Viral vectors, by activating innate immunity, cause global changes in the expression of epigenetic modifiers, favoring an open chromatin state1. Based on the recognition that activation of innate immunity increases epigenetic plasticity, we hypothesized that small molecule activators of toll-like receptor 3 (TLR3), together with external microenvironmental cues that drive EC specification, might be sufficient to induce transdifferentiation of fibroblasts into ECs (iECs). Here we show that TLR3 agonist Poly I:C, combined with exogenous EC growth factors, transdifferentiated human fibroblasts into ECs (in the absence of viral vectors or transcription factors). These iECs were comparable to HMVEC in immunohistochemical, genetic and functional assays, including the ability to form capillary-like structures and to incorporate acetylated-LDL. Furthermore, iECs significantly improved limb perfusion and neovascularization in the murine ischemic hindlimb compared to parental fibroblasts. Finally, using genetic knockdown studies, we find that the effective transdifferentiation of human fibroblasts to endothelial cells requires innate immune activation. This study suggests that manipulation of innate immune signaling may be generally used to modify cell fate. Our observations raise the question as to whether epigenetic plasticity and cell fate fluidity generally participate in the immune defense against pathogens. Because similar signaling pathways are activated by damage associated molecular patterns, epigenetic plasticity induced by innate immunity may play a fundamental role in transdifferentiation during wound healing and regeneration. Finally, this study is a first step toward development of a small molecule strategy for therapeutic transdifferentiation in vivo.

W-2158
NUCLEAR RECEPTOR GENE EXPRESSION ALTERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS DURING HEPATIC DIFFERENTIATION

Shiota, Goshi
Tottori University, Yonago, Japan

Aim: Human induced pluripotent stem cells (hiPSCs) are expected to be an effective cell source of regenerative medicine for liver disease. Recently, however, hepatic differentiation propensity of hiPSCs has been reported. Therefore, it is important to know the characteristics of hiPSCs with hepatic differentiation propensity that would enable selection of hiPS cell clones generated from individual patients for regenerative medicine. In this study, we screened 28 hiPS cell lines using 3 step differentiation method to identify the hiPS cells with hepatic differentiation propensity. Since nuclear receptors (NRs) regulate essential biological processes including differentiation, development, and liver metabolism, we conducted nuclear receptor gene expression analysis of hiPSC lines during hepatic differentiation. Methods: Differentiation toward definitive endoderm of 28 hiPSC lines was induced by activin A for 5 days and differentiation toward hepatoblasts was induced by FGF-4 and BMP-2 for 5 days. Differentiation toward hepatocytes was induced by HGF for 5 days, followed by OSM and DEX for another 5 days. Hepatic differentiation levels were assessed by real-time PCR analysis of specific marker. Finally, we confirmed 48 nuclear receptor gene expressions during hepatic differentiation by real-time PCR analysis. Results: Consistent to the previous report, all lines could not differentiate uniformly into endoderm lineage. Differentiation propensity toward endoderm was affected by donor of origins but not by reprogramming methods or cell type of origins. Among 28 hiPSC lines, HiPS-RIKEN-2B and HiPS-RIKEN-2F cells showed hepatic differentiation propensity. NRs were categorized as five patterns by expression profiles during hepatic differentiation. Seven NRs such as COUP-TF1, RARα, PPARγ, PR, PNR, TLX and GR were identified as the genes of which expression gradually goes up with differentiation. Seven NRs were identified the patterns of which expression goes up from STEP 1 to STEP 3, while of which expression goes down day 0 to STEP 1. Five NRs of which expression goes down from STEP 1 to STEP 3, while of which expression goes up from day 0 to STEP 1 were identified. Finally, eleven NRs of which expression was transiently enhanced at STEP 2, and the other 18 NRs have no particular patterns of expression from day 0 to STEP 3. Conclusion: HiPS-RIKEN-2F exhibited the most potent hepatocyte differentiation propensity among 28 hiPSC lines. Seven NRs of which expression gradually goes up with hepatic differentiation were higher in HiPS-RIKEN-2F cells than hepatic differentiation resistant cells. These data provide us useful information on molecular mechanism of hepatic differentiation propensity of hiPS cells.

W-2159
AN ACCESSIBLE RESOURCE FOR CGMP GENERATION OF HUMAN IPS CELLS AND THEIR DERIVATIVES FOR CELL THERAPY APPLICATIONS

Ni, Yuhui, Lu, Xiaowei, David, Michelle, **Singer, Matthew**, Chuang, Joseph, Wang, Jiwu
Allele Biotechnology and Pharmaceuticals, Inc., San Diego, CA, USA

The ability to reprogram human fibroblasts of different genetic backgrounds to induced pluripotent stem (iPS) cells holds great promise for the development of autologous cell therapies. Recently, we have optimized a key technology for the generation of clinically-suitable human iPS cells that utilizes delivery of transcription factors via synthetic mRNA; this technology avoids the concerns of other

reprogramming methods with regards to clinical suitability, such as random genomic integration by DNA-based (e.g., episomal) technologies, or the physiological cell stress of whole-virus infection (i.e., Sendai). Using these mRNA-iPSCs, we have improved neural differentiation by fine-tuning relevant signaling pathways with small molecules and synthetic mRNA. We have achieved highly efficient, very rapid and nearly synchronous differentiation of mRNA-iPSCs to neural progenitor cells in adherent culture, and we are now focused on creating specific subtypes of neurons with similar efficiency. Our long term goal is to create neurons of specific subtypes that can make functional synapses, preferably in a 3D culture system. However, one remaining roadblock to the translation of any iPS cell or its derivatives to clinical applications is the lack of established protocols and facilities for their production according to current Good Manufacturing Practice (cGMP). Here we also describe such a resource: we have established a 13,000 sq. ft. facility in San Diego, California (USA) that will be GMP-certified for the generation of human iPS cells and their derivatives using our synthetic mRNA platform. This facility will be available for both academic and industry research and development projects, and will provide a validated GMP environment for users on a one-off basis. Our goal for this facility is to help drive efforts that will result in novel cell therapies for unmet human needs in the health industry.

W-2160
A NEW INDUCTION METHOD FOR THE CONTROLLED DIFFERENTIATION OF HUMAN IPS CELLS USING FROZEN SECTIONS

Tadokoro, Susumu[1], Tokuyama, Reiko[1], Tatehara, Seiko[1], Ide, Shinji[1], Umeki, Hirochika[1], Fukushima, Tatsuhiro[1], Miyoshi, Keiko[2], Noma, Takafumi[2], Satomura, Kazuhito[1]

[1]Department of Oral Medicine and Stomatology, School of Dental Medicine Tsurumi University, Yokohama, Japan, [2]Tokushima University, Tokushima, Japan

The establishment of induced pluripotent stem cells (iPSCs) has had a profound impact on both basic biology and clinical medicine. Currently, human iPSCs have been expected as a promising cell source for cell-based regenerative therapy. However, iPSCs themselves also have some problems that need to be overcome. One of these issues is the establishment of a reliable and efficient strategy for inducing the differentiation of iPSCs under complete control. Currently available methods to induce iPSCs differentiation depend on providing appropriate environmental factors such as culture media, substrates, growth factors and differentiation factors. To select and determine the appropriate factors necessary or essential for the differentiation of iPSCs is very difficult, confusing, and time and money consuming. Another issue to be solved is to establish a simple method for assessment of the integrity/quality of iPSCs. It is well known that the potency of iPSCs differs among clones of iPSCs. In addition, some iPSCs form tumors when transplanted *in vivo*. From these facts, a simple, effective and efficient method has been expected for the evaluation of the quality of iPSCs toward the realization of safe and reliable regenerative medicine. In this study, we hypothesized that it is possible to induce the controlled differentiation of iPSCs by using frozen sections of tissues/organs which are targets for regeneration. First, we cultured iPSCs generated from oral mucosal cells on frozen sections of liver, brain and spinal cord for 9 days. iPSCs were also cultured on cover glass as a control. As a result, iPSCs cultured on frozen section of liver showed relatively large and polygonal morphology like hepatocytes. In contrast, iPSCs cultured on frozen section of brain/spinal cord showed dendritic morphologies like neuronal cells. Immunocyochemistry reveled that iPSCs on liver sections dominantly expressed hepatocytic markers (α-fetoprotein and α1-antitrypsin) and that iPSCs on brain/spinal cord sections dominantly expressed neural markers (glial fibrillary acidic protein and cyclic nucleotide phosphodiesterase). After some fields were arbitrarily selected under microscope, the number of AFP/AAT-positive or GFAP/CNPase-positive iPSCs was counted. The percentage of AFP/AAT-positive cells in the whole iPSCs was statistically higher on the liver sections compared with brain/spinal cord sections or cover glasses. In contrast to this, the percentage of GFAP/CNPase-positive cells in the whole iPSCs was statistically higher on the brain/spinal cord sections compared with liver sections or cover glasses. Importantly, some other iPSCs generated from other type of cells, i.e. human dental pulp cells and skin fibroblasts, also denoted the same tendency when they were cultured on frozen sections of liver, brain or spinal cord. These facts strongly suggested that iPSCs could differentiate into a specific cell lineage in response to certain factors containing in frozen sections of tissues/organs. More interestingly, the efficacy of induced differentiation of iPSCs on the frozen sections was noted to be significantly different among clones of iPSCs. Judging from these facts, the induction method for the controlled differentiation of human iPS cells using frozen sections reported in the present study could be useful as a simple and effective measures for inducing the differentiation of iPSCs and evaluating the quality of iPSCs.

W-2161
BOVINE TROPHECTODERM CELL LINES INDUCED FROM BOVINE FIBROBLASTS WITH REPROGRAMMING FACTORS

Talbot, Neil C.[1], Sparks, Wendy O.[1], Ealy, Alan D.[2], Powell, Anne M.[1], Caperna, Thomas J.[1], Blomberg, Le Ann[1], Garrett, Wesley M.[1], Donovan, David[1]

[1]Animal Bioscience and Biotechnology Laboratory, Beltsville Agricultural Research Center, Beltsville, MD, USA, [2]Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

Bovine trophectoderm (TE) cells were induced [induced bovine trophectoderm-like (iBT)] from bovine fetal liver-derived fibroblasts, and other bovine fetal fibroblasts, after viral-vector transduction with either 6 or 8 reprogramming factors (RF), including POU5F1, KLF4, SOX2, MYC, NANOG, LIN28, SV40 large T antigen, or hTERT. Light and electron microscopic analysis showed the induced epithelial cells had morphology typical of bovine TE cells. The cells formed epithelial monolayers with interspersed domes, often had lipid droplets, and displayed cell polarity with apical microvilli and lateral cell unions of desmosomes and tight junction-like elements. Semi-quantitative RT-PCR and antiviral activity assay showed that all of the independently colony-cloned cell lines expressed interferon-tau (IFN-τ) at passage 1 or 2. Subsequent measurement at later passage levels (≥ passage 8) of IFN-τ expression, including by immunoblot assay, showed that more than half of the iBT cells lines had stop expressing IFN-τ. Messenger RNAs specific to TE differentiation and function were found in the iBT cell lines, and 2D-gel analysis of cellular proteins showed an expression pattern similar to that of TE cell lines derived from bovine blastocysts. Integration of the virally transduced human RFs, POU5F1, KLF4, SOX2, and NANOG were detected by PCR in the iBT cell lines and their expression was variable, except hMYC was expressed in all the cell lines. Assay of endogenous bovine RF expression showed POU5F1 and MYC were expressed in all iBT cell lines, SOX2 in none, and KLF4 and NANOG in half of the cell lines. The results demonstrate that bovine TE can be induced via RF expression from bovine liver-derived fibroblasts, although other fibroblast populations, e.g., derived from fetal muscle, could also result in TE, but at lower frequencies.

W-2162
COMPARABLE GENERATION OF ACTIVIN-INDUCED DEFINITIVE ENDODERM VIA ADDITIVE WNT OR BMP SIGNALING IN ABSENCE OF SERUM

Teo, Adrian, Valdez, Ivan, Dirice, Ercument, Kulkarni, Rohit
Joslin Diabetes Center, Boston, MA, USA

There is considerable interest in the differentiation of human pluripotent stem cells (hPSCs) into definitive endoderm (DE) and, subsequently pancreatic cells for in vitro disease modeling and potential cell replacement therapy. Several current protocols use fetal bovine serum (FBS) which contains poorly-defined factors to induce DE formation. Here, we compared Wnt and BMP in their ability to co-operate with Activin signaling to promote DE formation in a chemically defined medium. Varying concentrations of Wnt3a, glycogen synthase kinase (GSK)-3 inhibitors CHIR99021 and 6-bromoindirubin-3'-oxime (BIO), and BMP4 could independently co-operate with Activin to effectively induce DE formation even in absence of serum. However, Wnt3a ligand was ineffective in suppressing E-CADHERIN/CDH1 and pluripotency factor gene expression unlike GSK-3 inhibitors or BMP4. Our findings indicate that both Wnt and BMP effectively synergize with Activin signaling to generate DE from hPSCs, though Wnt3a requires additional factors to effectively suppress the pluripotency program inherent in hPSCs. Overall, the various DE-inducing growth factor combinations that we have optimized demonstrate the ability to derive DE in chemically defined conditions without the need for serum.

W-2163
TARGETING THE HUMAN DYSTROPHIN GENE WITH TALENS AND CRISPR

Tremblay, Jacques P., Chapdelaine, Pierre, Rousseau, Joël
Universite Laval, Quebec, QC, Canada

The long-term aim of our research group is to develop an autologous cell therapy for Duchenne Muscular Dystrophy (DMD). Since the satellite cells of DMD patients are close to senescence, we eventually aim at transplanting myoblasts obtained by the differentiation of iPSCs derived from the patient own fibroblasts. We have already established a protocol to induce the differentiation of hiPSCs in myoblasts and these cells were successfully transplanted in the muscles of immunodeficient mice. However, the DMD iPSCs or the cells derived from them have to be genetically corrected. We are thus investigating two technologies (the TALENs and the CRISPR system) to correct the dystrophin gene. We have already produced 8 TALENs targeting exon 54 of the human dystrophin gene and we have identified the best pair able to induce Double Strand Breaks (DSB) in this exon in 293T cells. These DSB were repaired by Non Homologous End Joining (NHEJ) resulting in micro-insertion or micro-deletions (INDELs). The presence of these INDELs were detected by PCR amplification of the exon 54, heating and slow cooling. The presence of DNA mutations led to the formation of miss-paired DNA strands. These miss-pairing were cut with the Surveyor enzyme, leading to the presence of 2 additional bands in an agarose gel. However, when nucleofected in the myoblasts of a DMD patient, these TALENs did not produced INDELs that were detectable with the Surveyor enzyme. We have also screened 5 gRNA targeting the exon 54 of dystrophin. Several of these gRNA were co-transfected with an active Cas9 gene in 293T cells. The complex formed by the gRNA, the Cas9 and the DNA led to a DSB of the dystrophin gene repaired by NHEJ resulting in INDELs, detected with the Surveyor enzyme. Moreover, we have also transfected the gRNA and the Cas9 genes in the 293T cells containing a surrogate plasmid expressing the mCherry gene and containing an out of frame EGFP gene due to presence the sequence of exon 54 targeted by the gRNA. The transfection of a plasmid containing the gRNA and Cas9 led to a DSB of that plasmid and the restoration of the reading frame of the EGFP gene following repair by NHEJ. These gRNA/Cas9 was also induced DSB in the dystrophin exon 54 in myoblasts of a DMD patient. These DSB were repaired by NHEJ resulting in INDELs, which were detected with the Surveyor enzyme. Thus the recent results obtained with the CRISPR system are very encouraging. We are currently trying to transplant the genetically corrected myoblasts in muscles of an immunodeficient mice.

W-2164
DIRECTED FATE CONVERSION TO HUMAN GABAERGIC NEURONS USING TRANSCRIPTIONAL DRIVERS

Vadodaria, Krishna[1], Mertens, Jerome[2], Jappelli, Roberto[1], Lianna, Fung[1], Soltani, Sheila[1], Wright, Rebecca[1], Gage, Fred H.[1]
[1]Salk Institute for Biological Studies, La Jolla, CA, USA, [2]The Salk Institute for Biological Studies, La Jolla, CA, USA

GABAergic interneurons in the forebrain play important roles in regulating network activity and their dysfunction has been implicated in neuropsychiatric disorders such as autism, schizophrenia, and epilepsy. Patient-derived neuronal cultures based on induced pluripotent stem (iPS) cells and induced neurons (iN) offer distinct ways for investigating the pathophysiology of neuropsychiatric disorders in vitro. Using genetic overexpression of a MGE-derived transcriptional driver, we enhanced GABAergic differentiation using both iPS-derived neurons, and induced neurons directly converted from human fibroblasts. This strategy provides unique ways of enriching disease-relevant neuronal subtypes such as GABAergic interneurons in vitro, thus enabling the examination of neuronal subtype-specific defects that may not be found in mixed neuronal cultures.

W-2165
FOUR FACTORS CONTROL SELF-RENEWAL OF CORTICALLY SPECIFIED HUMAN MULTIPOTENT NEURAL CELLS

Varga, Balazs V., Faiz, Maryam, Nagy, Andras
Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

The regulation of neural stem cell self-renewal is poorly understood; however it has consequences in normal and pathological brain. Multipotent neural stem cells have been shown to require FGF and EGF signals for in vitro self-renewal. Nonetheless prolonged maintenance of cortically specified cells in the presence of FGF and EGF cannot preserve important in vivo properties - like differentiation potential to glutamatergic projection neurons - of the cerebral cortex . FGFR1, R2, and R3 triple knockout mouse cortices are specified correctly, but fail to proliferate normally. Furthermore, FGFR signaling alone is not sufficient to maintain cortical neural stem cells in vitro. On the contrary neural tissue-specific ablation of the mayor EGF receptor ErbB1 does not compromise normal brain development. These observations directed us to investigate potential role of other key signaling pathways in cortical human neuroectodermal stem cell (hNECs) self-renewal. We show the requirement for FGFR activity together with GSK3, BMPR and TGFbR inhibition (4F condition) to be sufficient to maintain cortical potential of rosette stage multipotent neural cells. In the presence of 4F, hNECs can respond to EGF, SHH and LIF, however, these signals are dispensable for self-renewal. Compared to rosette stage cells or later stage neural stem cells maintained in vitro in the presence FGF and EGF signals, hNECS preserve cortical specification and multipotency over long-term proliferation in 4F condition. Upon withdrawal of 4F, cells differentiate into mainly glutamatergic

projection neurons and to smaller degree GABAergic interneurons, astrocytes and oligodendrocyte progenitors. hNECs can integrate into the normal adult mouse brain, survive for > 90 days and differentiate in situ into neurons and glia cells.

W-2166
LINKING OXIDATIVE STRESS TO CELL FATE- IPSC-BASED DISEASE MODELING IDENTIFIES NEW THERAPEUTIC TARGET IN RETICULAR DYSGENESIS

Weinacht, Katja[1], la Marca, Giancarlo[2], Felgentreff, Kerstin[3], DeVine, Alexander[4], Schambach, Axel[5], Notarangelo, Luigi[6]
[1]Pediatric Hematology/Oncology, Boston Children's Hospital Boston, Boston, MA, USA, [2]Department of Clinical Chemistry and Pharmacology, Meyer Children's Hospital, Florence, Italy, [3]Childrens Hospital Boston, Boston, MA, USA, [4]Boston Children's Hospital, Boston, MA, USA, [5]Hannover Medical School, Hannover, Germany, [6]Division of Immunology, Children's Hospital Boston, Boston, MA, USA

Reticular Dysgenesis (RD) is one of the most serious forms of severe combined immune deficiency (SCID). It is characterized by complete absence of circulating lymphocytes and neutrophils. In addition, patients suffer from sensorineural hearing loss. Before newborn screening for SCID was implemented, the majority of patients succumbed to infection long before hematopoietic cell transplantation (HCT) could be attempted. To this date, the prognosis for RD remains grim. RD is caused by mutations in the mitochondrial ADP-generator Adenylate Kinase 2 (AK2). AK1 is a cytosolic protein that may compensate in various tissues for the lack of AK2. However, AK1 is not expressed in leukocytes and the stria vascularis of the inner ear [1]. While this observation may explain where AK2 defects manifest, the molecular mechanisms how AK2 defects take effect, remain largely obscure. Significant obstacles to elucidating disease pathology have been the lack of a suitable animal models and the unavailability of patient specimens. Using skin fibroblasts from a RD-patient we have recently identified at Boston Children's Hospital [2], we have generated induced pluripotent stem cells (iPSC) with defined homozygous AK2-mutation. In-vitro myeloid differentiation of AK2-mutated iPSCs recapitulates the characteristic maturation arrest at the promyelocyte stage observed in-vivo in patients with this condition. AK2 is expressed in the intermitochondrial space where it mediates the reaction AMP + ATP α 2 ADP. Generation and maintenance of adequate levels of ADP in the intermitochondrial space are required to support ATP synthase activity. Using Mass Spectrometry, we have shown that in the absence of AK2, the AMP/ADP ratio is markedly increased in iPS-derived myeloid cells, indicating a critical role of AK2 in maintaining ADP supply. Based on this data, we hypothesized that in patients with RD, ADP-depletion in myeloid progenitors would lead to stage 4 respiration, a well defined state in mitochondrial biology, in which the ATP-synthase lacks substrate and decreases its activity. This causes a reduction in proton flux from the intermitochondrial space back into the matrix, transient rise in membrane potential, and an escalation in the formation of reactive oxygen species (ROS). The cell responds by activating "inducible uncoupling", the opening of alternative proton pores, which allows proton flux back into the matrix, bypassing the ATP-synthase and foregoing the use of energy stored in the proton gradient. While this represents a cellular rescue mechanism in response to acute oxidative stress, extended oxidative-stress-induced uncoupling will eventually lead to a decline in proton gradient and membrane potential and ultimately in demise of the cell. To test this hypothesis, we have added Glutathione, the primary endogenous cellular antioxidant, to the culture conditions. We also tested G-CSF and all-trans-retinoic acid (ATRA), agents known to promote promyelocyte differentiation to mature neutrophils in other conditions. While G-CSF had no, and ATRA clearly deleterious effects on myeloid maturation in AK2-mutated cells, Glutathione led to a significant improvement in differentiation, allowing development of mature neutrophils in-vitro. Our results suggest that cell fate in RD is linked to oxidative stress and identify antioxidants as a possible therapeutic approach that may help reduce early mortality due to severe infections in patients with RD.

W-2167
ORGAN REGENERATION OF FUNCTIONAL GUT (IGUT) FROM MOUSE INDUCED PLURIPOTENT STEM CELLS

Yamada, Takatsugu, Ueda, Takeshi, Nakamoto, Takayuki, Kanehiro, Hiromichi, Nakajima, Yoshiyuki
Department of Surgery, Nara Medical University, Kashihara, Japan

Induced pluripotent stem (iPS) cells have the pluripotency to differentiate into broad spectrum derivatives of all three embryonic germ layers. The potential for directed differentiation of iPS cells into specific cell types has become very exciting because of the new scientific insights and possibility of using such cells for therapeutic purposes. However, the organ differentiation ability of iPS cells to organize a complex and functional "organ," which is composed of a variety of cell types, has not yet been demonstrated. We demonstrate that mouse iPS cells have the ability to organize a complex gut-like organ with motor function in vitro by a three-dimensional hanging drop culture system (6 days) to form spherical multicellular aggregates, embryoid bodies (EBs), and a subsequent outgrowth culture system. This "induced gut (iGut)" showed spontaneous contraction (Day7), rhythmic contractions (Day14) and highly coordinated peristalsis accompanied by a transportation of contents (Day21). The iGut with peristalsis-like contractions exhibited the periodic movements of back and force in the closed lumen. Ultrastructural analysis identified that the iGut had large lumens surrounded by three distinct layers (epithelium, connective tissue and musculature). Immunoreactivity for c-Kit, a marker of interstitial cells of Cajal (ICCs, enteric pacemaker cells), was observed in the wall of the lumen and formed a distinct and dense network. The neurofilament immunoreactivity was identified to form large ganglion-like structures and dense neuronal networks. The iGut was composed of all the enteric components of three germ layers: epithelial cells with microvilli (endoderm), smooth muscles, ICCs (mesoderm), and enteric neurons (ectoderm). This is the first to show the in vitro differentiation potential of iPS cells into particular types of functional "organs." This work not only contributes to understanding the mechanisms of incurable gut disease through disease-specific iPS cells, but also facilitates the clinical application of patient-specific iPS cells for novel therapeutic strategies such as patient-specific "organ" regenerative medicine in the future.

W-2168
LONG-TERM EXPANSION OF PROLIFERATIVE ENDODERMAL PROGENITOR CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Zhang, Ranran, Takebe, Takanori, Zheng, Yunwen, Taniguchi, Hideki
School of Medicine, Yokohama City University, Yokohama, Japan

Background: Endoderm and its derivatives including islet cells and hepatocytes have been efficiently recapitulated by the differentiation of human induced pluripotent stem cells (iPSC), which could be hopefully used toward treatment of diseases such as diabetes or liver dysfunction, respectively. However, the generation and expansion of endodermal progenitor cells (EPC) with unlimited proliferative capability *in vitro* are still not yet well established. Selective induction of endoderm could be achieved under the activin A treatment in serum free conditions, and the effect of activin A in inducing

definitive endoderm is enhanced when additional Wnt3a is present, however the generated CXCR4+C-Kit+ EPC under additional Wnt3a treatment for over 3 days loss of self-renewal capability. To solve these issues, we use the optimized differentiation conditions to produce the CXCR4+C-Kit+ EPC with additional combination of other factors including BMP4, VEGF and FGF2. Methods and Results: A step-wise protocol was adapted to induce definitive endoderm cells. Human iPSC were plated onto matrigel-coated dishes before the initiation of differentiation. The cells were induced to differentiate by culturing in the RPMI/1640 supplemented with B27, Wnt3a and Activin A for one day; then the medium was switched to RPMI/1640 supplemented with B27 and a combination of Activin A, BMP4, bFGF and VEGF. Finally, the cells were differentiated in SFD supplemented with Activin A, BMP4, bFGF and VEGF. The CXCR4+C-Kit+ EPC isolated from induced definitive endoderm cells are confirmed to have the capability of *in vitro* expansion for up to passage 20 with a stable expression of definitive endoderm markers of FOXA2 and SOX17. Further differentiation of endoderm lineages with derived CXCR4+C-Kit+ EPC are achieved by producing C-peptide secreting pancreatic β-cells and albumin-secreting hepatocytes. Conclusion: We could successfully expand and maintain the derived EPC in vitro under our optimized differentiation protocol. And the isolated EPC cells showed the potential of further differentiating into endodermal lineages including pancreatic β cells and hepatocytes. Thus, targeted *in vitro* maintenance and differentiation of endodermal progenitor cells are great potential for cell replacement therapy, toxicology, and further insight into endodermal derived organ transplantation.

W-2169
DEFINED EXTRACELLULAR MATRIX PROTEINS FOR CARDIAC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS: ESSENTIAL ROLE OF FIBRONECTIN

Zhang, Jianhua, Tao, Ran, Wilson, Gisela F., Annis, Douglas S., Mosher, Deane F., Kamp, Timothy J.
Medicine, University of Wisconsin - Madison, Madison, WI, USA

Extracellular matrix (ECM) plays multiple important roles in development, and we have previously described a matrix sandwich protocol for efficient cardiac differentiation of human pluripotent stem cells (hPSCs) which combines sequential applications of Matrigel ECM and growth factors (Activin A, BMP-4, bFGF) (Zhang et al. Circ Res 2012). However, Matrigel is an incompletely defined and variable mixture of ECM proteins and other components. The purpose of the present study is to test defined ECM proteins in the matrix sandwich protocol to determine both the optimal and essential ECM proteins for promoting cardiogenesis. Human laminin (LN) (isoforms of LN111 and LN521), fibronectin (FN) and collagen IV were tested by applying each ECM proteins on the bottom and the overlay of the matrix sandwich culture. Cardiac differentiation was measured by flow cytometry of $cTnT^+$ cells present at day 15 (Figure). Regardless of bottom ECM, FN overlay resulted in robust cardiac differentiation. In contrast, LN111 or LN521 overlay blocked cardiac differentiation completely. Interestingly, both FN and LN111 bottom coating enabled efficient cardiogenesis without overlay. To determine if FN is essential for cardiogenesis in this differentiation protocol, we tested FN blocking antibodies. Blocking FN fibrillogenesis in the matrix sandwich protocol inhibited cardiac differentiation in a concentration-dependent manner as measured by flow cytometry of $cTnT^+$ cells. When hPSCs were plated on LN111 and differentiated without matrix overlay, FN blocking antibodies also inhibited cardiogenesis, suggesting endogenously produced FN is essential. Immunolabeling of hPSCs plated on LN111 for FN showed a time-dependent increase of FN ECM. FN blocking antibodies also inhibited cardiac differentiation in the small molecule GiWi cardiac differentiation protocol (Lian et al., PNAS, 2012). These results indicate that FN plays an essential role in cardiogenesis of hPSCs. Because the major integrin binding receptor for FN is α5β1, we tested the effect of a blocking antibody specific for β1 integrin (P5D2) in the matrix sandwich protocol. When added at day 0, P5D2 showed significant blocking of cardiac differentiation yielding no cardiomyocytes. Taken together, we conclude that FN plays essential role in cardiac differentiation of hPSCs, and is regulated by integrin β1 signaling. Electrophysiological characterization of the FN/FN derived CMs demonstrated APs with nodal, atrial and ventricular-like features. These results identify several combinations of defined ECM proteins that can support robust cardiogenesis in the matrix sandwich protocol and furthermore, point to an essential role of fibronectin in cardiac differentiation of hPSCs.

IPS CELLS

W-2171
CLINICALLY SAFE INTEGRATION- AND XENO-FREE HUMAN IPSCS GENERATED BY MRNA TRANSFECTION METHOD

Hwang, Dong-Youn
CHA University School of Medicine, Seongnam, Kosovo, Republic of

The generation of integration- and xeno-free iPSCs is absolutely required for cell replacement therapy to treat a variety of incurable diseases. Until now, repid progress has been made in developing the methods of generating chromosome integration-free iPSCs, such as episomal plasmid transfection and Sendai viral-mediated gene delivery. Recently, the mRNA transfection method has also emerged as an efficient way to establish footprint-free iPSCs. In an effort to generate clinically compliant iPSCs, we sought to combine the mRNA transfection method with a xeno-free culture condition for human pluripotent stem cells (hPSCs) which was established in our laboratory.Human dermal fibroblasts were transfected multiple times with mRNAs encoding reprogramming factors and were maintained in our xeno-free hPSC culture condition. The hESC-like colonies appeared approximately 20 days were isolated and expanded in the same xeno-free hPSC culture condition to establish iPSC lines. Extensive characterization of the iPSCs demonstrated that they expressed pluripotent cell markers, retained pluripotent capability, showed similar global gene expression pattern to hESCs, and displayed normal karyotypes.This study demonstrated that therapeutically applicable xeno- and integration-free iPSCs can be generated by mRNA transfection method in combination with the xeno-free hPSC culture condition we established and will provide an opportunity for cell replacement therapy in the foreseeable future. This work was supported by grants from the Stem Cell Research Program (2010-0020347) and 2012M3A9C7050130 from the MSIP, and A120254-1201-0000200 from the Ministry of Health and Welfare, Korea

W-2172
EFFICIENT GENERATION OF TRANSGENE-FREE AND XENO-FREE IPSC FROM HUMAN CORD BLOOD CELLS AND THEIR DIFFERENTIATION INTO FUNCTIONAL DERIVATIVES

Park, Tea Soon, Zimmerlin, Ludovic, Zambidis, Elias
Institute for Cell Engineering and Department of Pediatric Oncology, Johns Hopkins School of Medicine, Baltimore, MD, USA

IPSC technology provides new opportunities to attain personalized regenerative therapies. However, current low reprogramming

efficiencies, lack of GMP compliance, and highly variable and limited differentiation into fully functional long-term engraftable lineages are critical challenges for the development of safe translational strategies. Although several groups have described the generation of xeno-free (XF)-iPSC, the optimization of these systems to clinical scale and the derivation of functionally-competent lineage committed cells have yet to be validated. We originally reported efficient bulk reprogramming of human myeloid progenitors (MP) to hiPSCs using a non-integrating episomal method. It was also more recently reported by others that murine MPs represent a permissive and "privileged" somatic donor cell capable of rapid, efficient, and nonstochastic reprogramming. We have validated the enhanced functional performance of human MP-iPSC-derived vascular progenitors (VP) using our established hematovascular differentiation system. Unlike standard hiPSC lines, MP-iPSC-derived VPs exhibited an immature phenotype with diminished somatic memory retention, senescence and DNA damage sensitivity, and demonstrated reliable long term engraftment into an ischemia-reperfusion mouse retina model. We have recently discovered that MP-iPSCs lack lineage skewing to non-hemato-vascular lineages with superior endodermal and ectodermal differentiation potencies. Here, we present the adaptation of this novel stromal priming MP-iPSC reprogramming system using defined XF and feeder-free (FF) reagents. Since, human cord blood (CB) cells were more amenable to reprogramming than adult blood progenitors, we initiated our validation of XF conditions using this donor source. Human CD34+ CB cells were thawed in RPMI supplemented with XF KnockOut Serum Replacement and expanded and differentiated into CD33+CD45+ myeloid progenitors (MP) for 3 days in XF StemSpan hematopoietic expansion medium with human recombinant protein of FLT3 ligand, TPO, kit-ligand (FTK). Following transfection using a single episomal vector expressing the four Yamanaka factors (SOX2, OCT4, KLF4, c-MYC), CB cells were co-cultured on irradiated XF human mesenchymal stromal cells. Reprogrammed MPs were initially transferred onto Synthemax-R plates in FTK-supplemented StemSpan-XF, and further cultured in Essential 8 medium. Distinct hESC-like colonies emerged 12 days following episomal plasmid nucleofection. Single XF-MP-iPSC clones were manually transferred on human vitronectin-coated plates in E8 medium. All established cell lines were confirmed to express pluripotency-associated markers by flow cytometry and Q-RT-PCR with robust differentiation into all 3 germ layers in NOD/SCID teratoma assay, and possessed normal karyotypes. Additionally, XF-MP-iPSC lines efficiently differentiated in vitro into hematovascular, neural and definitive endodermal lineages at levels that were comparable or superior to hESC and conventional CB-iPSC for all 3 lineages. We propose that the intrinsic properties of human MPs (e.g., rapid and efficient reprogramming, high quality and robust differentiation capacities) make them an ideal donor cell type for adaptation to GMP-compliant techniques. Thus, MPs represent an attractive source of permissive, easily acquired patient donor cells for further development of clinically relevant GMP-compliant regenerative approaches.

W-2173

MATISSE™ EPISOMAL REPROGRAMMING SYSTEM WITH INDIVIDUAL IDENTICAL EXPRESSION CONTROL ELEMENTS PACKAGED INTO A SINGLE CASSETTE AND REPROGRAMMING OF HUMAN SOMATIC CELLS

Paul, Sharan[1], Alapatt, Philomena[1], Dansithong, Warunee[2], Scoles, Daniel[2], Sams, Gary[1], Pulst, Stefan M.[2]

[1]Progenitor Life Sciences, Salt Lake City, UT, USA, [2]Department of Neurology, Clinical Neurosciences Center, University of Utah, Salt Lake City, UT, USA

Induced pluripotent stem cells (iPSCs) are invaluable tools for translation research. Despite the existence of multiple reprogramming methods most strategies have certain drawbacks. Progenitor Life Science's Matisse™ Reprogramming Technology overcomes impediments of traditional iPSC methods by developing all-in-one novel episomal reprogramming vector (RV). In this system reprogramming factors (RFs) (OCT3/4, SOX2, c-MYC, and KLF4) are arranged with individual attenuated CMV promoter (267bp) and polyA sequences into a single cassette, designated as pPuro(CMV_{mt1})-cMKSO (Matisse reprogramming system). This RV allows coordinated expression of all the four factors in transfected human somatic cells, which bypasses multistep screening procedures required to ensure homogeneous cultures. The use of the Matisse™ Reprogramming System also improves efficiency and time required for reprogramming. We used this RV to reprogram lymphoblastoid (LB) cells (EBV-immortalized B-lymphocytes) and human skin fibroblasts by electroporation or transfection methods without the use of feeder cells. Especially LB cells are hard to reprogram using commercial reprogramming approaches currently available. EBV-B cell line and BJ fibroblasts -derived iPSCs showed ES cell morphology, EBV-B cell line and BJ fibroblasts -derived iPSCs showed ES cell morphology, and expressed pluripotent cell-specific genes. The ability to reprogram banked patient EBV-transformed cell lines or skin fibroblasts efficiently offers an unprecedented opportunity to generate faithful genetic disease models and also provide a translational platform for therapeutic drug development.

W-2174

EFFICIENT GENERATION OF INDUCED PLURIPOTENT STEM CELLS (IPSCS) DERIVED FROM PARKINSON'S DISEASE (PD) STUDY PATIENT FIBROBLAST LINES USING CYTOTUNE®-IPS 2.0 SENDAI REPROGRAMMING KIT IN THE ESSENTIAL 8® FEEDER-FREE MEDIA SYSTEM

Piekarczyk, Marian S.[1], Piper, David R.[1], Schuele, Birgitt[2], Vogel, Kurt[1]

[1]Research and Development, Thermo Fisher Scientific, Madison, WI, USA, [2]Research and Development, Parkinson's Institute and Clinical Center, Sunnyvale, CA, USA

The absence of cellular models for Parkinson's Disease (PD) represents a major bottleneck and unmet need in PD research. Patient-derived induced pluripotent stem cells (iPSCs) offer exciting potential in cell therapy and *in vitro* disease modeling. Efficient reprogramming of patient somatic cells to iPSCs in feeder-free conditions plays a key role in realizing this potential. Many reprogramming methods have been optimized for use with numerous cell lines, but lead to technical challenges for researchers in converting adult or disease somatic cells to iPSCs consistently and efficiently. The CytoTune®-iPS 2.0 Sendai Reprogramming Kit uses Sendai virus and polycistronic vectors to reprogram somatic cells into induced pluripotent stem cells (iPSCs) which provides a more robust reprogramming efficiency, lower cytotoxicity, and faster viral clearance to generate integration-free iPSCs in feeder-free conditions. In this study, fibroblasts from skin biopsies of

two related Parkinson's disease (PD) study patients were reprogrammed in feeder-free conditions to iPSCs using Life Technologies CytoTune®-iPS 2.0 Sendai Reprogramming Kit. These iPSCs are transgene-free and karyotypically normal, express known pluripotency markers and are able to differentiate into embryoid bodies that present the three germ layer lineages: ectoderm, mesoderm, and endoderm. Gene expression analysis distinguishes these iPSCs from their parental fibroblasts and clusters them together with control Gibco® iPSCs and H9 ESCs. Given the efficiency, speed and ease of reprogramming of these adult, disease-related fibroblasts in feeder-free conditions, the CytoTune®-iPS 2.0 Sendai Reprogramming Kit can be applied to large scale reprogramming of multiple disease lines in an automated fashion to provide significant impact for researchers worldwide.

W-2175
REPROGRAMMING OF DONOR CELLS AND DIFFERENTIATION TO DEFINITIVE ENDODERM CELLS

Roberts, Casey[1], Chen, Silvia[2], Sachs, Patrick[3], Ogle, Roy C.[3]
[1]Eastern Virginia Medical School, Norfolk, VA, USA, [2]LifeNet Health, Virginia Beach, VA, USA, [3]School of Medical Diagnostic and Translational Sciences, Old Dominion University, Norfolk, VA, USA

Often iPSCs are produced from dermal fibroblasts; however, a variety of other cell types are successfully used. Human osteoblasts have not been previously used to establish iPSCs. Here, we show that iPSCs can be derived from human osteoblasts without de-differentiation and readily differentiate into derivatives of all three germ layers. Bone chips from donor tissue were processed to remove bone marrow components. The cells (OB11) displayed characteristic osteoblastic gene expression of RunX2, Col1A1, osteocalcin, BMP2, and osteopoetin at or greater to commercially available osteoblastic cells (NHOst, Lonza) when assayed by qRT-PCR. OB11 cells, also, had an up regulation of Oct4 and hTert compared to adult fibroblasts as determined by qRT-PCR. Furthermore, upon treatment with bone differentiation media (0.1uM dexamethasone, 10mM beta-glycerol phosphate, 50uM ascorbic 2-phosphate for 21 days) the OB11 cells differentiated into mineralizing osteocyte-like bone cells, confirmed by Alizarin Red S staining. The OB11cells were reprogrammed using Sendai viral vectors containing the Yamanaka factors (Life Technologies). The iPSCs were characterized for pluripotency markers with immunofluorescence, flow cytometry, and qRT-PCR. The OB11 iPSCs strongly expressed Sox2, Oct4, Nanog, Tra-1-81, Tra-1-60, and alkaline phosphatase. To determine if the iPSCs were able to generate derivatives of multiple germ layers, an embryoid body (EB) technique was employed. The EB-derived cells had positive expression for all 3 germ layers, including Tuj1 and Sox1 (ectodermal cell type markers), FoxA2 and AFP (Alpha fetoprotein, endodermal cell type markers), Actin A2 and SMA (smooth muscle actin, mesodermal cell type markers). They clearly showed higher expression of the mesodermal markers. This was confirmed with the hPSC scorecard panel (Life Technologies), which showed that the cells had potential for all three germ layers, but preferentially differentiated to mesodermal and ectodermal lineages. Since endoderm was the germ layer lineage observed with lowest frequency, we focused on testing the ability of the OB11 iPSCs to generate definitive endoderm, pancreatic beta cells, and hepatocytes. The iPSCs were treated with 50ng/mL Activin A and 1X B27 supplement for three days of direct differentiation (no formation of EB); the iPSCs generated definitive endoderm (DE) with high percentages of the cells expressing endodermal markers CXCR4 and Sox17. The DE cells were maintained in culture for over 35 days and high percentages of the cells continued to express CXCR4 and Sox17. The cells also continued to proliferate throughout the culture and when differentiated further, were able to generate proinsulin+ GLUT2+ pancreatic beta-like cells and HNF4+ hepatocyte-like cells. Human osteoblasts are readily reprogrammed to iPSCs. The iPSCs can efficiently generate DE cells capable of forming mature beta-like and hepatocyte-like cells.

W-2176
WNT SIGNALING IS ESSENTIAL FOR REPROGRAMMING TO PLURIPOTENCY

Ross, Jason T., Willert, Karl H.
University of California San Diego, La Jolla, CA, USA

Despite many years of intensive research in the field of reprogramming and induced pluripotency, relatively little is know about the role of the extracellular environment (cell-cell, cell-matrix and cell-soluble factor interactions) in this process. The recent discovery that somatic cells can be converted to pluripotent stem cells by exposure to sublethal stimuli has brought to the forefront the importance of the extracellular environment during reprogramming. We have performed an in-depth analysis of the developmental WNT signaling pathway in reprogramming. Using both small molecule inhibitors and mutations of WNT signaling components, including disease-associated mutations, we find that WNT signaling is essential for cellular reprogramming and generation of induced pluripotent stem (iPS) cells. Interfering with the activity of PORCN, which is required for processing and subsequent secretion of WNT proteins, potently reduces reprogramming rates, an effect that is rescued through exogenous activation of WNT/β-catenin signaling. Furthermore, blocking endogenous WNT signaling either through AXIN overexpression or small hairpin RNA-mediated knockdown of the WNT receptor FZD7 reduced the number of iPS cell colonies, suggesting that reprogramming requires an endogenous WNT/β-catenin signaling loop. Consistent with our hypothesis that WNT signaling plays a critical role during reprogramming, we find that the efficiency of iPS cell generation is significantly enhanced through ectopic activation of the pathway, including through the addition of purified Wnt3a protein and R-Spondin or the overexpression of β-catenin, especially in the absence of the WNT target gene (and reprogramming factor) MYC, indicating that WNT's mechanism of action is not entirely through MYC. In contrast, treatment with purified Wnt5a, a protein ligand known to antagonize canonical WNT signaling, significantly impairs reprogramming. Finally, we find that activation and inhibition of WNT/β-catenin signaling at early stages of reprogramming promotes and impairs iPS cell formation, respectively. From this study we conclude that WNT signaling is essential for successful reprogramming to pluripotency and may represent a major pathway for signal transduction from the extracellular environment to influence the reprogramming process.

W-2177
NEUROSPHERES-DERIVED FROM HIPSCS AUGMENTS MOTOR AND SENSORY FUNCTIONS IN SPINAL CORD INJURY PARAPLEGIC SCID MICE MODELS.

Sabapathy, Vikram[1], Murugan, Durai[1], Samuel, Rekha[2], Tharion, George[1], Kumar, Sanjay[1]
[1]Centre for Stem Cell Research, Vellore, Tamil Nadu, India, [2]Christian Medical College, Vellore, India

Spinal cord injury is a debilitating disorder that results in the progressive loss of motor and sensory functions. At present there is no proper cure for the treatment of spinal cord injury patients. Preliminary studies indicate the fact that cell therapy might serve a treatment option for patients suffering from spinal cord injury. The patient's autologous bone-marrow mesenchymal stromal cell's (MSCs) poses limited *in vitro* expandability, driving the need to find alternate cell source. Pluripotent stem cells might serve as an alternate source for cell therapy due to

its ability to differentiate efficiently into multiple neuronal subtypes. In this study, we have utilized perinatal human placenta-derived MSCs (hPD-MSC) for generation of iPSCs using integration-free nucleofection protocol. In-order to make the reprogramming clinically compatible we have utilized autologous MSC feeder for derivation of iPSCs. Treatment of cell undergoing reprogramming with dual kinase inhibitor and LIF effectively augmented the reprogramming efficiency with up regulation of PTyr705 STAT3, epithelial markers (beta catenin and e-cadherin), surface markers (Tra 1-81, Tra 1-60 and SSEA4) and endogenous core pluripotecy factors (Oct3/4, SOX2 and Nanog). Apart from the regular pluripotent marker we have observed increase in P-cadherin expression after reprogramming. The derived iPSCs were further subjected to rigorous *in vitro* and *in vivo* characterization. We have successfully used Tra 1-81 live stain and transient nanog-GFP based transfection method for selecting the fully reprogrammed colonies. For transplantation studies, we have created SCID mice spinal cord injury, paraplegic models with the help of compression injury at T10 level. Our preliminary data with transplantation of iPSCs derived neurospheres (iNSCs) suggest that it effectively augment neural regeneration with motor and sensory recovery. There was no adverse effect of cell therapy in the SCID mice like formation of teratoma after transplantation of iPSCs derived neurospheres. Functional recovery was evaluated using trans-cranial stimulations. Results also indicated that transplantation of iPSCs derived iNSCs led to increased neuronal myelination with reduced inflammation. Physiological data suggests that transplanted mice exhibited moderate bladder control and improved weight gain than the non-transplanted mice. Further, bone density results indicated significant bone loss in the non-transplanted mice compared to the control mice. Interestingly, there was no significant difference between transplantation of hPD-MSCs and iNSCs in terms of neural regeneration, remyelination and functional recovery. Besides, the hPD-MSCs transplanted mice exhibited better suppression of the inflammation when compared to the iNSCs. Additionally, in this study, we have successfully employed ICG based cell labeling method in order to *in vivo* track the cells using simple optical interface technique.

W-2178
UTILIZING A NOVEL MICRORNA DETECTION PLATFORM FOR PROFILING MICRORNA ACTIVITIES IN PRIMARY MOUSE EMBRYO FIBROBLASTS THROUGH THE GENERATION OF INDUCED PLURIPOTENT STEM CELLS

Santos, Mark[1], Zhang, Wenying[1], Asbrock, Nick[1], Koong, Victor[2], Hsu, Matthew[1]

Research and Development, EMD Millipore Corporation, Temecula, CA, USA, [2]EMD Millipore Corporation, Temecula, CA, USA

Cellular differentiation is a fundamental process in developmental biology. Embryonic stem cells (ES cells) must have the ability to differentiate into more specialized cell types or tissues for the body to respond to certain situations in which more advanced cell types are required for the body to function optimally. The reprogramming of ES cells has been highly investigated and their potential in regenerative therapies should not be underestimated. However, the use of ES cells has been somewhat controversial, leading to the generation of induced pluriopotent stem cells (iPS cells) as a viable alternative. Although we are aware that transcription factors Oct4, Klf4, Sox2, and c-Myc are indicated in the reprogramming process of mouse embryonic fibroblasts (MEFs) to iPS cells, to date the way in which these transcription factors influence this transition is still not clearly understood. MicroRNAs (miRNAs) are small, non-coding RNAs which have emerged as key targets which modulate gene expression. They have been implicated to control the fate of MEFs and have the ability to directly influence the reprogramming of pluripotency factors Oct4, Klf4, Sox2, and c-Myc. By having the ability to monitor and profile which miRNAs are indicated in iPS generation from MEFs we can more clearly understand the molecular mechanisms involved in this ubiquitous process. Here we describe a unique miRNA detection platform where we are able to profile and clearly identify which miRNAs are involved in the reprogramming process of mouse embryo fibroblasts to iPS cells. Using miRNA encoded hydrogel particles for detection, we were able to profile a host of miRNA targets in both MEFs as well as iPS cells by flow cytometry. Moreover, we also screened which miRNAs are involved during the transition phase between each cell type as well. By implementing our hydrogel particle technique, we now have the ability to screen up to 68 unique miRNA targets per sample across a 96 well plate within one assay run, lending us the opportunity for providing higher content analyses of which miRNA targets are intimately involved during this differentiation process, as well as identify other miRNAs which can potentially impact this transition as well. In the literature, many miRNA targets have been identified and their results indicate that specific miRNAs such as the miR-290-295 cluster, miR-21, miR-29a, miR-302, and the let-7 family of miRNAs all have emerged as important regulators of ES cell self-renewal, pluripotency, and differentiation. By using our novel platform for miRNA profiling and detection we have confirmed which miRNA signatures are present, but now also have the capability to profile more miRNAs to uncover any other potential miRNA targets indicated in this differentiation process which is not readily feasible using either RT-PCR or microarray techniques alone. In short, we have identified a platform to profile, identify, and characterize which miRNAs are present in the MEF to iPSC cell transition and how they function to regulate multiple signaling networks involved in iPS cell generation in a quick, inexpensive, and reliable way is integral when discovering clinically applicable reprogramming strategies.

W-2179
INDUCED PLURIPOTENT STEM CELLS HAVE SUPERIOR IMMUNOMODULATORY PROPERTIES COMPARED TO BONE MARROW DERIVED STEM CELLS IN VITRO

Schnabel, Lauren V.[1], Abratte, Christian M.[2], Schimenti, John C.[2], Felippe, M. Julia B.[3], Cassano, Jennifer Michelle[4], Southard, Teresa L.[2], Fortier, Lisa A.[3]

[1]North Carolina State University, Raleigh, NC, USA, [2]Biomedical Sciences, Cornell University, Ithaca, NY, USA, [3]Clinical Sciences, Cornell University, Ithaca, NY, USA, [4]Cornell University, Ithaca, NY, USA

The immunogenic properties of induced pluripotent stem cells (iPSCs) are currently being investigated as it has become evident that banked iPSCs will be needed for most clinical applications due to cell generation time and time associated with screening for both efficacy and safety. The immunogenic and immunomodulatory properties of mesenchymal stromal cells (MSCs) have been, and continue to be investigated for many of the same reasons and it has been demonstrated that they possess significant immunomodulatory properties. The purpose of this study was to evaluate the immunogenic and immunomodulatory properties of iPSCs compared to adult bone marrow-derived MSCs using modified mixed leukocyte reactions. Mouse embryonic fibroblasts (MEFs) were isolated from C3HeB/FeJ and C57BL/6J mice. Lentiviral and *PiggyBac* iPSC lines were generated via reprogramming of MEFs from each strain and validated via teratoma assay. Bone marrow-derived MSCs were isolated from each strain and validated. MEFs, iPSCs, and MSCs were immunophenotyped for MHC I and II expression. Leukocytes were isolated from splenocyte suspensions and modified one-way mixed leukocyte reactions (MLRs) were performed using MHC-matched and mismatched responder

leukocytes (CFSE-labeled) and stimulator leukocytes, MEFs, iPSCs, or MSCs. Responder leukocytes were examined at 4 different concentrations. In order to assess the immunogenic potential of the cells, MEFs, iPSCs, and MSCs were used as stimulator cells for responder leukocytes. In order to assess the immunomodulatory properties of the cells, MEFs, IPSCs, and MSCs were cultured in the presence of stimulator and responder leukocytes. MHC-matched stimulator leukocytes were used to establish baseline T cell proliferation and MHC-mismatched stimulator leukocytes were used as positive MLR controls. MEFs were considered the negative control in the immunomodulatory potential studies. Proliferation of gated CFSE-labeled responder T cells was evaluated via CFSE attenuation using flow cytometry. Relative T cell proliferation was calculated as the fold change from that of the MHC-matched MLR with stimulator leukocytes. Data were normalized by log transformation and analyzed with ANCOVA followed by the Tukey test for multiple comparisons. Significance was set at $p < 0.05$. MEFs had a phenotype of MHC I positive (low) and MHC II negative, while iPSCs had a phenotype of both MHC I and II negative. MSCs were MHC I positive and MHC II negative. As predicted based on MHC class II expression, all of the cell types tested had low immunogenicity when either MHC-matched or mismatched with the responder leukocytes. Our comparisons revealed that iPSCs generated through both lentiviral and piggyBac reprogramming methods had similar immunogenic properties as MSCs but had more potent immunomodulatory effects than MSCs. Co-culture of MHC-mismatched leukocytes with MHC-matched iPSCs resulted in significantly less responder T cell proliferation than observed for MHC-mismatched leukocytes alone and at more responder leukocyte concentrations tested than was observed for co-culture of MHC-mismatched leukocytes with MHC-matched MSCs. In addition, MHC-mismatched iPSCs were able to significantly reduce responder T cell proliferation when co-cultured with MHC-mismatched leukocytes, while MHC-mismatched MSCs were not. These results provide important information when considering the use of iPSCs in place of MSCs in both regenerative and transplantation medicine.

W-2180
AN EFFECTIVE, COST EFFICIENT MEANS OF ASSESSING HIPS PLURIPOTENCY: QUANTIFYING THE CELLULAR RESPONSE TO DNA DAMAGE

Secreto, Frank J.[1], Li, Xing[2], Bruinsma, Elizabeth[1], Hawse, Gresin[3], Perales-Clemente, Ester[4], Rasmussen, Boyd[3], Miller, Jennifer[3], Nelson, Timothy J.[5]

[1]General Internal Medicine Cardiac Transplant, Mayo Clinic, Rochester, MN, USA, [2]Program for Hypoplastic Left Heart Syndrome, Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN, USA, [3]Program for Hypoplastic Left Heart Syndrome, Mayo Clinic, Rochester, MN, USA, [4]Cardio Vascular Diseases, Mayo Clinic, Rochester, MN, USA, [5]Program for Hypoplastic Left Heart Syndrome, Pediatric and Adolescent Medicine, Mayo Clinic, Rochester, MN, USA

Successful implementation of future human stem cell therapies will largely depend upon the uniform quality of the cells in question. Human Induced pluripotent stem cells (hIPS) hold much promise as a potential therapeutic agent for an array of maladies, and the production of large repositories of these cells at several institutions is currently underway. However, an efficient means of quality control is needed in order to determine whether a clone is suitable for therapeutic applications. Pluripotency of hIPS cells is routinely determined by a number of techniques, including detection of cell surface markers such as SSEA-3/4 and TRA-1-60/1-80, expression of reprogramming genes, clonal morphology and teratoma formation in mice. These techniques, while useful in determining the pluripotent ground state of a limited number of clones, are cumbersome and expensive when applied to large numbers of samples. A scalable, functional assay that could reliably determine the quality of hIPS cells, regardless of tissue source or cellular origin, would provide a valuable tool. We have previously demonstrated that mouse IPS cell pluripotency is directly correlated with sensitivity to the DNA damaging agent etoposide, and herein we propose that such sensitivity provides an efficient and superior means to monitor functional pluripotency of hIPS clones. Initially, we examined five hIPS clones by morphology, pluripotent gene expression profile (SOX2, OCT4, cMYC), presence of SSEA-3 and TRA-1-60 and etoposide sensitivity. Morphology characteristic of undifferentiated colonies was directly correlated with low etoposide EC50 values (< 100 nM) and a higher percentage of apoptotic cells. However, SSEA-3 and TRA-1-60 were readily detectable in all five hIPS clones regardless of either cellular morphology or pluripotent gene expression. To confirm the pluripotency of selected hIPS clones, two clones with the lowest EC50 values were injected into the testis capsule of athymic nude mice. Teratoma formation was confirmed in all mice following dissection at week 8. Building off of our small scale study, we subsequently assessed the pluripotent potential of 91 hIPS clones by RNA expression analyses employing an expanded pluripotent gene expression panel (REXO1, SALL4, TDGF1, SOX2, POU5F1, cMYC and ZFP42), FLOW analysis for SSEA-4 and TRA-1-60 and sensitivity to etoposide. Differences in overall RNA expression patterns were determined by principal component analysis (PCA). Clones exhibiting significantly different RNA PCA profiles compared with the collective data set also displayed a significant decrease in etoposide sensitivity (Increased EC50 value) relative to the EC50 mean (71.5 nM). Notably, co-expression of SSEA-4 and TRA-1-60 (77.3% mean positive) was not significantly altered in clones with high etoposide EC50 values or outlier RNA PCA profiles. These results demonstrate that quantitative differences in DNA damage response between hIPS clones could replace expensive and less reliable biomarkers currently used to determine pluripotency of hIPS clones.

W-2181
PREMATURE TERMINATION OF REPROGRAMMING IN VIVO LEADS TO CANCER DEVELOPMENT THROUGH ALTERED EPIGENETIC REGULATION

Semi, Katsunori, Onishi, Kotaro, Yamamoto, Takuya, Tanaka, Akito, Yamanaka, Shinya, Woltjen, Knut, Yamada, Yasuhiro

CiRA, Kyoto University, Kyoto, Japan

Cancer is believed to arise primarily through accumulation of genetic mutations. Although induced pluripotent stem cell (iPSC) generation does not require changes in genomic sequence, iPSCs acquire unlimited growth potential, a characteristic shared with cancer cells. Here, we describe a murine system in which reprogramming factor expression in vivo can be controlled temporally with doxycycline (Dox). Notably, transient expression of reprogramming factors in vivo results in tumor development in various tissues consisting of undifferentiated dysplastic cells exhibiting global changes in DNA methylation patterns. The Dox-withdrawn tumors arising in the kidney share a number of characteristics with Wilms tumor, a common pediatric kidney cancer. We also demonstrate that iPSCs derived from Dox-withdrawn kidney tumor cells give rise to nonneoplastic kidney cells in mice, proving that they have not undergone irreversible genetic transformation. These findings suggest that epigenetic regulation associated with iPSC derivation may drive development of particular types of cancer.

W-2183

ESTABLISHMENT OF DISEASE MODEL USING INDUCED PLURIPOTENT STEM CELLS DERIVED FROM NIEMANN-PICK DISEASE TYPE C

Soga, Minami[1], Hamasaki, Makoto[1], Yoneda, Kaori[2], Nakamura, Kimitoshi[2], Matsuo, Muneaki[3], Irie, Tetsumi[4], Endo, Fumio[2], Era, Takumi[1]

[1]Department of Cell Modulation, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan, [2]Department of Pediatrics, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan, [3]Department of Pediatrics, Saga University, Faculty of Medicine, Saga, Japan, [4]Department of Clinical Chemistry and Informatics, Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan

Cellular models of intractable disease are essential tools for studying the molecular mechanism of these diseases and developing new therapies. But collection of biomaterial samples from patients with intractable diseases are difficult because of the limited numbers of patients and the inaccessibility of target cells. Because induced pluripotent stem cells (iPSCs) display multipotency and unlimited proliferation capacity *in vitro*, disease-derived iPSCs are expected to overcome these problems. Niemann-pick disease type C (NPC) is a lysosomal storage disease associated with mutations in *NPC1* or *NPC2*. These mutations disrupt this transporting system, resulting in the abnormal accumulation of free cholesterol and glycolipids in lysosomes. As enlargement of liver is one of major symptoms of NPC patients, here, we established four iPSCs lines from skin fibroblasts of two patients carrying different *NPC1* mutations using non-integrating Sendai virus, and then induced to differentiate into hepatocyte-like cells (HLCs). The efficiency of differentiation was similar between the normal-derived iPS cell and the NPC-derived iPS cell lines. But we found extremely high levels of free cholesterol accumulation and cellular dysfunctions in NPC-derived HLCs. 2-Hydroxypropyl-β-cyclodextrin (HPBCD) treatment, which is known as effective for reducing of cholesterol accumulation in *NPC1*-defective cells, reduced free cholesterol accumulation and recovered cellular dysfunctions in NPC-derived HLCs. Our data suggests that the transgene-free NPC-derived iPSC lines provide an appropriate cellular model, and are powerful tools for studying the molecular mechanism of diseases and developing new therapeutic agents.

W-2184

RNA-SEQ TO IDENTIFY NOVEL MARKERS FOR NEURAL TISSUE DIFFERENTIATION

Sun, Yongming A.[1], Giorda, Kristina[1], Frey, Elizabeth[2], Taylor, Madison[1], Barron, Tori[2], Piper, David[2], Meredith, Gavin[1]

[1]Thermo Fisher Scientific, South San Francisco, CA, USA, [2]Life Technologies, Madison, WI, USA

Neural tissue differentiated and cultured from patient-derived stem cells is expected to revolutionize the treatment of patients with brain and spinal injuries and diseases. Critical for these cellular therapies is accurate control and monitoring of differentiation but current methods for such cell typing are limited to qPCR and immunocytochemisty (ICC) which is not sufficient to discriminate between the numerous (likely >100,000) possible neural cell-types. RNA (transcriptome aka RNA-Seq) profiling permits the characterization and discovery of much-needed novel markers. To define the temporal transcriptional signature of neural stem cells, cultured human embryonic stem cells (H9) were compared to induced neural stem cells (NSCs) at d0, d7 and d14. ICC was performed on the putative NSC pools at d7 and d14 for markers of pluripotency (Oct4) and neural differentiation (nestin, Sox1, and Pax6) and H9 cells were stained on d14 for markers of pluripotency (Oct4 and SSEA4). Total RNA was isolated over the time course from the undifferentiated and differentiated cells. Ion Torrent libraries were created to profile expression of miRNAs and whole transcriptomes for each cell population. Multiplexed Proton sequencing and Torrent Suite Software analysis yielded ≥2.5 million small RNA reads and ≥29 million whole transcriptome reads per sample. Cluster analysis of the RNA-Seq profiles indicates that the cell populations have characteristic molecular signatures. Among genes that are decreased in induced cells are OCT4 (POU5F1), JARID2, NANOG , consistent with the differentiation of iPSCs into neurons. Among genes that showed increased expressions are NTRK2, POU3F2, and a number of HOX family genes. We also find lincRNA are involved in cell differentiation.

W-2185

IPSC AND FIBROBLAST RESOURCES FOR ADULT ONSET NEURODEGENERATIVE DISEASE BIOLOGY AND DISEASE MODELING - THE NATIONAL INSTITUTE OF NEUROLOGICAL DISORDERS AND STROKE (NINDS) REPOSITORY

Sutherland, Margaret

NIH/NINDS, Rockville, MD, USA

In 2006, the generation of induced pluripotent stem cells (iPSCs) from somatic cells through retroviral-driven expression of four embryonic transcription factors (c-Myc,Oct4, Sox2 and Klf4) initiated a revolution in resource development for therapeutic discovery. Development of iPSC lines from fibroblasts of patients with early onset neurological disorders such as, Spinal Muscular Atrophy, Friedreich's Ataxia, Rett syndrome, Fragile X syndrome, and Angelman syndrome demonstrated that the genetic defects carried by these cells could model certain aspects of the disease phenotype. In 2009, through the American Recovery and Reinvestment Act (ARRA), NINDS sponsored three consortia, to develop well characterized publically available, induced pluripotent stem cell (iPSC) lines for Huntington's Disease (HD) and familial forms of Parkinson's Disease (PD), Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Degeneration (FTLD). This consortium approach enabled rapid resource development and the initial identification of cellular phenotypes associated with late onset neurodegenerative disease in iPSC-derived neuronal and astrocyte cultures. In 2011, a limited competition NINDS Funding Opportunity Announcement (FOA) was released to enable the existing consortia to continue generating non-integrating iPSC lines, isogenic lines and reporter lines, which are distributed through the NINDS Repository at Coriell. The California Institute for Regenerative Medicine (CIRM), and non-government organizations including; the Amyotrophic Lateral Sclerosis Association (ALSA), the Association for Frontotemporal Degeneration (AFTD), CHDI, the Hereditary Disease Foundation (HDF), the Huntington's Disease Association of America (HDSA), the Michael J. Fox Foundation (MJFF), and the Parkinson's Disease Foundation (PDF) in collaboration with NINDS contributed to the second round of funding for this iPSC consortia effort. There are currently 162 fibroblast lines and 61 iPSC lines available through the NINDS Repository. In workshops with industry representatives and consortia members, challenges that currently hinder therapeutic development for central nervous system disorders (CNS) were identified and included: 1) a poor understanding of disease biology; 2) paucity of tractable central nervous system targets; 3) the poor predictive value of animal models; 4) difficulty in delivering drugs across the blood brain barrier; and 5) lengthy and large clinical trials. Although the discovery and validation of disease relevant phenotypes in differentiated patient-derived iPSC cell lines is still in its infancy, preliminary results from the four consortia suggest that human iPSC cell lines may provide valuable

tools to aid efforts in both understanding disease biology and in the development of new therapeutics for neurodegenerative diseases. As of May 2013, the NINDS Repository has distributed over 630 fibroblast lines and 220 iPSC lines to academic and industry investigators worldwide.

W-2186
MOLECULAR ANALYSIS TOOLS FOR EPIGENOMIC CHARACTERIZATION OF PLURIPOTENT STEM CELLS

Sylakowski, Kyle[1], Fergus, Jeffrey[2], Quintanilla, Rene H.[2], Lakshmipathy, Uma[2]

[1]Biological Sciences, California State University San Marcos, San Marcos, CA, USA, [2]Cell Biology and Stem Cell Sciences, Thermo Fisher Scientific, Carlsbad, CA, USA

Induced pluripotent stem cells (iPSCs) offer an ideal platform to generate patient-derived cell models to dissect basic biology and for therapeutic use, either in drug screening or cell replacement. As iPSCs are generated from various genetic backgrounds using different derivation and culture conditions, it is essential to carryout comprehensive characterization to ensure their identity, functionality, and genetic stability. Molecular analysis platforms offer a quantitative, accurate, and fast alternative to current cellular methods of confirming pluripotency. We had reported earlier on the TaqMan® hPSC Scorecard™, a comprehensive gene expression panel for rapid characterization of pluripotent stem cells. In this study, a similar PCR-based approach was utilized to quantitatively detect methylation status of specific genomic loci in pluripotent stem cells. Methylation specific primers were designed to 6 different loci that are known to be either methylated or unmethylated in the pluripotent state; specificity was confirmed in undifferentiated and differentiating H9 ESCs. In order to extend the study, iPSCs were derived from several donor phenotypes using a simplified workflow utilizing the Epi5™ iPSC Reprogramming kit in combination with the Lipofectamine®3000 Reagent transfection system. H9 ESCs and the generated iPSC clones will be used to generate Oct4-GFP reporter lines using targeted insertion and GeneArt ® Precision TALs technology, thus enabling the creation of reference standards from high quality pluripotent cells. Creation of pluripotent reporter ESC and iPSC from diverse sources provides a valuable tool to generate homogeneous population of cells for the creation of reference standards and comparison of molecular expression signature with alternate enrichment methods based on surface marker expression. Generation of standardized tools and technologies enables thorough characterization of pluripotent stem cells, and is critical for their downstream application in regenerative medicine.

W-2187
REPROGRAMMING LYMPHOBLASTOID CELL LINES (LCLS) INTO INDUCED PLURIPOTENT STEM CELLS (IPSC) RESULTS IN RECOVERY OF INDIVIDUAL VARIATION IN GENE EXPRESSION

Thomas, Samantha[1], Caliskan, Minal[1], Pritchard, Jonathan[2], Gilad, Yoav[1]

[1]Human Genetics, University of Chicago, Chicago, IL, USA, [2]Departments of Genetics and Biology and Howard Hughes Medical Institute, Stanford University, Stanford, CA, USA

Cell lines are often used in genomic studies because of their convenience and amenability to experimental manipulation. However, they do not always recapitulate the physiological properties of their primary tissue counterparts. Of particular concern to studies of gene regulation is the loss of individual gene expression signatures during the maintenance of cell lines. Caliskan et al. demonstrated that after even just one freeze thaw cycle, lymphoblastoid cell lines (LCLs) lose the majority of inter-individual variation in gene expression observed in the primary B cells from which the LCLs were derived. In this study, we ask whether reprogramming the LCLs to induced pluripotent stem cells (iPSCs) can recover the individual gene expression patterns lost during the freeze thaw cycling of LCLs. To do so, we collected whole genome gene expression data from iPSCs generated from three independently derived LCLs from each of the six individuals used in the Caliskan et al. study. Prior to reprogramming, the LCLs had undergone seven freeze-thaw cycles and expression correlations between individuals were just as strong as those within replicates of the same individual. Because much of the epigenome is reset during the reprogramming process, we expect to unmask native genetically regulated gene expression patterns and recover the individual variation lost during the freeze-thaw process. Evidence that iPSCs derived from LCLs better recapitulate the individual's original gene expression signature will firmly establish them as superior models for studies of gene regulation

W-2188
DIVERGENT REPROGRAMMING ROUTES LEAD TO ALTERNATIVE STEM CELL STATES

Tonge, Peter[1], Corso, Andrew[2], Monetti, Claudio[2], Puri, Mira C.[3], Hussein, Samer M.[4], Rogers, Ian[5], International, PG Consortium[2], Nagy, Andras[2]

[1]Lunenfeld Tanenbaum Research Institute, Toronto, ON, Canada, [2]Lunenfeld Tanenbaum Research Institute, Toronto, ON, Canada, [3]Samuel Lunefeld Research Institute, Toronto, ON, Canada, [4]Mount Sinai Hospital Samuel Lunenfeld Research Institute, Toronto, ON, Canada, [5]Mt. Sinai Hospital, Toronto, ON, Canada

Somatic cells can be reprogrammed to acquire a pluripotent stem cell (iPSC) fate through the over-expression of key transcription factors and can fulfill the strictest of all developmental assays observed for ESCs by generating completely iPSC-derived embryos and mice. However, it is not known whether there are additional classes of pluripotent cells. We have explored alternative outcomes of somatic reprogramming by fully characterising reprogrammed cells independent of preconceived definitions of partial or fully reprogrammed iPSC states. Global gene expression profiling revealed two distinct cohorts of cell lines, which we classify by morphological appearance as Compact colony forming cells (C-class) and Fuzzy colony forming cells (F-class). F-class cell lines can be serially subcloned whilst exhibiting a stable transcriptome, demonstrating the capacity for self-renewal and stability of the F-class cell state. We have found that the F-class cells possess the potential to efficiently generate differentiated cell types representative of all three germ layers in vivo, and can be directed to terminally differentiated cell types in vitro. The emergence of nanog-positive F-class lines is dependent on the expression of the four factors with a necessity for high levels of expression above those seen in ESCs. We demonstrate the ability to genetically modify F-class cells by generating homozygous knockouts of nanog, revealing that nanog is not required for the F-class cell state. Exposure of F-class cells to the DNA methyltransferase inhibitor 5-Aza-deoxycytidine (Aza) is toxic at active concentrations. In contrast, inhibition of histone deacetylases (HDACi) induces an F-class to ESC-like cell state transition, acquiring an ESC-like morphology and transcriptional profile. Following HDACi treatment, F-class derived cells can be maintained as transgene-independent cells capable of contributing to chimeras when cells are aggregated with preimplantation stage embryos. The F-class cells activate a unique set of genes that are associated with the loss of inhibitory epigenetic marks (H3K27me3 and/or CpG methylation), with many ESC associated genes (Lefty1, Cldn6, Gbx2) actually acquiring inhibitory CpG methylation during reprogramming to the F-class state. To our

knowledge, this is the first report to identify epigenetic changes that actively propel reprogramming cells towards an alternative pluripotent state. In summary, this study comprehensively characterizes the diversity of reprogrammed cell states. We demonstrate that by maintaining highly elevated reprogramming factor expression, mouse embryonic fibroblasts go through unique epigenetic modifications to arrive at a stable, Nanog-positive, alternative pluripotent state. In doing so, we prove that the pluripotent spectrum can encompass multiple unique cell states.

W-2189
THE DEVELOPMENT OF SCALE-UP BIOREACTOR SYSTEM FOR HUMAN INDUCED PLURIPOTENT STEM CELL STIRRED SUSPENSION CULTURE

Wada, Masanori[1], Matsuura, Katsuhisa[2], Ishikawa, Yoichi[1], Shimizu, Tatsuya[3], Okano, Teruo[3]
[1]ABLE Corporation, Tokyo, Japan, [2]Institute of Advanced Biomedical Engineering and Science (TWIns) / Department of Cardiology, Tokyo Women's Medical University, Tokyo, Japan, [3]Institute of Advanced Biomedical Engineering and Science (TWIns), Tokyo Women's Medical University, Tokyo, Japan

[Introduction] Induced pluripotent stem (iPS) cells are promising cell sources for regenerative medicine. The stirred suspension culture using a bioreactor system is an efficient method for the large scale expansion of human iPS cells. On the basis of our hypothesis that a low share stress and the uniform medium flow in a vessel are important for the suspension culture of human iPS cells, we showed the bioreactor system which is capable of the stirred suspension culture of the inoculated single iPS cells in the aggregates condition at the last this conference. We designed the bioreactor which expanded culture volume to 1000mL, and tried scale up culture from 100mL this time. [Material and method] At first we designed the stirrer including delta shape paddle impeller for 1000mL bioreactor. This impeller similar as 100mL bioreactor prevents a turbulent flow and agitates medium by laminar flow. The optical DO sensor and pH electrode were used for maintain the culture condition. The single cell suspension were prepared from 2D on-feeder culture and inoculated into 100mL bioreactor as passage 1 (2x10^5cells/mL). The cell aggregates were collected after the stirred suspension culture for 4 to 5 days, and dissociated into the single cells by using Accumax. The culture period was adjusted by proliferation of cell. The obtained single cells were re-inoculated into 2 or 3 of 100mL bioreactor and stirred suspension culture was performed as passage 2. After the culture of passage 2, obtained single cell suspension re-inoculated into 1000mL bioreactor as passage 3. The number of cells, and the number and size of aggregates were measured and the undifferentiated property of the expanded iPS cells was evaluated with the flow cytometric analysis. [Results and discussion] The large scale culture using the delta shape paddle impeller enabled to create a lot of aggregates 200 to 300 micrometer diameter in the cultivation for 4 to 5 days. Furthermore the number of cells increased 5 times (1x10^9cells) to the number of inoculated cells. The similar cell growth rate was observed for 4 to 5 days in each culture after the passage. The flow cytometric analysis revealed that more than 95% of population holded undifferentiated ability in all the passages. Collectively, we established the methods for the expansion of human iPS cells and scale up the culture size to 1000mL with undifferentiated state.

W-2190
REPROGRAMMING EFFICIENCY AND GENETIC STABILITY OF INDUCED PLURIPOTENT STEM CELLS FROM LI-FRAUMENI SYNDROME PATIENTS WITH MUTATED TP53

Weltner, Jere[1], Rivlin, Noa[2], Trokovic, Ras[1], Lund, Riikka Johanna[3], Lahesmaa, Riitta[4], Malkin, David[5], Rotter, Varda[2], Otonkoski, Timo[1]
[1]University of Helsinki, Helsinki, Finland, [2]Weizmann Institute of Science, Rehovot, Israel, [3]Turku Centre for Biotechnology, University of Turku and Abo Akademi University, Turku, Finland, [4]Turku Centre for Biotechnology, Turku, Finland, [5]University of Toronto, Toronto, ON, Canada

P53 controls cellular responses to genetic instability and limits the efficiency of reprogramming to pluripotency. Li-Fraumeni syndrome (LFS) is a hereditary cancer syndrome caused most commonly by heterozygous germline TP53 mutations. To determine the impact of heterozygous TP53 background on the reprogramming efficiency and genetic stability of human cells, we have reprogrammed fibroblasts from Li-Fraumeni syndrome patients to induced pluripotent stem cells (iPSC) and analyzed their genetic stability. Fibroblasts from three donors, two of which had a truncating frame shift mutation (P128fs) and one which had a missense mutation (Y163C) , were reprogrammed using both Sendai viral induction with OCT4, SOX2, KLF4 and C-MYC (Cytotune), and retroviral induction with OCT4, SOX2 and KLF4. Three Sendai viral iPSC clones form each donor cell line were cultured further and analyzed for copy number variations (CNV) in passages 4, 8 and 12 using Affymetrix SNP6 array and compared to previously published data. Six additional clones of the Y163C iPSC were analyzed by G-banding at passage 15. Sendai viral inductions did not reveal obvious differences in reprogramming efficiency between LFS and healthy control cells. However, when fibroblasts were reprogrammed using the 3-factor retroviral system, the reprogramming efficiency was significantly higher in the Y163C cells (Y163C 0.21%, P128fs 0.007%, ctrl 0.013%; P<0.001). The LFS iPSCs expressed pluripotent cell specific markers, differentiated to all three germ layers and were free of Sendai viral episomes. Total CNV numbers did not differ between LFS iPSCs and controls in early passage samples (p. 4-5 and 7-9). However, at passage 11 to 13, the LFS iPSCs contained significantly more CNVs than controls (median 187 vs 112.5, P=0.007), suggesting a less stable genotype in long term culture. Furthermore, two of the three Y163C lines contained large chromosomal aberrations. Additional 6 iPSC lines were induced from the Y163C donor and karyotypically analyzed by G-banding. Of these lines 3 out of 6 were found to contain mosaic cell populations with trisomy 1. In summary, we have reprogrammed Li-Fraumeni syndrome patient fibroblasts to iPSCs. The Y163C appears to act in a dominant fashion, leading to increased reprogramming efficiency and causing genetic instability in long term culture. The LFS iPSCs also frequently show chromosome 1 trisomy recapitulating chromosomal aberrations often detected in normal human pluripotent cell cultures. The $P53^{Y163C}$ iPSC provide a valuable model for further studies of genomic stability and carcinogenesis.

W-2191
GENERATION OF NAIVE-LIKE PORCINE INDUCED PLURIPOTENT STEM CELLS

Williams, Kaylyn R.[1], Villafranca, Maria Cristina[1], Eyestone, Willard H.[2]
[1]Biomedical and Veterinary Sciences, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA, USA, [2]Large Animal Clinical Sciences, Virgina-Maryland Regional College of Veterinary Medicine, Blacksburg, VA, USA

Induced pluripotent stem cells (iPSCs) can be made by forced

expression of Oct4, Sox2, Klf4, and c-Myc in somatic cells. However, the process is inefficient and the mechanisms not fully understood. Most porcine iPSCs (piPSCs) reported are the more differentiated "primed" rather than "naïve" state, spontaneously differentiate in culture, and require persistent exogenous reprogramming factor expression to maintain pluripotency. We hypothesized that using an inducible expression vector, optimizing culture conditions and reducing DNA methylation at key promoters would more efficiently yield naïve, stable piPSCs independent of exogenous factors. The goal of this project was to 1) generate piPSCs, then 2) validate reagents with mouse cells. The reprogramming vector used was a doxycycline (dox)-inducible polycistronic lentiviral vector with a reverse tetracycline transactivator companion. Mouse iPSCs (miPSCs) were produced by transfecting primary mouse embryonic fibroblasts with lentivirus, followed by culture in Serum Replacement (SR) Media + leukemia inhibitory factor (LIF) + dox for 22 days. Expansion without dox indicated that cells sustained stemness via endogenous expression of pluripotent genes. miPSCs had high alkaline phosphatase activity and stained positively after immunofluorescence (IF) for stem cell markers Oct4, Sox2, and Nanog and negatively for human/primed markers Tra-1-61 and Tra-1-80. Polymerase Chain Reaction (PCR) confirmed endogenous and no exogenous factor expression. The same method was used to make piPSCs, with addition of fibroblast growth factor (FGF) to culture media. Colonies were a mix of round, naïve-like and flat, primed-like colonies. Only round colonies were picked. During expansion, colonies retained naïve morphology with dox but died without it, indicating insufficient expression of endogenous pluripotency genes. piPSCs expanded to passage 6 with little spontaneous differentiation. Cells had weak alkaline phosphatase activity. IF was inconclusive for Nanog and negative for Tra-1-61 and Tra-1-80. Oct4 and Sox2 were omitted due to confounding exogenous expression. PCR for endogenous expression is in process. Future experiments will address the effect of LIF versus FGF on the number of naïve versus primed colonies, and the effects of inhibition of glycogen synthase kinase 3B (GSK3B) and extracellular signal regulated kinase (ERK1/2), knockdown of Mbd3, and overexpression of Tet on the efficiency and quality of piPSC production.

W-2193
IN VITRO TERATOMAS, A NOVEL IPSC QUALITY ASSAY, REVEALS DIFFERENTIATION BIAS OF IPSC LINES

Wolvetang, Ernst[1], Fortuna, Patrick RJ[1], Ovchinnikov, Dmitry. A.[1], Whitworth, Deanne J.[2], Thakar, Nilay Y.[3], Pera, Martin F.[4]
[1]Australian Institute for Bioengineering and Nanotechnology, Brisbane QLD, Australia, [2]School of Veterinary Science, Gatton QLD, Australia, [3]University of Queensland, Brisbane QLD, Australia, [4]University of Melbourne, Melbourne VIC, Australia

The efficiency of human pluripotent stem cells (hPSC) to differentiate into cells representative of the three germlayers and their propensity to revert to/or remain in an undifferentiated state following differentiation can vary greatly between lines. Although surrogate pluripotency assays for assessing hPSC pluripotency have been reported (such as embryoid body and transcriptome based methods), the gold standard for hPSC remains the *in vivo* teratoma assay where undifferentiated hESCs or hiPSCs are injected into immune-deficient mice. This assay suffers from a non-predictable "take-rate", is not quantitative and requires costly animal husbandry. Here we report on the development of a cost-effective, *in vitro* teratoma assay that is cheap, high-through-put and quantitatively assesses the pluripotency and tumorigenic propensity of hESC and iPSC lines. We have validated this assay with hESC, viral and integration-free hIPSC, genetically abnormal hPSC and nulli-, uni- and pluripotent human EC-lines. Remarkably, undirected *in vitro* differentiation of hESCs and hiPSCs matches the complexity (or lack thereof), both histologically and transcriptionally, of *in vivo* teratomas. The in vitro teratoma assay should prove to be a valuable tool for assessing the quality of hPSC destined for clinical applications and, given its simplicity and low cost, has the potential to be adopted widely.

W-2194
REGENERATION OF HUMAN NATURAL KILLER T CELLS THROUGH IPSC TECHNOLOGY: POTENTIAL FOR TUMOR IMMUNOTHERAPY

Yamada, Daisuke, Vizcardo Sakoda, Raul, Endo, Takaho, Fujii, Shin-ichiro, Koseki, Haruhiko
Integrative Medical Sciences (IMS), RIKEN, Yokohama, Japan

Invariant natural killer T (*i*NKT) cells are characterized by the expression of an invariant Vα24-Jα18 paired with Vβ11 in humans (Vα14-Jα18 paired with Vβ8/7/2 in mice), that recognizes glycolipids, such as α-galactosylceramide (α-GalCer), presented on the MHC class I-like molecule, CD1d. *i*NKT cells act as innate T lymphocytes and serve as a bridge between the innate and acquired immune systems. *i*NKT cells augment anti-tumor responses by producing IFNγ, which acts on NK cells to eliminate MHC-non-restricted (MHC^-) target tumor cells, and on $CD8^+$ cytotoxic T lymphocytes to directly kill MHC-restricted (MHC^+) tumor cells. Both of these tumor cell types are simultaneously present in cancer patients, and at present *i*NKT cells are only the cell type capable of eliminating them. Based on these findings, it had developed *i*NKT cell-targeted adjuvant immunotherapies with strong anti-tumor activity in humans. However, two-thirds of patients were ineligible for this therapy due to the limited numbers of *i*NKT cells in their bodies. In order to overcome this problem, we applied iPSCs to regenerate *i*NKT cells. In this study, we first examined that efficient iPSCs induction conditions in human circulating T cells and found that additional forced expression of *SV40* together with Yamanaka factors (OSKM; *OCT4, SOX2, KLF4, c-MYC*) by sendai virus (SeV) vector accelerate human iPSCs induction. We then succeeded in establishing iPSCs from human *i*NKT cells (NKT-iPSCs) and the Vα24-Jα18 paired with Vβ11 genomic rearrangement in NKT-iPSCs were confirmed by conventional PCR and direct sequencing. Furthermore, we showed that NKT-iPSCs can differentiate to NKT (iPS-NKT) cells by co-culture with OP9/OP9DLL1. We will discuss about potency for tumor immunotherapy using regenerated *i*NKT cells derived from NKT-iPSCs.

W-2195
PREDICTION OF DIFFERENTIATION TENDENCY OF HUMAN PLURIPOTENT STEM CELLS TOWARD ENDODERM

Yanagihara, Kana[1], Okamura, Minako[1], Kanie, Kei[2], Kato, Ryuji[2], Furue, M K.[1]
[1]National Institute of Biomedical Innovation, Osaka, Japan, [2]Graduate School of Pharmaceutical Sciences, Nagoya University, Nagoya, Japan

Human pluripotent stem cells (hPSCs) including human embryonic stem cells (hESCs) and human induced pluripotent cells (hiPSCs) are able to replicate indefinitely and rapidly. Therefore, hPSCs-derived hepatocytes are useful as a tool for drug toxicity screening. We have previously developed efficient differentiation methods of hPSCs into functional hepatocyte-like cells. However, hPSCs showed variation in differentiation efficiencies into hepatocytes. A method to select a suitable lines from plenty cell lines is required. Previous study demonstrated that the variation in differentiation efficiency into hepatocytes is attributable to the genetic background of the donor cells. Therefore, we hypothesized that gene expression profiles

in undifferentiated hPSCs might be able to predict differentiation efficiency into hepatocytes. In this study, gene expression profiles in undifferentiated state, autonomously differentiated state via embryoid body (EB) formation, and hepatocyte-like cells of one hESC line, and two hiPSC lines (4 clones) were compared. Bioinformatics analysis revealed that there was little correlation between undifferentiated hPSCs and their EB. Our results have shown that hiPSC clones derived from one donor cell line showed variation in gene expression profiles and differentiation efficiency into hepatocytes, suggesting that gene expression in undifferentiated hPSCs might influence differentiation efficiency. Further bioinformatics analysis is ongoing now.

W-2196
FUNCTIONAL ANALYSIS OF PIG NANOG GENE AND ITS REGULATION IN PLURIPOTENT STEM CELLS

Yang, Fan[1], Wang, Huayan[2]
[1]Department of Animal Biotechnology, College of Veterinary Medicine, Northwest A and F University, Yangling, China, [2]College of Veterinary Medicine, Northwest A and F University, Yangling, China

Nanog is a member of homeobox gene and plays the important role to maintain pluripotency of embryonic stem cells. However, the molecular features and transcription regulation of the NANOG gene are not well investigated in domestic animals. In this study, we characterized pig NANOG gene by sequencing alignment, operative regulation of upstream regulatory region and functional expression in pluripotent stem cells. By genomic DNA sequence alignment, two dysfunctional NANOG pseudogenes, one at chromosome 1 (NANOGP1) and the other one at chromosome 5 (NANOGP2), were identifies. The coding sequence and 5' and 3' untranslated regions in NANOG2 show the high homology with the functional NANOG gene located at chromosome 1 (NANOG), but without the conserved homeodomain sequence. The transcriptome analysis confirmed that the NANOG RNA was derived from NANOG gene, but not from NANOGP2. Pig Nanog promoter (PNP) regions from NANOG (PNP-c1, 2.3kb) and NANOGP2 (PNP-c5, 2.5kb) were cloned, and constructed the reporter vectors. The activation of PNP-c1 was only observed at the reproductive tissue cells and pluripotent stem cells, while PNP-c5 activation was detected ubiquitously in both pluripotent stem cells and somatic cells. Within the PNP-c1, several putative transcriptional binding domains, such as Sox2-Oct4 composite site that responses to the regulation of Oct4/Sox2 complex, were identified. The result of serial deletions showed that a 283 bp PNP-c1 promoter was sufficient to direct the NANOG expression in pluripotent cells. Pig Nanog protein derived from chromosome 1 retains N domain, H domain, and C1/W/C2 domain. We identified that the H domain had a nuclear localization signal sequence that was required for NANOG to import into the cell nucleus, and removal of N domain increased NANOG transcriptional activity. Overexpression of NANOG could promote the downstream target genes OCT4 and SOX2 upregulation in pig iPS cells and fibroblasts. Our results confirm that NANOG from chromosome 1 encodes the functional NANOG that can interact with other pluripotency factors and maintain stem cell pluripotency.

W-2197
GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM HUMAN CIRCULATING MULTIPOTENT STEM CELLS

Yang, Han-Mo[1], Kim, Ju-Young[2], Lee, Ju-Eun[2], Jin, Sooryeonhwa[2], Lim, So-Jung[2], Kim, Baek-Kyung[2], Cho, Hyun-Jae[1], Kwon, Yoo-Wook[2], Hur, Jin[1], Cho, Hyun-Ju[2], Lee, Sahmin[1], Han, Jung-Kyu[1], Park, Young-Bae[1], Kim, Hyo-Soo[1]
[1]Department of Internal Medicine, Seoul National University Hospital, Seoul, Republic of Korea, [2]Seoul National University Hospital, Biomedical Research Institute, Seoul, Republic of Korea

Background: The discovery of induced pluripotent stem (iPS) cells has opened up new possibility of molecular understandings for development and therapeutic applications for patient-specific disease. One of the important issues for clinical applications is cell source. Human peripheral blood is one of the easy accessible cell sources. However, isolated peripheral blood cells have shown low gene transfection efficiency and inconveniences requiring specific methods to isolate. Here, we report a novel population of peripheral blood-derived stem cells, which can be easily reprogrammed to iPS cells. Methods and Results: We freshly isolated peripheral mononuclear cells (PBMC) from human peripheral blood and seeded on the fibronectin-coated plate. We observed adherent cells from as early as 5 days after the start of culture and those cells gradually formed colonies. We were able to isolate these cells with very high efficiency. Furthermore, we have also confirmed that these cells can be differentiated to osteogenic, adipogenic, and myogenic-lineage cells. Therefore, we named these cells circulating multipotent adult stem cell (CiMS). We were successful in generating iPS cells with these cells. These cells showed enhanced efficiency of gene transduction, compared to the human dermal fibroblast. We obtained reprogrammed colonies (CiMS-iPS) in 8 days after 4 factor virus transduction without feeder cells. We identified CiMS-iPS had similar features to embryonic stem cell in morphology, gene expression, epigenetic state and ability to differentiate into the three germ layers. We obtained more than 46 iPS cell lines from PBMC of patients with cardiovascular disease and normal volunteers. Conclusions: Our study showed new methods to isolate stem cells from peripheral blood and to generate iPS cells with high efficacy. This suggests that our new approach could be one of ideal methods for clinical application of iPS cells in future.

W-2198
TO CLONE OR NOT TO CLONE: GENERATION OF IPS CELLS BY BATCH SELECTION OF POOLED CULTURE

Yang, Wenli[1], Liu, Ying[1], Wagner, Thomas[2], Mordwinkin, Nicholas[3], Yan, Ruilan[1], Wu, Joseph C.[4], Rader, Daniel[1], Duncan, Stephen A.[2], Morrisey, Edward[1]
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]Medical College of Wisconsin, Milwaukee, WI, USA, [3]Stanford University, Menlo Park, CA, USA, [4]Stanford University School of Medicine, Stanford, CA, USA

Currently, the iPSC field faces two inherent rate-limiting hurdles that must be overcome in order to allow scale-up generation of large numbers of iPSC lines for population studies: 1) the requirement for manually picking and expanding clones and subsequent characterization; and 2) the lack of miniaturization of the cell culture methods. In order to develop higher throughput iPSC generation methods, we sought to bypass the labor-intensive manual colony picking method by applying a batch/pool purification strategy of generating of iPSCs using rigorous cell surface pluripotent marker(s) selection followed by additional characterization for pluripotency. To this end, we batch selected for pluripotent stem cells from a population of newly formed iPS cells by labeling cells with TRA-1-60 or SSEA-

4 antibodies, followed by magnetic activated cell sorting (MACS). To enrich for pluripotent cell populations, we performed 2 rounds of antibody selection over 3-4 passages. By applying this method, we were able to generate homogenous iPS cell pools with similar surface antigen expression profile as compared to iPSC clones derived in parallel from the same individual. We further compared pooled and cloned iPS cells and show that both cell groups retain stable expression of all pluripotency markers tested over long term passaging (up to 70 passages) and exhibit a normal karyotype. Importantly, we show that batch purified iPS cells are able to undergo both spontaneous and lineage specific differentiation in vitro with the same efficacy as clonally derived cells. Using an embryoid body formation assay followed by analysis of tri-lineage specific gene expression using hPSC Scorecard, we show that pools and clones have similar differentiation potential. Finally, iPS cell pools also converted into functional hepatocytes and vascular endothelial cells with similar efficiency and reproducibility as their clonally derived counterparts. Taken together, our results show that generation of iPSCs using the batch method may be a higher throughput alternative to the manual colony picking approach to iPSC derivation. Current studies are underway to address the important question of whether iPSC pools remain multi-clonal through serial passaging.

W-2199
AP-1 TRANSCRIPTION FACTOR JDP2 POTENCIATES THE GENERATION OF MEDULLOBLASTOMA CANCER STEM CELLS FROM INDUCED PLURIPOTENT STEM CELLS

Yokoyama, Kazushige[1], Ku, Chia-Chen[1], Lin, Shih-Han[1], Wuputra, Kenly[1], Lin, Ying-Chu[1], Lin, Chang-Shen[1], Miyoshi, Hiroyuki[2], Nakamura, Yukio[2]
[1]Kaohsiung Medical University, Kaohsiung, Taiwan, [2]RIKEN BRC, Tsukuba, Japan

Transcription factor Jun dimerization protein (JDP2) plays roles in cell cycle regulation, cellular senescence, nuclear reprogramming and oncogenesis through the epigenetic control involved in cascades of $p19^{Arf}$-Mdm2-p53-p21-cyclin/CDK or $p16^{Ink4a}$-cyclin/CDK-RB-E2F. Clinical studies of medulloblastomas indicate that JDP2 might be a tumor suppressor gene candidate because normal granule cells express significant levels of JDP2, whereas cancer cells do not. Thus, we generated three different induced pluripotent stem cells (iPSCs) from human medduloblastoma cancer cells (DAOY1) using Lenti-virus encoded standard 4 factors (4F; Oct4, Klf4, Sox2 and c-Myc), 2 factors (2F; Oct4 and JDP2) and I factor (JDP2). The original DAOY1 expressed three standard stemness genes like Oct4, Sox2 and Nanog, but did not show the alkaline phosphatase activity. By contrast, iPSCs expressed stemness genes and demonstrated the alkaline phosphatase activity. Moreover, we found that iPSCs enhanced the tumor progression as compared with DAOY1 in SCID mice. These results indicate that JDP2 is highly possible to play a critical role of tumor potentiating function of iPSCs to generate the cancer stem cells. We also found the role of JDP2 is concerned the signaling of Wnt signals such as the genes encoding LEF1 (lymphoid enhancer binding protein), TCF3. The cross talk of Wnt signal and LIF/JAK-STAT3-Oct4 will be critical for generation of cancer stem cells from medduloblastoma iPS cells.

W-2200
ETHNICALLY DIVERSE PLURIPOTENT STEM CELLS FOR DRUG DEVELOPMENT

Zakinova, Angela[1], Glenn, Victoria L.[1], Schell, John Paul[1], Pivaroff, Cullen G.[1], Tran, Ha T.[1], Altun, Gulsah[1], Lynch, Candace L.[1], Abdelrahman, Sara[1], Peterson, Suzanne[2], Fakunle, Eyitayo S.[2], Loring, Jeanne F.[2]
[1]Chemical Physiology, The Scripps Research Institute, La Jolla, CA, USA, [2]The Scripps Research Institute, La Jolla, CA, USA

Drug development is a lengthy and expensive process that can last more than 10 years and can cost in excess of $500 million. One reason for these exorbitant costs is "post-marketing drug failure" in which drugs are recalled after hundreds of millions of dollars have been spent getting the drugs to market. The primary reason for post-marketing drug failure is idiosyncratic drug induced liver injury (DILI). DILI may frequently be caused by genomic variations in genes encoding drug metabolizing enzymes as there is extensive evidence showing that these variations affect drug toxicity and efficacy. However, there is currently no practical means to screen candidate drugs in vitro for genome variation-associated toxicity early in the drug development pipeline. Current methods utilize in vitro testing, animal studies and human clinical trials, all of which are suboptimal for capturing population-based genetic variation. We propose that the development of a human induced pluripotent stem cell (iPSC) biobank that captures the most common genetic variations in drug metabolizing enzymes can provide the pharmaceutical industry with a renewable source of cells for early stage toxicology screens. We are building an ethnically diverse panel of iPSCs that can fulfill this need. We derived iPSCs from fibroblasts obtained from skin biopsies and from a multiethnic cohort of healthy individuals, including several Caucasians, African Americans, Asians and Middle Easterns. Using immunocytochemistry, embryoid body-based differentiation techniques and teratoma assays, we have demonstrated that the ethnically diverse iPSC lines analyzed thus far are truly pluripotent. In addition, extensive characterization by whole genome single nucleotide polymorphism (SNP) analysis indicates that the reported ethnicity of the lines matches that determined by SNP analysis. This project is ongoing, and we hope that it will lead to more efficient toxicity screening for drug development.

W-2201
DIFFERENTIAL ROLES OF LIN28A AND LIN28B IN REPROGRAMMING

Zhang, Jin[1], Ratanasirintrawoot, Sutheera[2], Wu, Zhaoting[1], Seligson, Marc T.[1], Cacchiarelli, Davide[3], Shinoda, Gen[1], Ross, Christian[5], Li, Hu[4], Asara, John[5], Scott, Ficarro[6], Marto, Jarrod[6], Cantley, Lewis[7], Daley, George[1]
[1]Boston Children's Hospital, Boston, MA, USA, [2]Harvard University, Cambridge, MA, USA, [3]Harvard University Department of Stem Cell and Regenerative Biology, Cambridge, MA, USA, [4]Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Rochester, MN, USA, [5]Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, 02115, USA, Boston, MA, USA, [6]Dana-Farber Cancer Institute; Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA, [7]Meyer Cancer Center, Weill Cornell Medical College and New York Presbyterian Hospital, New York, NY, USA

LIN28A and its paralog LIN28B are RNA binding proteins that are highly expressed in early embryogenesis, silent in most adult tissues, and re-activated in multiple human cancers. Both proteins bind and inhibit biogenesis of the let-7 family of tumor suppressor microRNAs, which in turn repress multiple genes involved in proliferation, cell

cycle, and mTOR signaling, and both proteins have been reported to bind directly to numerous mRNAs to influence translation. When added to the reprogramming cocktail of OCT4, SOX2, and NANOG, LIN28A enhances reprogramming efficiency, but whether LIN28B functions similarly to promote reprogramming, and by what precise mechanisms remains unanswered. We have observed that addition of either LIN28A or LIN28B can promote efficient reprogramming, implying functional redundancy. However, genetic deletion of either LIN28A or LIN28B also compromises reprogramming efficiency, and double deletion reduces reprogramming to very low levels, suggesting additive and perhaps independent modes of action. In time course reprogramming experiments, LIN28B becomes upregulated in somatic cells before LIN28A. Moreover, LIN28B but not LIN28A is upregulated in partially reprogrammed cells, with up-regulation of LIN28A a more critical indicator of the reprogrammed state. Loss of either LIN28A, LIN28B or both precluded cells from adopting an embryonic mode of glycolytic metabolism, suggesting that LIN28A and B cooperatively influence metabolic targets. Interestingly however, our data argue that the effect of the LIN28 proteins on the reprogramming of cellular metabolism occurs independently of effects on let-7. First, loss of LIN28A, but not LIN28B leads to up-regulation of let-7 in iPS cells and ES cells. Second, knock-down of LIN28A in DGCR8-/- ES cells, which do not produce mature let-7, nonetheless skews metabolism away from glycolysis to favor enhanced oxidative phosphorylation. Finally, LIN28 proteins bind a variety of mRNAs for critical metabolic enzymes that can account for the observed changes in the metabolism of somatic and pluripotent stem cells. These findings implicate the LIN28 proteins as partially redundant metabolic regulators in pluripotent stem cells and reprogramming.

W-2202
A HIGH THROUGHPUT PIPELINE TO CREATE IPSC-DIFFERENTIATED CARDIOMYOCYTES

Zhao, Chang
California State University San Marcos, San Marcos, CA, USA

Despite technological advances, there is still lack of efficient manner of generating cardiomyocytes to serve extensive downstream studies, such as genetic and functional analyses. The goals of our project are: 1) collect fibroblasts from a diverse cohort of 280 individuals, 2) generate iPSC lines from that cohort and, 3) differentiate these iPSC lines into cardiomyocytes for genetic analyses. We have obtained fibroblasts from a cohort of 278 subjects of varying ages (18-88) and ethnicities (all five subpopulations from the 1000 Genomes Project). To optimally reprogram fibroblasts to iPSCs, we chose Sendai Virus (SeV) due to its non-integrative property with human genome. The pluripotency of the iPSCs was checked by flow cytometry, using antibodies against the pluripotency markers SSEA4and TRA-1-81. We also validated and implemented the Illumina HumanCore Array in order to confirm: 1) Sample Identity, comparing SNPs from the iPSC with a matched germline (blood) DNA; 2) Genomic Stability, by examining the samples for copy number abnormalities (CNAs); 3) Genetic Ancestry, comparing data with that of self-reported ancestry; and 4) the presence of disease-related SNPs across the genome. We are optimizing conditions of the cardiac differentiation protocol to develop a pipeline of producing cardiomyocytes from iPSCs, with plating densities, media, passaging reagents, passage numbers taken into account. At present, we are able to differentiate with 80-90% efficiency, as evidenced by beating populations and validated using flow cytometry. Synchronized beatings are first observed at day 8-15, and mature cardiomyocytes are present at day 40+.Our forthcoming goals for this phase of the project are: 1) scale-up of the cardiomyocyte differentiation protocol, 2) develop robust quality control assays for these cardiomyocytes, and 3) to develop functional assays to measure cardiomyocyte electrophysiology. These assays will be the foundation for the genotype – phenotype comparisons we make in downstream genetic analyses. Funding resources: California Institute for Regenerative Medicine (CIRM) and the National Institutes of Health (NIH)

W-2203
IDENTIFICATION OF THE MITOCHONDRIA STATUS IN HUMAN INDUCED PLURIPOTENT STEM CELLS, HUMAN EMBRYONIC STEM CELLS AND THEIR DIFFERENTIATED COUNTERPARTS

Zhu, Lili[1], Gomez-Duran, Aurora[1], Saretzki, Gabriele[2], Lako, Majlinda[1], Armstrong, Lyle[1]
[1]*Institute of Genetic Medicine, Newcastle University, Newcastle, United Kingdom,* [2]*Institute for Ageing and Health, Newcastle University, Newcastle, United Kingdom*

Human somatic cells can be directly reprogrammed into human induced pluripotent stem cells (hiPSCs), which share the properties of self-renewal and pluripotency with Human embryonic stem cells (hESCs). Somatic cell reprogramming and pluripotent cell differentiation involve dramatic mitochondrial changes. For the promise of hiPSCs to be realised, it is necessary to investigate whether mitochondria number and function is regulated in the same way in the differentiated progeny of hiPSCs as in hESCs. In this work, we characterized mitochondria status in 6 pluripotent stem cells, including 2 hESC and 4 hiPSC, and in neural cells derived therefrom. We show that generally hiPSCs got similar mitochondrial status as hESCs; however, human iPSCs and their progeny differ from hESC in some features such as mitochondrial DNA copy number, ATP generation and oxygen consumption rate. Genome-wide transcriptome analysis shows a group of mitochondrial-related genes with distinct expression profile in hiPSCs and hESCs that may underline the observed changes. Together our work indicate that mitochondrial status should be checked before further use of hiPSCs in pathological studies, drug screening, and cell transplantation.

W-2204
INDUCTION OF PLURIPOTENT STEM CELLS FROM SIMIAN FOAMY VIRUS-INFECTED ADULT CYNOMOLGUS MONKEY MESENCHYMAL STEM CELLS

Xu, Lin[1], Sun, Xiaoting[1], Zhang, Yu Alex[2], Zhang, Jian[1], **Zou, Chunlin**[1]
[1]*Center for Translational Medicine, Guangxi Medical University, Nanning,Guangxi, China,* [2]*Cell Therapy Center, Xuanwu Hospital, Capital Medical University, Beijing, China*

Induced pluripotent stem (iPS) cells hold great promise for cell-based therapies in regenerative medicine. Thus it is essential to test their safety and efficacy in large animal models prior to clinical applications. Because of great similarities in genetic evolution, immune system, physiology and metabolism between non-human primates (NHPs) and humans, NHPs provide valuable experimental model systems for modeling human disorders and for developing therapeutic strategies. The present study aims to generate cynomolgus monkey iPS cells from adult mesenchymal stem cells (MSCs) by retroviral expression of Oct3/4, Sox2, Klf4, and c-Myc. However, previous studies have indicated that simian foamy virus (SFV) was highly prevalent in both captive and free ranging NHP populations. Our results also show that approximately 70% of bone marrow MSCs from adult (6-7 years old) cynomolgus monkeys are infected with SFV. SFV can significantly inhibit proliferation of adult cynomolgus monkey MSCs and these MSCs are difficult to grow after the third passage. Therefore, tenofovir, an antiviral agent, need to be added in the cell culture. Here, we test the effects of tenofovir on reprogramming of SFV-infected adult cynomolgus

monkey MSCs. Adult cynomolgus monkey MSCs transduced by the above four reprogramming factors, are treated with 5 or 10μM tenofovir which are added in the cell culture at various stages of reprogramming. We find that cynomolgus monkey embryonic stem (ES) cell-like colonies appear only under the condition that 5μM tenofovir is added into the culture at early stage (days 2 to 6 post transduction). Several colonies are picked and stably maintained in human ES medium. These cynomolgus monkey iPS cell lines show the expression of pluripotency markers, a normal karyotype, and pluripotency by both in vitro and in vivo assays. To our knowledge, this is the first report on iPS cell lines established from SFV-infected adult cynomolgus monkey MSCs. This study provides a foundation for autologous transplantation using iPSCs in treating nonhuman primate disease model. Supported by the National Basic Research Program of China (2012CBA01307, C. Zou), National Natural Science Foundation Project (81360123, C. Zou).

IPS CELLS: DISEASE MODELING

W-2205
PATH TO THE CLINIC: ASSESSMENT OF IPSC-BASED CELL THERAPIES IN VIVO IN A NON-HUMAN PRIMATE MODEL

Hong, So Gun, Winkler, Thomas, Wu, Chuanfeng, Guo, Vicky, Pittaluga, Stefania, Nicolae, Alina, Donahue, Robert, Metzger, Mark, Price, Sandra, Uchida, Naoya, Kuznetsov, Sergei A., Kilts, Tina, Li, Li, Robey, Pamela G., Dunbar, Cynthia
National Institutes of Health, Bethesda, MD, USA

Induced pluripotent stem cell (iPSC)-based cell therapies have great potential for regenerative medicine, but are also potentially associated with significant risks. Current rodent models are not optimal predictors of efficiency and safety for human clinical applications. Therefore, we developed a clinically relevant non-human primate model to assess the tumorigenic potential and *in vivo* efficacy of both undifferentiated and differentiated iPSCs, in the autologous setting without immunosuppression. Transgene-free rhesus macaque iPSCs (RhiPSCs) were derived using the Cre-excisable STEMCCA vector, leaving a short non-expressed vector DNA tag for subsequent tracking studies. RhiPSCs, adapted to culture on Matrigel or the chemically-defined synthetic surface SynthemaxII, retained their pluripotent phenotype. We used the RhiPSCs first to develop a clinically-relevant autologous teratoma model in the presence of an intact immune system, and second to evaluate the potential of clinical use of RhiPSC derived mesodermal stromal-like cells (RhiPS-SCs) for *in vivo* bone regeneration. RhiPS-SCs expressed typical surface markers (CD44, CD73, CD90, CD105, and CD166) and could further differentiate to the osteogenic lineage *in vitro*. $5X10^5$- $2X10^7$ cells from five independent RhiPSC clones were subcutaneously injected into both the donor autologous rhesus monkeys and immunocompromised NSG mice. Prior to implantation, cells were extensively washed and an autologous plasma clot method was used to support teratoma formation, omitting likely immune rejection of a xenogenic scaffold (e.g., Matrigel). Simultaneously, four independent autologous RhiPS-SCs mixed with hydroxyaptite/tricalcium phosphate (HA/TCP) ceramic particles were subcutaneously implanted to investigate *in vivo* bone formation. After 3-14 weeks, all RhiPSC implants at the cell doses used in this study resulted in teratomas in NSG mice. In autologous monkeys, undifferentiated autologous iPSCs indeed formed teratomas in a dose-dependent manner. However, teratoma formation is less efficient in autologous monkeys than in NSG mice. With cell doses of $5X10^5$, $2X10^6$, $5X10^6$, $1X10^7$, and $2X10^7$ RhiPSCs, 0/5, 0/5, 0/1, 4/10, and 3/4 implants resulted in teratomas in autologous rhesus monkeys, respectively. PCR for the vector LTR confirmed RhiPSC origin. Teratomas obtained from the autologous monkeys, but not from NSG mice, were accompanied by an inflammatory reaction including a significant lymphocytic infiltration in the tumor and the surrounding tissue, composed by a mixture of CD4+ and CD8+ T-lymphocytes, CD20+ B-lymphocytes and few CD56+ cells. Teratomas as well as the inflammatory reaction persisted up to at least 30 weeks post-injection. We performed OCT3/4 staining to investigate whether the immune response is related with residual endogenous pluripotency gene expression, and found no residual OCT3/4 positive cells were found in teratomas. In contrast, simultaneously implanted RhiPS-SCs in HA/TCP formed well defined bone trabeculae in the autologous monkeys, without evidence of teratoma formation or evident inflammatory reaction, up to 39 weeks post-implantation. These studies represent the first autologous large-animal teratoma model, and demonstrate the autologous regenerative potential of iPSCs to form functional bone tissue *in vivo.*

W-2206
UTILIZATION OF AN INDUCED PLURIPOTENT STEM CELL APPROACH TO EXAMINE SRCAP MUTATIONS IN FLOATING-HARBOR SYNDROME

Hood, Rebecca L.[1], Boycott, Kym M.[2], Stanford, William L.[3], Bulman, Dennis E.[4]
[1]Biochemistry, Microbiology, and Immunology, CHEO Research Institute, University of Ottawa, Ottawa, ON, Canada, [2]Genetics, CHEO Research Institute, University of Ottawa, Ottawa, ON, Canada, [3]Ottawa Hospital Research Institute, Ottawa, ON, Canada, [4]Pediatrics, CHEO Research Institute, University of Ottawa, Ottawa, ON, Canada

Floating-Harbor Syndrome (FHS) is a rare genetic disorder which is characterized by short stature, delayed osseous maturation, language deficits, and unique dysmorphic facial features. Utilizing an exome-sequencing approach, we identified heterozygous truncating mutations in SRCAP as the cause underlying FHS. Subsequent targeted Sanger sequencing has resulted in the identification of SRCAP mutations in a total of 50 FHS patients. The mutations identified were shown to be de novo in all instances where parental DNA was available. It is anticipated that these mutations result in widespread gene dysregulation; however the mechanisms and biological pathways underlying the FHS phenotype are currently unknown. As patient cartilage was unavailable for molecular characterization, we chose an induced pluripotent stem cell (iPSC) approach to examine the growth deficiency phenotype of FHS. FHS patient fibroblast and gender-matched wild-type fibroblast samples were therefore reprogrammed into iPSCs using an episomal vector system. iPSCs were subsequently characterized by pluripotency cell surface marker expression, in vitro embryoid body differentiation, and in vivo teratoma formation assays. Karyotype analysis was performed to ensure there were no chromosomal rearrangements after reprogramming. Our current efforts are directed at further characterizing these iPSC clones and differentiating them into cartilage. We can then use FHS patient-derived chondroprogenitors to elucidate the molecular mechanism underlying the bone pathogenesis of FHS.

W-2207
COMPOUND HETEROZYGOUS INHERITANCE OF NOTCH1 VARIANTS IMPAIRS CARDIOGENESIS IN PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS CREATED TO MODEL HYPOPLASTIC LEFT HEART SYNDROME

Hrstka, Sybil, Theis, Jeanne, Li, Xing, Olson, Timothy, Nelson, Timothy J.
Mayo Clinic, Rochester, MN, USA

Hypoplastic left heart syndrome (HLHS) is a congenital heart disease characterized by severe underdevelopment of the left ventricle, ascending aorta, and left heart valves. Multiple genetic components contribute to its pathophysiology and this complexity has precluded the creation of an animal model for disease modeling. Here, we established an in-vitro platform using patient-specific hiPSCs to rapidly assess the genetic etiology for heart chamber malformation focusing on comparisons within a parent-proband triad. The general approach integrates whole genome sequencing of individuals in a multiplex family comprised of an HLHS-affected patient and two relatives with milder congenital heart disease (CHD), filtering candidate genes based on our roadmap of heart development, and experimental validation of disease-causing genes in studies that assess gene expression throughout the directed cardiovascular lineage differentiation of hiPSCs. Initial screening identified two distinct variants of NOTCH1, a CHD-related gene previously implicated in aortic valve disease, and both variants were inherited by the proband. In vitro modeling of cardiac development with HLHS-affected and non-HLHS-affected hiPSCs has revealed that cardiogenesis is impaired in the HLHS-affected hiPSCs. Moreover, the HLHS-affected hiPSCs exhibit diminished expression levels of several Notch signaling pathway components during the early specification steps in the guided conditions for cardiac differentiation. Our findings are supportive of the hypothesis that the severe cardiac phenotype of HLHS can be attributed to mutation burden in NOTCH1 in conjunction with mutations in genes that modify Notch signaling.

W-2208
MEASURING CONNECTIVITY IN HUMAN IPS CELL DERIVED NEURONS USING A MULTI ELECTRODE ARRAY

Jones, Eugenia, Carlson, Coby B., Mangan, Kile P., Arnold, Brian, DeLaura, Susan, Thompson, Arne, Ott, Vanessa
Cellular Dynamics International, Madison, WI, USA

With the dramatic rise in the incidents of neurological diseases, there is a sense of urgency to develop better pharmaceuticals and therapies. However, a major challenge in neuroscience research and drug development is access to clinically-relevant cell models. Human induced pluripotent stem cell (iPSC)-derived neurons provide a novel model to help facilitate a better understanding of the mechanisms of neurological diseases in a physiologically relevant environment. Using the Axion Maestro multi-electrode array (MEA) platform, we have assessed neuronal activity and bursting behaviors in iPSC-derived neuronal cultures. This highly pure population of neurons are derived from a normal human donor. Herein, we describe the culture conditions, assay protocol, and analytical tools used to record the neuronal activity of human iPSC-derived neurons. We demonstrated dose-dependent responses to multiple glutamatergic- (APV, DNQX, Kainic Acid), as well as GABAergic- (Gabazine, L655-708), selective pharmacological agents for both over-all firing rates and bursting behaviors. Moreover, the complete blocking of glutamatergic activity (co-application of APV and DNQX) abolished all neuronal activity, indicating the dependence on synaptic connectivity. Our findings highlight the differences from primary rodent cultures on MEA and support the conclusion that iPSC-derived neuronal cultures display network activity that can be measured and modulated within one week in culture.

W-2209
ABERRANT NEURONAL DIFFERENTIATION OF NOONAN SYNDROME IPSCS IN VITRO

Ju, Younghee[1], Lee, Beom-Hee[2], Yoo, Han-Wook[2], Han, Yong-Mahn[1]
[1]Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Republic of Korea, [2]Department of Pediatrics, Asan Medical center, Seoul, Republic of Korea,

Embryonic development is finely controlled by both intrinsic and extrinsic mechanisms. Single-gene mutation may influence abnormal organogenesis during embryonic development. Noonan syndrome (NS) is one of genetically heterogeneous developmental disorders caused by hyper-activation of Ras-MAPK pathway. It is clinically characterized by congenital heart defect, facial dysmorphism, neurocognitive delay, feeding disability, learning problem, hearing loss, skeletal and hematological abnormalities. However, how activated Ras-MAPK pathway translates complex features of NS during human developmental process remains elusive. To elucidate cellular mechanisms, induced pluripotent stem cells of NS (NS-iPSCs) were generated from patient fibroblasts with SHP2-activating mutation. NS-iPSCs showed smaller and abnormal structures during embryoid body formation, eventually resulting in failure of neural differentiation. When Ras-MAPK signaling was inhibited, NS-iPSCs developed to neural rosettes and neural precursors with normal transcriptional profiles of neuronal genes. These results indicate that activated Ras-MAPK signaling may affect specification of neuronal fate during early embryonic development in Noonan syndrome.

W-2210
GENERATION OF CARDIOMYOCYTE FROM SYNOVIOCYTES OF PATIENTS WITH RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS VIA INDUCED PLURIPOTENT STEM CELL

Jung, Seung Min, Park, Chang Hee, Park, Sung-Hwan, **Ju, Ji Hyeon**
Catholic University, St Mary's Hospital, Seoul, Republic of Korea,

Objectives*:* Cardiovascular complication is a main cause of morbidity in arthritic patients. Cardiovascular complication in rheumatoid arthritis (RA) occasionally results from inflammatory insults and in osteoarthritis (OA) from degenerative aging process. To differentiate into cardiomyocytes from induced pluripotent stem cells (iPSCs) of the patients with RA and OA give more opportunity for disease modeling or cell replacement therapy in arthritis-associated cardiomyopathy. Methods: Total 4 clones of iPSCs were generated from synoviocytes of two RA patients and two OA patients using 4-in-1 lentiviral vector. iPSCs were checked for pluripotency with RT-PCR on Nanog, Oct4, Sox2, Klf4 and with immunofluorescence on Nanog, SSEA4, Oct4, and Tra-1-60. Established iPSC lines from 4 clones were differentiated into cardiomyocyte lineages (iPSC-CMs) using standard 3D EB differentiation protocols. To investigate whether cardiac cells were properly generated, immunofluorescence and calcium imaging were performed. Results: iPSC colonies were successfully generated from RA and OA synoviocytes. mRNA expression of Nanog, Oct4, ZPF-42, and Sox2 for pluripotency markers were elevated after reprogramming process. After 30 days of differentiation protocol for cardiomyocytes, RA-iPSC-cardiomyocytes and OA-iPSC-cardiomyocytes were generated. Immunofluorescence study and RT-PCR were positive results proving of mature cardiomyocyte. Calcium handling image was well observed at RA/OA-iPSC-cardiomyocytes.

Conclusions: To generate patient-specific cardiomyoctes in arthritic patient could be potential cell source of cell therapy when those patients should take diagnosis "in a dish" or cytotherapy against cardiomyocytes.

W-2211
STEM CELL-DERIVED MOTOR NEURONS FROM SPINAL AND BULBAR MUSCULAR ATROPHY PATIENTS

Kats, Ilona[1], Grunseich, Christopher[1], Zukosky, Kristen[2], Ghosh, Laboni[1], Harmison, George[1], Bott, Laura[1], Rinaldi, Carlo[1], Chen, Kelian[1], Chen, Guibin[3], Boehm, Manfred[4], Fischbeck, Kenneth H.[1]
[1]National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA, [2]Brown University Neuroscience, Providence, RI, USA, [3]National Institutes of Health, Bethesda, MD, USA, [4]NIH/ NHLBI, Bethesda, MD, USA

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is an X-linked neuromuscular disease caused by a polyglutamine repeat expansion in the androgen receptor. The disease is known to cause degeneration of motor neurons through a ligand-dependent toxic gain of function, but the exact mechanism is not known. Motor neurons differentiated from SBMA patient derived induced pluripotent stem cells (iPSCs) provide a model for characterizing the disease mechanism and designing potential therapy. iPSCs were generated from six patients and compared to three control lines. Motor neurons were differentiated from the iPSCs, treated with androgen, and assessed for disease relevant phenotypes. The expanded polyglutamine repeats were unstable during re-programming, with several lines showing expansion or contraction. Patient iPSCs expressed less androgen receptor than control lines but show androgen dependent stabilization and nuclear translocation with ligand exposure. Stem cell derived motor neurons showed immunoreactivity for HB9, Isl1, ChAT, and SMI-32, and were differentiated with similar efficiency between the patient and control lines, with no detectable difference appreciated following androgen treatment. Patient-derived motor neurons were found to have decreased HDAC6 levels and those with longer repeat lengths were shown to also have increased acetylated α-tubulin. This finding was confirmed in patient spinal cord sections and in stably transfected mouse cells. Another HDAC6-dependent process, perinuclear lysosomal enrichment, was disrupted in motor neurons from iPSC lines with longer repeat lengths. The observed reduction in androgen expression, increase in acetylated α-tubulin and decrease in HDAC6 provide opportunities for further investigation of the SBMA disease mechanism and potential treatment.

W-2212
CELLULAR MODELING OF CITRIN DEFICIENCY USING INDUCED PLURIPOTENT STEM CELLS

Kim, Yeji[1], Choi, Jung-Yoon[2], Lee, Beom-Hee[3], Yoo, Han-Wook[3], Han, Yong-Mahn[1]
[1]Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea,, [2]Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea,, [3]Department of Pediatrics, Asan Medical Center, Seoul, Republic of Korea,

Human induced pluripotent stem cells (iPSCs) are useful for studying cellular modeling of many genetic disorders. Citrin deficiency (CD) is a recessive genetic disorder which has a mutation on *SLC25A13* gene encoding citrin. Mutation on *SLC25A13* gene leads to adult-onset citrullinemia (CTLN2) as well as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). The patients show diverse symptoms in liver such as liver-specific urea cycle enzyme downregulation, abnormal amino acid accumulation, and hyperammonemia. However, cellular mechanisms of citrin deficiency have not been reported in detail yet. Here, we generated iPSCs from dermal fibroblasts of a citrin deficiency patient (CD-iPSCs) by ectopic expression of Yamanaka's factors. CD-iPSCs showed normal characters of human pluripotent stem cells. CD-iPSCs were differentiated into hepatocytes to understand pathophysiology of liver failure which is a typical feature of the disease. Hepatocytes derived from CD-iPSCs normally expressed hepatocyte-specific markers such as α-antitrypsin and albumin with functionality of LDL uptake, like hESC-derived hepatocytes. CD-iPSC-derived hepatocytes represented the disease phenotypes such as depletion of citrin protein and failure of urea production. Thus, we found that CD-iPSCs partly mimicked the disease symptoms. Cellular modeling of citrin deficiency using iPSCs will be useful for understanding unknown mechanisms of the disease.

W-2213
TARGETED INVERSION AND REVERSION OF THE BLOOD COAGULATION FACTOR 8 GENE IN HUMAN iPS CELLS USING TALEN

Kim, Dong-Wook
Department of Physiology, Yonsei University College of Medicine, Seoul, Republic of Korea,

Hemophilia A, one of the most common genetic bleeding disorders, is caused by various mutations in the blood coagulation factor VIII (F8) gene. Among the genotypes that result in hemophilia A, two different types of chromosomal inversions that involve a portion of the F8 gene are most frequent, accounting for almost half of all severe hemophilia A cases. In this study, we used a TALEN pair to invert a 140-kbp chromosomal segment that spans the portion of the F8 gene in human induced pluripotent stem cells (iPSCs) to create a hemophilia A model cell line. In addition, we reverted the inverted segment back to its normal orientation in the hemophilia model iPSCs using the same TALEN pair. Importantly, we detected the F8 mRNA in cells derived from the reverted iPSC lines but not in those derived from the clones with the inverted segment. Thus, we showed that TALENs can be used both for creating disease models associated with chromosomal rearrangements in iPSCs and for correcting genetic defects caused by chromosomal inversions. This strategy provides an iPSC-based novel therapeutic option for the treatment of hemophilia A and other genetic diseases caused by chromosomal inversions.

W-2214
MOTOR NEURON PROTEOME REVEALED BY QUANTITATIVE MASS SPECTROMETRY

Klim, Joseph, Williams, Luis, Eggan, Kevin Carl
Harvard University, Cambridge, MA, USA

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective loss of upper and lower spinal motor neurons. Our inability to isolate these faulty cells from patients and study them in vitro has impeded progress for decades. To identify the root cause of ALS and develop effective therapeutics, research efforts are needed that drive at the cellular basis of neural degeneration. To this end, our group has introduced an ALS-causing mutation into a human embryonic stem cell line. Specifically, zinc-finger nucleases were used to induce a dominant missense mutation (A4V) in the superoxide dismutase 1 gene (SOD1). Both cell lines can be differentiated into spinal motor neurons with high efficiencies. Using this isogenic pair of stem cell lines, we applied in-depth mass spectrometry-based proteomics to purified populations of stem cell-derived motor neurons. To facilitate relative quantification of proteins

from the isogenic samples, stable isotope labeling by amino acids in cell culture (SILAC) was employed. We demonstrate that effective incorporation of the heavy amino acids can be achieved during the differentiation process. Moreover, these experiments resulted in the identification of more than 6000 proteins expressed by motor neurons. Quantitative analysis revealed differences in proteins levels between the neurons with wildtype SOD1 and those with mutant SOD1. To take full advantage of mass spec-based proteomics, we also employed serial enrichment of to reveal differences in phosphorylation events. These investigations provide clues as to the molecular pathology of ALS and could yield new therapeutic targets.

W-2215
ALTERED EPIGENETIC AND TRANSCRIPTIONAL REGULATION OF INDUCED PLURIPOTENT STEM CELLS IN PATIENTS WITH HYPOPLASTIC LEFT HEART SYNDROME

Kobayashi, Junko[1], Yoshida, Masashi[2], Tarui, Suguru[1], Ishigami, Shuta[1], Okuyama, Michihiro[1], Nagai, Yusuke[3], Kasahara, Shingo[1], Naruse, Keiji[3], Ito, Hiroshi[2], Sano, Shunji[1], Oh, Hidemasa[4]

[1]Cardiovascular Surgery, Okayama University, Okayama, Japan, [2]Cardiovascular Medicine, Okayama University, Okayama, Japan, [3]Cardiovascular Physiology, Okayama University, Okayama, Japan, [4]Okayama University Hospital, Okayama, Japan

Background: Although a number of studies have uncovered heterozygous mutations in cardiac regulatory genes caused hypoplastic left heart syndrome (HLHS), the identified genetic variants may not be directly correlated with biological insights that potentially contribute to disease development. The genetic basis of patients with HLHS remains unknown and the lack of animal models to reconstitute the cardiac maldevelopment has hampered the study of this disease. Induced pluripotent stem (iPS) cells, which closely resemble embryonic stem (ES) cells, can be generated from human somatic tissues from a variety of diseases to recapitulate complex physiological phenotypes. Patient-specific iPS cells may provide an additional tool for studying human heart disease. The aim of this study is to determine the epigenetic and transcriptional network responsible for myocardial patterning and morphogenesis during cardiac development with respect to left ventricle hypoplasia in humans.
Methods: Cardiac progenitor cells (CPCs) were isolated from patients with written informed consent. Two-independent iPS cell lines were generated from both HLHS and BV heart-derived CPCs, respectively. Global gene expression of HLHS- and biventricle (BV) heart-derived iPS cells was analyzed to dissect the complex genetic circuits by using human ES and iPS cells (201B7) as controls. Real-Time RT-PCR, mutation analysis includes NKX2-5, HAND1, and NOTCH1, ChIP assay, and cardiac-specific gene promoter activity were examined and compared with these cells before and after myocardial lineage induction.
Results: We found one synonymous single nucleotide polymorphism in NKX2-5 and five in NOTCH1, respectively. No mutation was detected in these genes in all samples. Cardiac transcriptional factors such as NKX2-5 and HAND1, known to drive cardiac growth and morphogenesis through primary heart field development, were significantly downregulated in HLHS-derived iPS cells at 2 to 3 weeks after differentiation compared with their levels in control 201B7 iPS- and BV-iPS-derived cardiomyocytes. ChIP assay showed that a marked decrease in dimethylated histone H3-lysine 4 (H3K4me2) and acetylated histone H3 (acH3) was found within the NKX2-5 promoter regions in differentiated HLHS-derived iPS cells compared with those from BV patient. We also identified a significantly increased trimethylated H3-lysine 27 (H3K27me3) in the differentiated HLHS-derived iPS cells. In addition, NOTCH/HEY expressions were markedly reduced, those may be responsible for the obstruction in the regions of atrioventricular and outflow tract myocardium. To specify the target transcripts responsible for cardiac development of HLHS, cardiac troponin-T (TNNT2) promoter analysis was performed by using disease-specific CPCs and iPS cells. Obviously, TNNT2 promoter activity was suppressed in HLHS-derived CPCs and iPS cells compared with those from BV hearts before cardiac differentiation. These repressed promoter activities could be fully restored by transient transfection of NKX2-5, HAND1, and NOTCH1 genes into these stem/progenitor cells by synergistic fashion.
Conclusions: These findings suggest that patient-specific iPS cells may provide potential molecular insights to dissect the complex cardiac malformations in human. The epigenetic and transcriptional regulation of NKX2-5, HAND1, and NOTCH1 may play crucial a role in the development of myocardial growth, patterning, and morphogenesis in HLHS.

W-2216
CELLULAR STRESS RESPONSES OF A53T ALPHA-SYNUCLEIN IPS-DERIVED NEURONS REVEAL DEFECTS IN OXIDATIVE STATE, APOPTOSIS AND PROTEIN DEGRADATION

Kouroupi, Georgia[1], Taoufik, Era[1], Tsioras, Konstantinos[1], Bohl, Delphine[2], Antoniou, Nasia[1], Chroni, Dafni[3], Politis, Panagiotis K.[4], Stellas, Dimitris[5], Vekrellis, Kostas[4], Bregestovski, Piotr[6], Stefanis, Leonidas[4], Matsas, Rebecca[1]

[1]Laboratory of Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, Athens, Greece, [2]Unité Rétrovirus et Transfert Génétique, INSERM U622, Department of Neuroscience, Institut Pasteur, Paris, France, [3]Department of Biochemistry, Hellenic Pasteur Institute, Athens, Greece, [4]Division of Basic Neurosciences, Biomedical Research Foundation of the Academy of Athens, Athens, Greece, [5]Department of Cancer Biology, Biomedical Research Foundation of the Academy of Athens, Athens, Greece, [6]Epilepsy and Cognition Brain Dynamics Institute, INSERM U 1106, Aix-Marseille University, Marseille, France

Parkinson's disease (PD) is a common, progressive neurodegenerative disorder characterized by loss of dopaminergic neurons in the nigrostriatal pathway of the brain, resulting in motor and cognitive deficits. A major drawback hampering PD research is the lack of appropriate models and inaccessibility to live human neurons. Cellular reprogramming technologies and the generation of human induced pluripotent stem (iPS) cells now offer a unique opportunity to study disease pathogenesis and may have important implications in Regenerative Medicine. Here we report the generation of iPS cells from skin fibroblasts of Parkinsonian patients with a familial form of the disease and aged-matched unaffected individuals. The dominantly inherited G209A mutation in the α-synuclein gene SNCA encoding the A53T mutant α-synuclein protein (αSYN) was first identified in the Greek population and is associated with the appearance of an early form of PD which is prevalent in Greece (Polymeropoulos et al. Science 1997). Multiple iPS lines were generated and their pluripotency and karyotype integrity was confirmed using a number of in vitro and in vivo assays. As wild-type αSYN and the A53T mutant protein have a central role in PD we developed an iPS-based cellular model to study disease pathogenesis by directed differentiation of patient-derived iPS cells to dopaminergic precursors and neurons that fire action potentials. We found that the patient cells were more sensitive to cell death caused by exposure to various stress agents suggesting defects in mitochondrial function, protein degradation, oxidative state, apoptosis and exosome function. This model represents a new experimental system to investigate the mechanistic basis of neurodegeneration

caused by α-synuclein dysfunction with ultimate aim to develop novel therapeutics for PD.

W-2217
MODELLING CAMPOMELIC DYSPLASIA USING INDUCED PLURIPOTENT STEM CELLS

Kwon, Sarah[1], Chang, Wing Y.[2], Carpenedo, Richard[3], Manias Rothberg, Janet[4], Chen, Zhaoyi[4], Li, Betty[1], Kandel, Rita A.[5], Stanford, William L.[3]

[1]University of Toronto, Toronto, ON, Canada, [2]Stemcell Technologies, Vancouver, BC, Canada, [3]Ottawa Hospital Research Institute, Ottawa, ON, Canada, [4]University of Ottawa, Ottawa, ON, Canada, [5]Mount Sinai Hospital, Toronto, ON, Canada

The goal of this study is to dissect the molecular pathogenesis of Campomelic Dysplasia (CD), a skeletal malformation disease caused by heterozygous mutations affecting SOX9 expression. Since access to embryonic cartilage tissue is not feasible and various mutations can result in different phenotypic outcomes, induced pluripotent stem cells (iPSCs) were generated from two different CD patients and differentiated in vitro and in vivo towards chondroprogenitors and cartilage tissue, respectively. Patient fibroblasts were obtained from two CD patients encoded by different SOX9 mutations (referred to as CD short and CD long) as well as two healthy, gender-matched donor controls (WT iPSCs). Fibroblasts were reprogrammed to iPSCs through retroviral overexpression of OCT4, SOX2, KLF4, and C-MYC. Three iPSC clones from each CD patient and two clones from each healthy control were characterized and used in this study. Molecular differences between CD and WT iPSCs were identified through in vitro and in vivo differentiation methods. For in vitro methods, iPSCs were first differentiated towards mesendoderm fate using a combination of BMP4 and Activin A. Cells were further differentiated towards chondroprogenitors using a micromass culture approach. For in vivo methods, iPSCs were injected intramuscularly into immunodeficient mice. Teratomas formed 12-16 weeks later and cartilage tissues generated within the teratomas were analyzed and compared. Results: iPSCs generated from CD patient fibroblast samples expressed human embryonic stem cell surface markers, differentiated in vitro and in vivo into all three germ layers, and displayed normal karyotypes. During early differentiation towards mesendoderm, CD long iPSCs maintained an epithelial-like morphology and had reduced expression of mesendoderm and epithelial-to-mesechymal transition (EMT) genes compared to CD short and WT iPSCs. Further differentiation of iPSCs towards chondroprogenitors revealed that CD long iPSCs had reduced levels of proteoglycans and reduced expression of cartilage genes compared to CD short and WT iPSCs. All iPSC lines were able to form cartilage tissue expressing type II collagen and proteoglycans in vivo with no gross morphological differences between WT and CD tissues. Conclusions: iPSCs can be derived from patients with heterozygous mutations in SOX9, as SOX9 is not known to play a key role in pluripotency or reprogramming. CD short iPSCs differentiated efficiently towards mesendoderm and chondroprogenitors, similar to WT iPSCs. However, CD long iPSCs exhibited delayed differentiation towards mesendoderm, affecting their capacity to differentiate towards chondroprogenitors. This suggests SOX9 may play a role in specification to mesendoderm, one of the embryonic origins of cartilage tissue. We have shown that different mutations in SOX9 can lead to different phenotypic outcomes whose mechanisms can be investigated using iPSCs as a human in vitro model. Since SOX9 controls expression of key extracellular matrix genes found in embryonic and adult cartilage tissue, CD iPSC-derived chondroprogenitors can be used to elucidate pathways in which aberrant expression of SOX9 leads to improper expression of these genes. Further, we have established a differentiation protocol and set of assays that can be applied to modelling of other cartilage-associated diseases.

W-2218
AN INTEGRATED MULTI ELECTRODE ARRAY NANODEVICE FOR DRUG-INDUCED CARDIOTOXICITY SCREENING AND DRUG DISCOVERY

Kz, Kshitiz[1], Schwerdt, Helen[2], Choi, Youngsoo[3], Gray, Kevin[1], Kim, Peter[1], Laflamme, Michael[4], Chae, Junseok[2], Kim, Deok-Ho[5]

[1]Bioengineering, University of Washington, Seattle, WA, USA, [2]ECEE, Arizona State University, Tempe, AZ, USA, [3]Biomedical Engineering, University of Washington, Seattle, WA, USA, [4]Pathology, University of Washington, Seattle, WA, USA, [5]University of Washington Department of Bioengineering, Seattle, WA, USA

Safety pharmacological concerns are the major cause of drug attrition during clinical development, with cardiovascular-related toxicities (usually arrhythmias) accounting for most of the total. While late detection of cardiotoxicity is costly, there are additional concerns that the currently mandated hERG assay test results in well recognized false positives and false negatives, potentially limiting many efficacious drugs to be developed. Currently mandated hERG test is physiologically suboptimal since it checks for blockage of a single ion channel, account for most but not all drug induced arrhythmias. Recent advances in stem cell derived somatic cell system has spurred interest in screening for cardiotoxicity using human pluripotent stem cell derived cardiomyocytes. However, these cardiomyocytes are immature, have a fetal phenotype, and can only be probed as single cells, or as an unorganized multicellular ensemble not recapitulating the intrinsic organization of cardiac tissue. We have created a polymeric nanotextured surface to culture human pluripotent stem cell derived cardiomyocytes. Culturing human pluripotent stem cell derived cardiomyocytes on our nanogrooved surfaces promoted their maturation into a more adult-like cardiomyocyte phenotype, and organized them into the highly aligned architecture of mature myocardium. Additionally, we have integrated our nanotextured surface with a multi-electrode array (MEA) compatible with commercial amplifiers facilitating direct electrical recording of action potential propagation across the human heart tissue mimetic. The integrated MEA nanodevice can be used for drug cardiotoxicity screen, for drug discovery of anti arrhythmogenic compounds, as well as a research tool to study cardiac function in an in vitro human cardiac tissue mimetic.

W-2219
TIME COURSE GENE EXPRESSION PROFILING OF HUMAN IPS CELL MODELS OF WILLIAM SYNDROME THROUGHOUT NEURONAL DIFFERENTIATION

Lalli, Matthew A.[1], Park, J. Christine[1], Wang, Yidi[1], Budisteanu, Magdalena[2], Arghir, Aurora[3], Kosik, Kenneth S.[1]

[1]Neuroscience Research Institute, University of California, Santa Barbara, CA, USA, [2]Neuropediatric Pathology, Alexandru Obregia Clinical Hospital of Psychiatry, Bucharest, Romania, [3]Clinical Cytogenetics, Victor Babes National Institute of Pathology, Bucharest, Romania

Williams Syndrome (WS) is a neurodevelopmental disorder characterized by a 1.5 Mb deletion of ~26 contiguous genes resulting in a characteristic physical, cognitive and behavioral profile. Although the genotype and phenotype of this disorder are both well-characterized, genotype-phenotype correlations underlying the neurological aspects of the diseases remain largely unknown. Until now, animal models and atypical patient deletions have provided the best tools to study this disorder. The seminal technological breakthrough of generating patient-specific induced pluripotent stem cells (iPSCs) has enabled us

to generate stem cell models of WS from two WS individual's fibroblasts. We have profiled the transcriptomes of these cells at four time points as cells are differentiated from stem cell to induced neuron and we have identified which of the WS deleted genes are haplodeficient at the mRNA level at each stage. Remarkably, we detect differentially expressed (DE) genes between control and WS cells at the iPS cell stage, including 6 WS deleted genes. Global transcriptional consequences of this contiguous genetic deletion syndrome increase throughout differentiation and DE gene lists are enriched for key neurodevelopmental pathways. These findings suggest neuronal differentiation of our models of WS can help elucidate the molecular circuitry underpinning the neurological hallmarks of WS.

W-2220
UNDERSTANDING THE MOLECULAR BASIS OF FRAGILE X SYNDROME

Lee, Eveline[1], Boland, Michael[2], Nazor, Kristopher L.[3], Loring, Jeanne F.[4]

[1]Chemical Physiology, The Scripps Research Institute, La Jolla, CA, USA, [2]The Scripps Research Institute, La Jolla, CA, USA, [3]The Scripps Research Insitute, San Diego, CA, USA, [4]The Scripps Research Institute, La Jolla, CA, USA

Fragile X Syndrome (FXS), a genetically determined neurodevelopmental disorder, is the most common known cause of inherited intellectual disability and a leading single-gene cause of autism among boys. FXS is associated with the expansion of a trinucleotide CGG repeat in the 5'UTR of the FMR1 gene on the X chromosome, resulting in epigenetic silencing and the loss of expression of the fragile X mental retardation protein (FMRP). FMRP is an mRNA binding protein that is abundant in the brain and functions as a translational repressor. Importantly, approximately one third of mRNAs encoding presynaptic and postsynaptic proteins are targets of FMRP. Disruption of FMRP expression during neural development leads to dysregulation of protein synthesis in neuronal dendrites and synapses in the cortex, hippocampus and cerebellum; the net result of this aberrant regulation is a higher density of immature dendritic spines, which are abnormally elongated. An altered neural differentiation potential has been described recently for FXS-neural precursor cells (NPCs) in which gliogenesis is promoted relative to neurogenesis in the absence of FMRP; this suggests that the underlying molecular defects of FXS could arise as early as the progenitor stage and that studies aimed at early events at the cellular level might be more informative than current approaches involving adult patients or animals. We have generated a bank of biopsy-derived induced pluripotent stem cell (hiPSC) lines from FXS patients with varying clinical manifestations of the disease. FXS-hiPSCs display a neurogenic defect early during neuronal differentiation relative to control hESC and hiPSC lines. Accordingly, the two aims of this project are designed to map the molecular differences between control and FXS iPSCs during differentiation using established methods and a novel single cell approach. The clear phenotypic difference in neurogenesis between disease specific and normal iPSCs provides a robust framework to study Fragile X Syndrome. This will enable us to identify genes and pathways that are dysregulated early in FXS, which are potential targets for future therapies.

W-2221
PHENOTYPES IN HUMAN IPS CELL-DERIVED DOPAMINERGIC NEURONS WITH GLUCOCEREBROSIDASE MUTATION FROM MONOZYGOTIC TWINS DISCORDANT FOR PARKINSON'S DISEASE

Li, Aiqun[1], Woodard, Chris[1], Campos, Brian[1], Kuo, Sheng-Han[2], Nirenberg, Melissa[3], Nestor, Michael[1], Sulzer, David[2], Zimmer, Matthew[1], Lipnick, Scott[1], Chang, Stephen[1], Noggle, Scott[1]

[1]The New York Stem Cell Foundation Research Institute, New York, NY, USA, [2]Columbia University Medical Center, New York, NY, USA, [3]New York University Langone Medical Center, New York, NY, USA

Parkinson's disease (PD), a neurodegenerative disorder that disproportionately affects midbrain dopaminergic neurons, is attributed to a combination of genetic and environmental risk factors. Recently, we recruited a set of monozygotic twins of Ashkenazi Jewish background who remain discordant for PD after five years of disease in the affected twin. Whole-exome sequencing indicates that both twins carry the heterogeneous mutation of glucocerebrosidase (GBA N370S), suggesting increased genetic susceptibility to PD. To address the contribution of the GBA N370S mutation and why only one twin is suffering from PD, we derived induced pluripotent stem (iPS) cells, neural stem cells (NSCs) and characterized midbrain dopaminergic (mDA) neurons from the affected twin (PD-twin), unaffected twin (control twin), subjects with sporadic PD, and healthy controls. Using fluorescence-activated cell sorting (FACS), we dramatically improved the purity of NSCs (~ 90%) and mDA neurons (70 - 80%) compared with earlier studies. These robust techniques ensure standardized accuracy and reproducibility in the subsequent quantitative studies that include assessing transcriptome profile, determining α-synuclein (α-Syn) level, and measuring dopamine synthesis/release. In twins' GBA N370S iPS cell-derived mDA neurons, GBA activity was only ~ 50% of the controls and α-Syn protein level_not mRNA level_increased up to three-fold, indicating the impaired α-Syn degradation in neurons with GBA enzyme deficiency rather than post-transcriptional regulation. High-performance liquid chromatography (HPLC) analysis indicated that PD-twin's neurons had significantly reduced capacity to synthesize and release dopamine. RNA-seq showed that the levels of monoamine oxidase (MAO-A and MAO-B) expression in PD-twin-derived neurons were higher than those of the healthy twin. Encouragingly, delivery of both lenti-virus 7.2 wild-type GBA and 10 μM Rasagiline to the twin's neurons efficiently decreased α-Syn protein level and enhanced dopamine production. These findings reinforce the human 'PD-in-a dish' model and show potential for distinguishing between genetic and epigenetic PD factors and disclosing inherent properties of cells that are indicative of PD development. Moreover, this study suggests that glucocerebrosidase activators and MAO inhibitors might potentially help reduce the risk of PD in those with associated genetic risk factors.

W-2222
A PHENOTYPIC SCREEN USING INDUCED MOTOR NEURONS FROM C9ORF72 AMYOTROPHIC LATERAL SCLEROSIS PATIENTS IDENTIFIES PATHWAYS THAT MODULATE EXCITOTOXICITY AND PROTEIN TRAFFICKING AS THERAPEUTIC TARGETS

Li, Yichen[1], Shi, Yingxiao[1], Alworth, Sam[2], Wing-Yan, Yik[1], Ichida, Justin[1]

[1]Broad-CIRM Center, University of Southern California, Los Angeles, CA, USA, [2]DRVision Technologies LLC, Bellevue, WA, USA

Amyotrophic lateral sclerosis (ALS) is a fatal and incurable neurodegenerative disease that mainly targets the motor system. Frontotemporal dementia (FTD) is the second most common pre-

senile dementia and is characterized by degeneration of the frontal and temporal lobes. Accumulating evidence suggests that ALS and FTD overlap clinically, pathologically and genetically. Recently, a GGGGCC repeat expansion mutation in the C9ORF72 locus has been identified as the most common known cause of both ALS and FTD. However, the molecular mechanism of how the C9ORF72 mutation leads to neurodegeneration is still unknown, and it is difficult to create animal models carrying the GGGGCC repeat expansion due to the extremely long and repetitive nature of the insertion. We sought to study the disease mechanism and to seek for small compounds that can act as therapeutic agents by taking advantage of induced motor neurons (iMNs) made with direct lineage conversion of patient-specific cells. With retroviral delivery of specific transcription factors and co-culture with primary glia, we obtain motor neurons that display a morphology, gene expression signature, electrophysiology, synaptic functionality, in vivo engraftment capacity and sensitivity to degenerative stimuli that are similar to those of embryo-derived motor neurons (Son et al., Cell Stem Cell, 2011, 9:205). The repeat expansion in the C9ORF72 patient iMNs induces the formation of RNA foci and repeat-associated non-ATG (RAN) proteins in vitro, which are hallmarks of this form of ALS. Furthermore, when we used a Nikon Biostation live cell imager to conduct longitudinal tracking, we identified a severe, robust and reproducible survival phenotype from three pairs of patients and controls. Moreover, we could rescue the survival phenotype of C9ORF72 iMNs with CRISPR/Cas9 correction of the GGGGCC repeat expansion. Thus, the in vitro survival phenotype of iMNs reflects the C9ORF72 disease processes. Given the fact there is no drug discovery that directly applies known disease-sensitive patient motor neurons, we performed a phenotypic screen of 1000 therapeutic compounds using the disease-sensitive iMNs. To increase throughput, we utilized an innovative image analysis recipe using SVCell image analysis software (DRVision Technologies LLC) to automate the longitudinal tracking. The recipe applies teachable machine learning methods to enhance the image signal to noise and extract fine neuronal processes and classify cells using regulated decision trees. The screen re-discovered compounds that were already shown to be neuroprotective such as alsterpaullone. More importantly, we identified and validated hits that specifically promoted the survival of C9ORF72 iMNs but not control iMNs, including some that fully rescue ALS iMN survival. The C9ORF72-specific hits largely fall into pathways that modulate excitotoxicity and protein trafficking. Using longitudinal tracking, biochemical assays, and CRISPR-mediated genome editing, we have shown that C9ORF72 iMNs generated by direct lineage conversion exhibit the hallmark phenotypes of C9ORF72 ALS. By using CRISPR-mediated genome editing, our results indicate for the first time that induced neurons can recapitulate disease phenotypes. By exploiting the degenerative phenotype of these patient-specific induced motor neurons, we have found that perturbation of the pathways that modulate exicottoxicity and protein trafficking can specifically rescue the survival of C9ORF72 iMNs.

W-2223
CURE MOTOR NEURON: NOVEL IPSC APPROACH TO ADVANCE ALS RESEARCH

Lubitz, Sandra[1], Cavaleri, Fatima[1], Eggan, Kevin Carl[2], Rubin, Lee[3], Sternberger, Ina[1], Scheel, Andreas[1], Howd, Adrian[1]
[1]Evotec, Hamburg, Germany, [2]Harvard University, Cambridge, MA, USA, [3]Harvard University Department of Stem Cell and Regenerative Biology, Cambridge, MA, USA

ALS is a neurodegenerative disorder that affects both lower motor neurons in brainstem and spinal cord, and the upper motor neurons in the motor cortex. It is most commonly a sporadic disease, 5-10% of cases are familial and usually of autosomal dominant inheritance. Loss of motor neurons with both familial and sporadic ALS is relentlessly progressive, and most patients die within three to five years after symptom onset. ALS remains a devastating disease with limited therapeutic options. The high unmet medical need is compounded by a challenging drug discovery backdrop given the disease's heterogeneity and rapid clinical progression. Our strategic partnership with the HSCI aims to identify compounds that can prevent or slow the loss of motor neurons that occurs with the progression of ALS. The collaboration 'CureMotorNeuron' leverages human motor neuron assays based on ALS patient-derived induced pluripotent stem (iPS) cells that were developed by Dr. Lee Rubin and Dr. Kevin Eggan at Harvard, as well as Evotec's leading drug discovery infrastructure and expertise to identify compounds that will have therapeutic value against this life-threatening disease. This novel phenotypic screening approach involves a large array of iPSC-derived motor neurons from both familial and sporadic ALS patients - an approach that is often referred to as a 'clinical trial in a dish'. Our plan is to systematically screen for new mechanisms, targets and compounds that have the potential to be developed into new products that will modify and ideally halt the progression of ALS and potentially other motor neuron diseases.

W-2224
CARDIAC DISEASE MODELING USING ENGINEERED HUMAN IPS CELL-DERIVED CARDIOMYOCYTES

Mandegar, Mohammad A.[1], Yoo, Jennie[1], Judge, Luke M.[1], Nguyen, Trieu[1], Perez-Bermejo, Juan[2], Miyaoka, Yuichiro[2], Lizarraga, Paweena[2], Chan, Amanda[2], Huebsch, Nathaniel[3], Spindler, Matthew[1], Truong, Annie[1], Whitehead, Evan H.[3], Spencer, Ian[2], Qi, Lei S.[3], So, Po-Lin[2], Conklin, Bruce R.[2]
[1]Gladstone Institutes, San Francisco, CA, USA, [2]Gladstone Institute of Cardiovascular Disease, San Francisco, CA, USA, [3]University of California, San Francisco Center for Systems and Synthetic Biology, San Francisco, CA, USA

Cardiovascular disease is the leading cause of mortality in the United States. Rare genetic mutations that cause severe early onset inherited cardiac diseases provide clues to the pathogenic nature of more common forms of the disease. Common genetic variants are hypothesized to influence cardiac disease development in a much larger portion of the population and the clinical relevance of most common polymorphisms is undetermined. Understanding the biological consequences of these genetic variants is one of the major challenges of biomedical research. Our research is focused on using human cellular models to elucidate the molecular basis of how disease-associated mutations might lead to the development of inherited cardiomyopathies and arrhythmias. Induced pluripotent stem (iPS) cells provide an ideal system to model human cardiac disease phenotypes as they can be differentiated into cardiomyocytes (iPS-CM) with relative ease and at high efficiencies. To model cardiomyopathies, we are using transcription activator-like effector nuclease (TALEN)-¬assisted gene targeting in iPS cells to generate null lines in key genes associated with cardiomyopathy (e.g. MYBPC3). We have successfully generated both heterozygous and homozygous MYBPC3 knockout iPS cell lines and are currently in the process of determining cellular phenotypes using electrophysiology and cellular morphometric assays. Preliminary phenotypic results suggest a significant cell size difference between the WT and MYBPC3 null iPS-CM and the disruption of the sarcomeric integrity in the MYBPC3 null iPS-CM upon adrenergic simulation. No significant differences were observed in their contractile rate and calcium handling properties. To facilitate high-throughput phenotypic analysis of modulated genes, we have generated an iPS cell line that expresses a synthetic calcium biosensor (GCaMP) to measure electrophysiological activity in beating

cardiomyocytes. Additionally, we are constructing a fluorescently tagged sarcomeric alpha-actinin (ACTN2-GFP) reporter line that will be used for cellular morphometric assays using live cell imaging. Most recently we have adopted the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-dCas9 effector platform to enable gene activation and repression for cardiac disease modeling using iPS cells. Preliminary results in iPS cells show co-expression of the catalytically inactive Cas9 (dCas9) fused to the Krüppel associated box (KRAB) repressor domain with the appropriate guide RNA can efficiently repress the transcription of reporter genes (EGFP). We are in the process of generating an inducible dCas9-effector iPS cell line to enable both activation and repression of key disease-associated genes. By combining the isogenic disease models with the dCas9-effector we can directly test the effect of activating or inactivating putative disease modifier genes. Furthermore the inducible CRISPR-dCas9 effector in iPS cell reporter lines (GCaMP and ACTN2-GFP) could provide an invaluable tool to interrogate hundreds of putative disease-associated genes in a high throughput manner to unravel the molecular mechanisms underlying human cardiac disease.

W-2225
MODELING ASD WITH EARLY BRAIN OVERGROWTH USING INDUCED PLURIPOTENT STEM CELLS

Marchetto, Maria Carolina[1], Belinson, Haim[2], Yuan, Tian[3], Braga, Patricia Beltrão[4], Nunez, Yanelli[1], Pierce, Karen[5], Courchesne, Eric[5], Geschwind, Daniel[6], Wynshaw-Boris, Antony[2], Muotri, Alysson[5], Gage, Fred H.[1]

[1]Salk Institute for Biological Studies, La Jolla, CA, USA, [2]Case Western Reserve University, Cleveland, OH, USA, [3]University of California Los Angeles, Los Angeles, CA, USA, [4]University of São Paulo, São Paulo, Brazil, [5]University of California San Diego, La Jolla, CA, USA, [6]University of California Los Angeles, La Jolla, CA, USA

Autism spectrum disorders (ASD) are complex neurodevelopmental diseases that affect about 1% of children in the United States. Such disorders are characterized by deficits in verbal communication, impaired social interaction, as well as limited and repetitive interests and behavior. The precise mechanisms that cause autism, however, remain unknown. Recently, neuropathological imaging and genetic studies have provided important insights into ASD, and these studies have led to two major hypotheses for autism pathogenesis: altered brain growth and dysfunctional neuronal networks (Courchesne et al. 2003; Dementieva et al. 2005; Garber 2007). The major impediment to testing these and other hypotheses of autism is the lack of relevant animal and cell models. The direct study of live brain tissue from ASD patients is not feasible, and most animal models lack many key elements of ADS, Reprogramming of human somatic cells to a pluripotent state by over-expression of specific genes into induced pluripotent stem cells, or iPSCs (Takahashi et al., 2007) has provided an exciting opportunity to produce a relevant human cellular model of human complex neurogenetic diseases, and iPSCs have been generated for several neurological disorders and diseases, including Rett syndrome, a syndromic ASD (Marchetto et al. 2010). Here we show the generation and preliminary phenotypical analysis of 8 idiopathic autistic iPSC lines and 5 age/gender-matched control lines. We performed differentiation into neural progenitor cells (NPC) and mature neurons in culture and performed RNA expression analysis at different time points. We show molecular and phenotypical evidence that both cell proliferation and neuronal maturation are affected in our model system, directly confirming the two cellular phenotypes. Specifically, we detected altered cell cycle and altered levels of excitatory and inhibitory markers on neural cells at early stages of differentiation. Changes during neural development could be involved in the ASD pathology observed in the patients. Knowledge of whether altered brain growth and/or dysfunctional neuronal networks play a role in the development of autism will inform studies in human patients. These studies should provide important and novel insights into the development of ASD and will test whether cellular markers are present in patient-derived NPCs and neurons from iPSCs. Studying biological, neural basis of ASD would likely lead to the development of clinically useful biomarkers of risk for this disorder in young, pre-symptomatic infants, which may lead to the development of novel therapies.

W-2226
IDENTIFICATION OF GENE SETS DYSREGULATED BY MUTANT ACVR1 GENE CAUSING A RARE INTRACTABLE DISEASE, FIBRODYSPLASIA OSSIFICANCE PROGRESSIVA

Matsumoto, Yoshihisa[1], Ikeya, Makoto[1], Fukuta, Makoto[1], Hsiao, Edward[2], Hayashi, Yohei[3], Asaka, Isao[1], Otsuka, Takanobu[4], Conklin, Bruce R.[3], Toguchida, Junya[1]

[1]Kyoto University, Kyoto, Japan, [2]University of California - San Francisco, San Francisco, CA, USA, [3]Gladstone Institutes, San Francisco, CA, USA, [4]Nagoya City University, Nagoya, Japan

Fibrodysplasia Ossificans Progressiva (FOP) is a rare genetic disease characterized by progressive ectopic ossifications, which are often initiated after physiological stimuli. The responsible gene for FOP is the *ACVR1* gene, which is one of type I receptors for BMP. The replacement of one amino acid in ACVR1 seems to activate downstream genes regulating bone formation, although the precise mechanisms are still to be investigated. Application of patient-derived iPS cells will be useful for the research of this disease, because harvesting target tissues from patients is strictly prohibited because tissue damage accelerates the ectopic ossification. Recently we have reported that iPSCs derived from FOP patients showed enhanced osteogenic and chondrogenic differentiation properties (Matsumoto et al, Orphanet J Rare Dis 2013). Because experiments were done under the culture condition without exogenous BMP signals, these data may support the hypothesis that the mutant ACVR1 can be activated via BMP-independent mechanisms. However, the precise molecular pathway responsible for the enhanced differentiation properties are unknown, which requires to investigate how and when the ACVR1 is activated during the differentiation process. Here we addressed this issue by a new approach using the differentiation system through the neural-crest cell lineage (Fukuta et al, submitted). Multi-potent neural crest cells (NCCs) were efficiently induced from iPSCs, from which multipotential mesenchymal stromal cell (MSC)-like cell population can be obtained. Therefore we are able to analyse the signal pathway at each of iPSC, NCC, MSC, and terminally differentiated stage. In addition, to minimize the effect of genetic background other than ACVR1 mutation, we established genetically rescued cell lines from parental FOP-iPSCs by the homologous recombination.Using this new approach, we have found that the abnormal signal pathway in FOP-derived cells switched on even before the differentiation step. Searching the differentially expressed genes at the precursor stage between FOP- and rescued FOP-derived cells identified a set of genes, which may be a candidate for targets of molecular therapy for this intractable disease.

W-2227
ISOGENIC DISEASE MODELING OF HEREDITARY NEUROPATHIES CAUSED BY ABERRANT DNA METHYLATION

Merkle, Florian[1], Schier, Alexander[2], Eggan, Kevin Carl[2]
[1]Harvard University, Cambridge, MA, USA, [2]Harvard University, Cambridge, MA, USA

It is well established that aberrant DNA methylation can cause neuronal death, but the mechanisms underlying this neurodegeneration are still poorly understood. In particular, specific mutations in the DNA methyltransferase DNMT1 have recently been shown to cause the similar but distinct neurodegenerative diseases HSAN1E and ADCA-DN. A broad spectrum of neurons degenerate in these autosomal dominant diseases, but they are characterized by dramatic loss of sensory neurons. Using the Cas9/CRISPR system we have introduced causal point mutations into the DNMT1 locus in human embryonic cell lines. These mutant lines and their otherwise isogenic parental control lines can differentiated into a broad spectrum of neuron types, including sensory neurons, in order to probe the methylation and gene expression changes that may underlie their neurodegeneration.

W-2228
ISOLATION OF SINGLE-BASE GENOME-EDITED HUMAN IPS CELLS WITHOUT ANTIBIOTIC SELECTION

Miyaoka, Yuichiro, Conklin, Bruce R.
Gladstone Institute of Cardiovascular Disease, San Francisco, CA, USA

Human genetics can be challenging to study in vitro, since the difference between health and disease can be determined by a single point mutation. Furthermore, traditional methods to generate mutant cell lines use antibiotic resistance markers, leaving a genetic "scar" that can interfere with studying the resulting phenotype. The advent of human induced pluripotent stem (iPS) cells and site-specific nucleases has revolutionized our ability to engineer isogenic "scarless" mutant human iPS cell lines that exactly reflect patients' mutations. A major challenge of scarless mutagenesis is that isolating the rare recombinant iPS clones is difficult without antibiotic selection. Using high levels of nuclease, or increasing nuclease activity to circumvent this problem, also runs the risk of reducing the fidelity of mutagenesis. Indeed, a critical measure of success in genome editing is the fidelity of mutagenesis, which is dictated by two key factors: the specificity of each nuclease complex, and the number of nuclease complexes in each cell. Unlike many biochemical reactions with millions of substrates, nucleases need to target just two genomic loci in each diploid cell. Any excess nucleases may only serve to increase off-target damage. Therefore, genome engineering is faced with a logistical challenge: scarless mutagenesis with the highest fidelity results in rare mutagenic events, but isolating a rare mutant cell amidst the hundreds of otherwise identical cells is exceedingly difficult. To solve this problem, we developed an efficient method that allows for precise base-by-base genome editing in cells followed by efficient detection, sib-selection, and isolation of mutant clones.

IPS CELLS: EPIGENETICS

W-2229
CHARACTERIZING REGULATORY VARIATION USING IPSCS DERIVED FROM HAPMAP INDIVIDUALS

Banovich, Nicholas E.[1], Thomas, Samantha[1], Burnett, Jonathan E.[1], Ward, Michelle C.[1], Kagan, Courtney L.[1], Pavlovic, Bryan J.[1], Gallego Romero, Irene[1], Pritchard, Jonathan K.[2], Gilad, Yoav[1]
[1]Human Genetics, University of Chicago, Chicago, IL, USA, [2]Department of Biology, Stanford University, Palo Alto, CA, USA

The study of human regulatory variation has been hindered by the limited availability of primary tissue samples. The advent of induced pluripotent stem cells (iPSCs) provides the potential for unprecedented access to many human tissues from a single individual, and has the potential to revolutionize studies of gene expression regulation. To this end we have generated a panel of iPSCs from 70 Yoruba HapMap individuals. This panel has previously been the subject of numerous studies characterizing gene regulation in a single somatic cell type, making them extremely attractive candidates for additional studies of gene regulation. We have collected RNA and DNA from these iPSCs to perform a number of assays including RNA sequencing (RNA-seq), genome wide DNA methylation profiling, as well as assay for transposase-accessible chromatin (ATAC) sequencing. These data allow us to examine multiple facets of gene regulation including, mRNA expression, epigenetic modifications, transcription factor binding, chromatin accessibility, and nucleosome positioning. Additionally, we have differentiated ten of these individuals to endoderm and collected material for the assays described above. This study provides a valuable first step into the characterization of human gene regulation using iPSCs.

W-2230
STUDIES ON DMOG INDUCED CHANGES IN THE ACTIVITY OF GENES INVOLVED IN REPROGRAMMING OF HUMAN NEURAL STEM CELLS UNDER ATMOSPHERIC AND LIMITED OXYGEN CONCENTRATION

Ilona, Szablowska-Gadomska, Martyna, Podobinska, Katarzyna, Drela, Krystyna, Domanska-Janik, **Buzanska, Leonora**
NeuroRepair Department, Mossakowski Medical Research Center, Warsaw, Poland

The previous studies of our group have indicated beneficial role of the low, similar to physiological, oxygen level conditions for human neural stem cell reprogramming. We were successful in inducing pluripotency in HUCB-NSC (Human Umbilical Cord Blood - Neural Stem Cell) using the set of reprogramming proteins (Oct4-9R, Klf4-9R and Sox2-9R) as well as small molecules influencing cell chromatin structure through activating/repressing epigenetic marks (RG-108 and TSA) at 5% oxygen level with starting population maintained in serum free condition. Looking for molecular mechanism(s) underlying acquisition of pluripotency in low 5% oxygen level we falsified the hypothesis that prolyl hydroxylase inhibitor (DMOG)-induced cellular accumulation of Hypoxia Inducible Factors (HIF's) may directly influence the expression of genes involved in HUCB-NSC reprogramming. To reveal the possible mutual molecular relationship between pluripotency regulatory network and epigenetic process the expression of OCT4, NANOG, HDAC1, HDAC2, DNMT3a, DNMT3b as well as HIF1α, HIF2α and HIF3α genes are examined by quantitative RT-PCR (qRT-PCR) at atmospheric (21%) and lowered (5%) oxygen level and at different serum conditions. To quantify the relative expression of tested genes specific primers and SYBER Green detection

system or TaqMan Gene Expression Assays were used. Our data indicate, that at both tested oxygen concentrations HUCB cells express HIF1α and HIF2α, but not HIF3α genes on similar, relatively high level. The low oxygen was beneficial for the proliferation of HUCB-NSC and activation of OCT4 and NANOG genes regardless serum condition, however DMOG treatment did not affect these responses. Moreover DMOG treatment didn't cause the activation of pluripotency genes at atmospheric oxygen tension in any serum conditions, though in the presence of serum significantly elevated expression of HIF1α and HIF2α. This suggest that the mechanisms of activation of pluripotency genes during reprogramming at low oxygen level is not directly linked to the accumulation of HIF transcription factors. The lack of response may be connected with constitutive high expression of HIF1α and HIF2α observed in HUCB NSC starting population. Our preliminary study of the other possible mechanism responsible for epigenetic regulation of pluripotency genes revealed, that the expression HDAC1, HDAC2, DNMT3a and DNMT3b was significantly elevated in neural-stem-cell-derived iPSC in cells growing in serum free and low oxygen conditions as compared to the control, starting population. These studies indicated that oxygen stress induced activation of pluripotency regulatory network in HUCB NSC line model is highly linked with the activation of genes involved in epigenetic regulation however may operate through HIF-independent mechanism.

Sponsored by grant from Polish MSRHE Nr 2011/01/B/N23/05401, MMRC statutory funds and EIT+ BioMed 5.4 Project

W-2231
VITAMIN C INDUCES TET1-DEPENDENT DNA DEMETHYLATION DURING SOMATIC CELL REPROGRAMMING

Guo, Lin[1], Chen, Jiekai[2], Zhang, Lei[3], Xu, Guo-liang[4], Pei, Duanqing[5]
[1]Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences., Guangzhou, China, [2]Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China, [3]Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China, [4]Institute of Biochemistry and Cell Biology,CAS, Shanghai, China, [5]Guangzhou Institute of Biomedicine and Health CAS, Guangzhou, China

Vitamin C, which is also known as L-ascorbic acid, is an important nutriment for human. Vitamin C is famous for preventing and treating scurvy, and also participating in antioxidant and catecholamine synthesis. One of the important roles of vitamin C is as a cofactor of the Fe2+ and α-ketoglutarate dependent dioxygenase. Many epigenetic enzymes, such as histone demethylases and DNA demethylases, are dioxygenases. Recently studies showed that, the epigenetic states of the cells could be changed in the present of vitamin C. Here we report that TET1 (ten-eleven translocation) protein can either positively or negatively regulates somatic cell reprogramming under the modulation of vitamin C. TET1 deficiency enhances reprogramming, and its overexpression impairs reprogramming in the presence of vitamin C by regulating the mesenchymal-to-epithelial transition (MET) progress. However, without vitamin C TET1 shows no effect on MET process and promotes somatic cell reprogramming. The new insight of vitamin C's functions on TET1 modulation suggests the extensive roles of vitamin C in cellular epigenetic regulation. Given the vital role of vitamin C in human health, our results may inspire future work on this micronutrient in epigenetic regulation of both physiological and pathological processes.

W-2232
THE ROLE OF SOMATIC MEMORY AND X-CHROMOSOME ACTIVATION IN HUMAN INDUCED PLURIPOTENT STEM CELLS WITH HIGH DIFFERENTIATION POTENCY

Huo, Jeffrey, Park, Tea Soon, Talbot, C. Conover, Zimmerlin, Ludovic, Baylin, Stephen, Zambidis, Elias
Johns Hopkins School of Medicine, Baltimore, MD, USA

The ideal patient-specific human induced pluripotent stem cell (hiPSC) for clinical use would efficiently generate all tissue types with minimal interline variability or lineage skewing. Until now, most induced pluripotent stem cells have demonstrated significant limits and variabilities in their multi-lineage differentiation potency. Furthermore, many standard hiPSC have been suggested to possess potentially cancer-like epigenetic changes during their reprogramming from adult differentiated cells. Finally, increasing evidence implicates derangements in hiPSC X-chromosome regulation as an obstacle to functional pluripotency. These problems represent a challenge for the full therapeutic realization of hiPSC technology. We have developed a plasmid-based, non-integrated episomal reprogramming approach that incorporates a "priming" stage with bone marrow stromal cells, and generates hiPSC lines from myeloid donor cells with unprecedented efficiencies. We found that these high-quality, low passage, myeloid progenitor-derived hiPSC lines efficiently differentiate to every non-hematopoietic lineage that has been tested thus far, while retaining high hematopoietic differentiation potency. Analysis of genome-wide expression and methylation microarray data on ~50 different human pluripotent stem cell lines revealed genetic and epigenetic characteristics setting apart these "stromal-primed" myeloid hiPSC with highly universal differentiation potency, from previous generations of hiPSC with more limited differentiation capacity. Derivation methods that led to hiPSC with greater differentiation potency also led to hiPSC with a greater degree of X-chromosome activation. Furthermore, we found that "stromal-primed" myeloid-progenitor derived hiPSC largely escaped the acquisition of aberrant hypermethylation signatures previously observed in standard reprogramming methods. Finally, these highly-potent "stromal-primed" myeloid-progenitor derived hiPSC had more complete erasure of hematopoietic transcriptional and epigenetic donor marks during reprogramming. Together, these results show that stromal-primed" reprogramming of myeloid progenitors resulted in hiPSC with a characteristic higher epigenetic quality and more complete reprogramming to pluripotency, that parallels their more universal differentiation potency. These data suggest that future screening and selection of hiPSC quality should be based on identifying hiPSC with fully completed reprogramming to pluripotency, and attempts to "harness" lineage-specific somatic memory to potentiate or augment lineage-directed differentiation should be avoided.

CHROMATIN IN STEM CELLS

W-2233
MIR-326 AND ITS HOST GENE BETA-ARRESTIN1 ARE EPIGENETICALLY SILENCED AND CONTROL PROLIFERATION AND HEDGEHOG/GLI SIGNALLING IN NEURONAL AND MEDULLOBLASTOMA STEM CELLS

Miele, Evelina[1], Po, Agnese[2], Cucchi, Danilo[2], De Smaele, Enrico[2], Canettieri, Gianluca[2], Mai, Antonello[2], Mastronuzzi, Angela[3], Locatelli, Franco[3], Levrero, Massimo[2], Gulino, Alberto[1], Ferretti, Elisabetta[2]
[1]University of Rome Sapienza and Istituto Italiano di Tecnologia, Rome, Italy, [2]University of Rome Sapienza, Rome, Italy, [3]Bambino Gesu Children Hospital, Rome, Italy

Neuronal stem cells (NSC) and medulloblastoma stem cells (CSC) have been reported to share gene expression features and recent studies have highlighted the crucial role of miRNAs in stem cells and signaling pathway deregulation in tumors. We have previously identified medulloblastoma microRNAs profile. Moreover, we showed that miR-326 is strongly downregulated in tumors and is a repressor of the Hedgehog/Gli signalling. We aimed to further define the miR-326 role in NSC and CSC. We found that miR-326 cooperates with its host gene β-arrestin1 as a tumor suppressor locus, which is lost in tumor as well as in stem cells. We showed that miR-326/β-arrestin1 locus blocks NSC and CSC proliferation by a transcriptional activation of p27, a major determinant of cell cycle exit. Moreover miR-326/β-arrestin1 locus affects the activity of the Hedgehog/Gli signalling at multiple levels. In detail, β-arrestin1 inhibits Hedgehog/Gli through the modulation of Gli1 K518 acetylation while the intragenic miR-326 controls Gli2 and smoothened, activatory molecules of the pathway. The expression of β-arrestin1 and miR-326 is tightly controlled by histone epigenetic changes in NSC and CSC. Indeed, epigenetic drugs are able to reactivate the miR-326/β-arrestin1 locus and suppress CSC in vitro and in vivo. Kaplan-Meier analysis performed on medulloblastoma tumors (n=53) with up to 5 years follow-up shows that patients with lower levels of β-arrestin1 have a significant reduced survival ($p < 0.005$). Our results suggest that miR-326 and its host gene β-arrestin1 epigenetically silencing is a common feature across multiple medulloblastoma subtypes. Our study revealed a new microRNA/host gene network in NSC and CSC and proposes β-arrestin1 as tumor suppressor and prognostic marker for medulloblastoma patients, susceptible to be re-expressed by epigenetic treatment.

W-2234
SET DOMAIN CONTAINING 5, A DIVERGENT TRANSCRIPT WITH ROSA26, IS ESSENTIAL FOR EMBRYOGENESIS, CELL SURVIVAL AND CHROMATIN MAINTENANCE

Osipovich, Anna[1], Gangula, Rama[1], Magnuson, Mark A.[2]
[1]Molecular Physiology and Biophysics, Vanderbilt University Medical Center, Nashville, TN, USA, [2]Vanderbilt University Medical Center Molecular Physiology and Biophysics, Nashville, TN, USA

SET-domain containing proteins play a vital role in regulating gene expression during development through modifications in chromatin structure. Here we show that SET domain containing 5 (Setd5) is divergently transcribed with Gt(ROSA26)Sor (or ROSA26) from a bidirectional promoter, that ROSA26 expression positively regulates Setd5, and that Setd5 is essential for mammalian development. Setd5-deficient embryos exhibit severe defects in neural tube formation, somitogenesis, and cardiac development, have aberrant vasculogenesis in embryos, yolk sacs, and placentas; and die between embryonic days (E) 10.5-E11.5. Setd5-deficient embryonic stem cells have impaired proliferation, increased apoptosis, defects in cell cycle progression, diminished ability to be differentiated into cardiomyocytes, and have increased levels of global histone acetylation and altered histone methylation patterns. In addition, SETD5 co-immunoprecipitates with multiple components of the NcoR/SMRT repressive complex suggesting that it is necessary for this multicomponent nuclear complex to regulate chromatin accessibility and gene transcription.

W-2235
DETECTION OF DIFFERENCES IN HISTONE METHYLATION MARKS IN VERY PRIMITIVE SUBSETS OF HUMAN HEMATOPOIETIC CELLS BY INTRACELLULAR FLOW CYTOMETRY

Rabu, Gabrielle Marie[1], Knapp, David JHF[1], Kannan, Nagarajan[1], Miller, Paul Harry[1], Pellacani, Davide[1], Wei, Lisa[1], Kimura, Hiroshi[2], Hirst, Martin J.[3], Eaves, Connie[1]
[1]Terry Fox Lab BC Cancer Agency, Vancouver, BC, Canada, [2]Graduate School of Frontier Biosciences,, Osaka University, Suita, Japan, [3]Centre for High Through-put Biology, Department of Microbiology, University of British Colombia and Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada

Gene regulation through covalent histone modification contributes to cell fate changes as multipotent cells differentiate. Sequencing techniques now allow high resolution measurement of histone marks, but these currently require large input cell numbers, and thus can reflect only average values of the entire population tested. Intracellular flow cytometry offers a potentially simple and fast alternative for measuring different amounts of specific marks in individual cells of defined phenotypes, although sequence-specific profiles are not thereby generated. K562 cells were induced to differentiate with either 20 nM phorbol 12-myristate 13-acetate for 48 hr, or with 0.6 mM sodium butyrate for 4 days. Control and treated cells were lysed for Western blot (WB) analysis or fixed for intracellular flow cytometry. Monoclonal antibodies against H3k4me3, H3k9me3, H3k9me2 and H3k27me3 were used for WB analysis or after non-covalent labeling with fluorochromes (Zenon® labeling kit, Life Technologies) for flow cytometry. These antibodies combined with a panel of surface markers were also used to stain subsets of $CD34^+$ cells from 4 different pools of human cord blood (CB) cells. Median fluorescence intensity (MFI) values obtained by flow cytometry and the relative intensity of differentially precipitated histones visualized on WBs of untreated and differentiated K562 cells were similar for all 4 anti-histone mark antibodies tested. MFI results for different CB subsets from all 4 pools tested were also consistent (mean pair wise Rho = 0.68-0.98, SD = 0.02-0.2). Two pairs of antibodies (H3k9me3 and H3k27me3; and H3k9me3 and H3k4me3) showed correlated staining profiles suggestive of non-specific binding, although this was not evident in all cell populations. Nevertheless, a subset (means = 10%, SD = 7%) of the $CD34^+38^-45RA^-CD90^+CD49f^+$ cells in all 4 pools analyzed showed a consistently lower H3k27me3 MFI and a higher H3k9me3 MFI than the bulk $CD34^+CD38^-CD45RA^-CD90^+CD49f^+$ cells. Flow cytometric measurements of $CD34^+$ CB cells co-stained with additional surface markers and 4 antibodies to specific histone marks identified a minority sub-population within the $CD34^+38^-45RA^-CD90^+CD49f^+$ cells that display a distinct staining pattern. These results suggest flow cytometric approaches may be useful to analyze bulk epigenetic changes in rare cell types.

W-2236
TEMPORARY ACCUMULATION OF 5-CARBOXYLCYTOSINE INDICATES INVOLVEMENT OF ACTIVE DEMETHYLATION IN LINEAGE SPECIFICATION OF NEURAL STEM CELLS

Ruzov, Alexey, Wheldon, Lee M.
University of Nottingham, Nottingham, United Kingdom

5-Methylcytosine (5mC) is an epigenetic modification involved in regulation of gene activity during differentiation. Tet dioxygenases oxidize 5mC to 5-hydroxymethylcytosine, 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). Both 5fC and 5caC can be excised from DNA by thymine-DNA glycosylase (TDG) followed by regeneration of unmodified cytosine via base excision repair pathway. Despite evidence that this mechanism is operative in embryonic stem cells, the role of TDG dependent demethylation in differentiation and development is currently unclear. Here we demonstrate that widespread oxidation of 5hmC to 5caC occurs in postimplantation mouse embryos. We show that 5fC/5caC are transiently accumulated during lineage specification of neural stem cells (NSCs) in culture and in vivo. Moreover, 5caC is enriched at the cell type-specific promoters during differentiation of NSCs, and TDG knockdown leads to increased 5fC/5caC levels in differentiating NSCs. Our data suggest that active demethylation contributes to epigenetic reprogramming determining lineage specification in embryonic brain.

W-2237
ROLE OF SIRT1 ON REGULATING GENOMIC IMPRINTING AND GERMLINE GENES IN MURINE PLURIPOTENT STEM CELLS

Lim, Jisun[1], Heo, Jinbeom[2], Jeong, Jaeho[2], Kang, Hyunsook[1], **Shin, Dong-Myung**[1]
[1]Department of Biomedical Sciences, University of Ulsan College of Medicine, Seoul, Republic of Korea, [2]University of Ulsan College of Medicine, Seoul, Republic of Korea,

Genomic imprinting is epigenetic process responsible for paternal-specific, mono-allelic expression of so-called imprinted-genes. It is well-known that imprinted genes play a crucial role in embryogenesis, fetal growth, the totipotential state of the zygote, and the pluripotency of developmentally early stem cells. Recently, we found that epigenetic changes in selected genomic imprinted genes (e.g., Igf2-H19, RasGgrf1) govern the quiescent status in a population of very small embryonic-like (VSEL) stem cells in murine bone-marrow (BM). Of importance, the DNA methylation in these imprinted loci was increased during the ageing of VSELs. Here, we show that SIRT1, a class III histone deacetylase regulates the DNA methylation status in some imprinted loci in the murine pluripotent stem cells. By screening the single cell cDNA library for VSEL isolated from young (4-weeks) and old (18-months) mice, we found that the expression of several epigenetic factors (Suz12, Cbx4, Phc1, Ash1L, Bmi1, Ring1b, SIRT1, HDAC7, Dnmt3a, Dnmt3b) significantly changed during VSEL ageing. When siRNA targeted to them was transfected into murine embryonic stem cells, SIRT1 specifically affected the DNA methylation of Igf2-H19 and Kcnq1 imprinted loci. By analyzing the transcriptome between $SIRT1^{+/+}$ and $SIRT1^{-/-}$ ESCs, several imprinted genes (Meg3, SNRPN, Dlk1, Lit1, H19, Mrkn3, Ndn, H13, and Axl) and germ-line related genes was repressed in the SIRT1 deficient ESCs. The repression of them was rescued by the expression of SIRT1 cDNA construct in $SIRT1^{-/-}$ ESCs. Furthermore, we found that the DNA methylation in the affected genes was significantly increased in $SIRT1^{-/-}$ ESCs and it was also rescued by the expression of SIRT1 cDNA. Taken together, we demonstrate the SIRT1 is a novel epigenetic factor controlling the DNA methylation mediated genomic imprinting and germ-line genes in pluripotent stem cells. Thus, it could be important to investigate the molecular nature of SIRT1 mediated protection of DNA methylation and its significance of the pluripotent stem cells.

W-2238
EPIGENETIC REGULATION IN HUMAN ES CELLS - THE BRG1 CHROMATIN REMODELING COMPLEX AND THE MIR-302 FAMILY

Wade, Staton L., Ward, James M., Archer, Trevor K.
NIEHS, National Institutes of Health, Research Triangle Park, NC, USA

Stem cell self-renewal and pluripotency are controlled by a complex, robust network of signal transduction pathways, transcription factors and epigenetic regulators. While numerous studies have addressed the complex crosstalk between the regulators of this pluripotency network, few direct connections have been made between the chromatin modifiers and miRNAs that are critical for maintenance of the ES cell state. Here we present evidence that the Brg1 chromatin remodeling complex is directly regulated by the miR-302 family in human ES cells. Using gain-of-function and loss-of-function assays, we show that miR-302 directly represses the BAF170 and BAF53a subunits of the Brg1 complex. Loss of miR-302 during differentiation leads to derepression of these subunits, suggesting this regulation dictates changes in complex composition that occur during differentiation. Furthermore, our data indicate that maintaining appropriate levels of the BAF170 subunit is critical for hES cell self-renewal and pluripotency. Gene expression analysis using microarray and RNA-seq technology revealed that BAF170 directly regulates genes involved in Nodal signaling, and is thus important for ES cell self-renewal. Nodal signaling also has a role in endodermal differentiation, and our data suggest that BAF170 may be required for efficient differentiation toward endodermal lineages. Our findings support a role for miR-302-mediated changes in the Brg1 complex in maintaining pluripotency through suppressing endodermal differentiation and suggest that relief of this inhibition is important for human endodermal lineage specification. This work places crosstalk between chromatin remodelers and miRNAs at the center of cell fate decisions during early human development. Further, it provides mechanistic insight into the essential role of the Brg1 complex in balancing stem cell pluripotency and differentiation. As the endodermal lineage gives rise to cells of the liver and pancreas, understanding these mechanisms will aid in the use of stem cell therapies for liver disease, metabolic syndromes and diabetes.

EDUCATION AND OUTREACH, ETHICS AND PUBLIC POLICY, HISTORY AND SOCIAL ISSUES

W-2239
STEM CELLS IN SOCIAL MEDIA: IMPLICATIONS FOR PUBLIC POLICY

Robillard, Julie[1], Kwon, Brian[2], Illes, Judy[3]
[1]National Core for Neuroethics, University of British Columbia, Vancouver, BC, Canada, [2]Department of Orthopaedics, University of British Columbia, Vancouver, BC, Canada, [3]University of British Columbia, Vancouver, BC, Canada

Social media is used by millions of people worldwide, and is reshaping where and how conversations take place about advances in biomedical research. While a growing portion of patients, families and health care providers are turning to the Internet to find information about

stem cells, little is known about the information that is shared among stakeholders through social media. To address this gap, we will deliver the first empirical analysis of the stem cell discussion taking place on the popular social media platform Twitter in two specific contexts: spinal cord injuries and aging-associated neurodegenerative diseases (Alzheimer, Parkinson). We used data mining techniques to collect Twitter microposts ("tweets") containing the words "stem cell" and a key words relating to either spinal cord or Alzheimer/Parkinson over a period of 6 months. Our search strategy yielded a total of 2,873 tweets (1,381 for spinal cord injury, 1,792 for Alzheimer/Parkinson), each of which constitutes a unit of analysis. After developing a coding guide specifically for each data set, we are conducting an in-depth analysis of the users, patterns of use, and major themes in the conversation about stem cells. The presentation will describe our novel methodology for assessing the discourse about stem cells in social media and will feature preliminary data from the content analysis. By delivering empirical evidence about the conversations about stem cells on Twitter, we will add the growing voice of social media users to the policy table and inform a broad range of stakeholders about the impact of this new form of public engagement.

W-2240
DEVELOPING EDUCATIONAL RESOURCES TO ADVANCE ETHICAL UMBILICAL CORD BLOOD BANKING AND RESEARCH: A CANADIAN PERSPECTIVE

Beak, Carla, **Isasi, Rosario**, Knoppers, Bartha Maria
Centre of Genomics and Policy, McGill University, Montreal, QC, Canada

As the therapeutic use of cord blood stem cells in transplantation continues to grow, so too does the use of cord blood in research. From studies to improve cord blood collection, manufacturing and storing processes; to studies of the utility of cord blood to treat various hematopoietic and non-hematopoietic disorders; to the use of cord blood cells to derive pluripotent stem cells - cord blood research is making important contributions to the scientific and clinical advancement of the stem cell field. This research requires a consistent, ethically sourced supply of cord blood samples. Obtaining an ample supply of such samples has been a challenge for the research community. In 2013 Canadian Blood Services (CBS) launched the National Public Cord Blood Bank (NPCBB) which collects, tests and stores cord blood units for potential patient matching and use in transplantation. As part of their services, CBS has developed a system by which samples that are not suitable for storage and transplantation are available - nationally and internationally - to the scientific community for biomedical research purposes. To contribute with capacity building of Research Ethics Boards (REBs), who will be tasked with ensuring this research protects donors, we developed educational resources designed to assist REBs in the evaluation of research protocols which utilize cord blood samples. The "REB Primer on Research and Cord Blood Donation" (the Primer), outlines key ethical and legal considerations and identifies Canadian normative documents that are relevant to the use of cord blood in research. It also introduces the NPCBB and describes the systems CBS has implemented to address governance issues. The Primer is intended to assist REBs in evaluating the ethical acceptability of research protocols, facilitate harmonized decision making by providing a common reference, and highlight the role of REBs in governance frameworks. However, it was written to be accessible to the general public and may serve a broader purpose to increase public awareness of cord blood banking and the policies and procedures public systems have put in place to protect donors. In addition, the Primer highlights the ethical and legal issues germane to the research use of cord blood samples globally: informed consent, privacy, and governance. As such, while developed for the Canadian context, the Primer can serve as a model for other countries to achieve similar goals. By presenting the Primer we hope to illustrate how the development of such educational tools can facilitate the ethical implementation and governance of programs related to stem cell research.

W-2241
INFORMED CONSENT IN CLINICAL TRIALS USING STEM CELLS: SUGGESTIONS AND POINTS OF ATTENTION FROM AN INFORMED CONSENT TRAINING WORKSHOP WITH SIMULATED PATIENTS

Kusunose, Mayumi[1], Muto, Kaori[1], Sean, Philpott[2]
[1]*The Institute of Medical Science, The University of Tokyo, Tokyo, Japan,* [2]*The Union Graduate College, Schenectady, NY, USA*

Informed consent is an essential requirement of ethical research involving human participants. True informed consent has three key components: full disclosure, understanding, and voluntariness. In practice, this is usually accomplished by providing and discussing with potential research participants with a lengthy document that describes what the study is about and how it will be conducted. Far too often, however, this fails to meet the standards of informed consent. Particularly for clinical trials of complex new interventions like induced pluripotent stem cells (iPS), many patients and the public are unfamiliar with the nature of these trials. Recently, Japan's Ministry of Health, Labor and Welfare emphasized the need for increasing public understanding of clinical trial in "New 5-Years Clinical Trial Activation Plan." Under the aforementioned circumstance, the world's first clinical trial using induced pluripotent stem (iPS) cells was approved in Japan in 2013. As clinical trials with iPS cells have attracted the attention of the media and aroused high expectations in patients, a workshop on informed consent in clinical trials using stem cells was conducted in Tokyo in February, 2014.. Here, we report details of the workshop and discuss attention points of informed consent regarding stem cell research. Applications for the workshop were accepted from Japanese research groups that have been planning to conduct stem cell clinical trials in the near future. Seven people -- all of who have roles in conducting or supporting the informed consent process for prospective subjects or preparing consent forms in their research groups -- participated in the four and one-half hour workshop. This workshop used a novel approach to teach how to obtain informed consent from study volunteers: the use of role-playing exercises using simulated patients. Two consent forms were used in the workshop: "Transplant of Chondrocytes Derived from Autologous Bone Marrow Mesenchymal Stem Cells for Cartilage Damage in Knee Joint (Phase I Trial)," which is educational material, and "Clinical research on autologous iPS cell-derived retinal pigment epithelium sheet transplantation for exudative age-related macular degeneration," which is a real consent form from the clinical trial. As identified by participants at the workshop, one of the key challenges with obtaining true informed consent was ensuring equitable subject selection, particularly for potentially vulnerable patients enrolled in clinical trials using stem cells ("stable ethics"). Other issues identified include ensuring that study volunteers understand that they can withdraw from the study at any time, and monitoring and ensuring the long-term safety of patients. Most important, particularly for studies involving cutting-edge interventions like the use of stem cells, researchers must understand that they cannot always adequately address patient questions and concerns during the initial recruitment and consent process, so must continue to engage study volunteers in discussions of trial design, conduct and outcomes throughout the research process.

W-2242
CHALLENGES IN THE TRANSLATION AND COMMERCIALIZATION OF CELL THERAPIES

Levine, Aaron D.[1], Dodson, Brittany P.[2]
[1]*Georgia Institute of Technology, Atlanta, GA, USA,* [2]*School of Public Policy, Georgia Institute of Technology, Atlanta, GA, USA*

Cell therapies generally and stem cell therapies more specifically offer significant potential to improve the practice of medicine and provide benefits to patients who currently have limited or no treatment options. Ideally, these innovative therapies can complement existing small molecule, biologic and device approaches – forming a so-called fourth pillar of medicine – and allowing clinicians to identify the best treatment approach for each patient. Despite this potential, cell therapies are substantially more complex than small molecule or biologic interventions. This complexity poses challenges for scientists and firms developing cell therapies and regulators seeking to oversee this growing area of medicine. In this project, we retrospectively examine the development of seven cell therapies – including three autologous interventions (Epicel, Carticel and Provenge) and four allogeneic interventions (Apligraf, Dermagraft, Prochymal and Osteocel Plus) – with the aim of identifying common challenges and promising strategies to help scientists, firms and regulators successfully bring new cell therapies to market. We complement this analysis with a series of qualitative interviews with experts in various aspects of the cell therapy industry, including people working in academia and industry as well as those working in relevant portions of the financial sector. Our analysis, developed through review of existing literature collected from company documents, newspapers, journals, analyst reports and similar sources, and refined through analysis of the qualitative interviews, identified several common challenges that cell therapy firms must address in both the pre- and post-market stages. Key pre-market challenges included identifying and maintaining stable funding to see firms through lengthy developmental timelines and uncertain regulatory processes. These challenges are not unique to cell therapies, of course, but the novelty of cell-based interventions complicates these efforts compared to small molecule or biologic interventions. The atypical nature of cell therapies also led to post-market difficulties, including challenges navigating the reimbursement process and convincing providers to change their treatment approaches. In addition, managing the cost of producing, storing and distributing cell therapies at scale was a challenge that started pre-market and continued into the post-market phase. We conclude by identifying key pitfalls and best practices applicable to the development of future cell and stem cell therapies.

W-2243
THE IMPLICATION OF THE UNPATENTABILITY OF HUMAN EMBRYONIC STEM CELL BASED INVENTIONS IN THE EUROPEAN UNION ON THE JUSTIFICATION OF HUMAN EMBRYONIC STEM CELL BASED THERAPIES

Faltus, Timo
University of Leipzig, TRM-Leipzig, Leipzig, Germany

In the aftermath of the European Court of Justice's (CJEU) decision case of *Brüstle v Greenpeace* of October 2011 that patent claims encompassing human embryonic stem cells (hESCs) were patent-ineligible in the European Union on public order and morality grounds a rash of stories has appeared predicting the destruction or exodus of hESC research in Europe. Irrespectively, whether these prediction s are justified, amazingly it has not been examined so far, whether this decision has an implication on the justification of hESC based therapies at least in Europe. The ruling in the *Brüstle v Greenpeace* case follows a challenge by Greenpeace over a patent granted to German scientist *Oliver Brüstle* for the technique to derive nerve cells from hESCs; an invention which could later be potentially useful for the treatment of neurodegenerative diseases. The CJEU ruling held that processes requiring the prior destruction of human embryos, or their prior use as base material, cannot be patented. This holding applies even if - as is the case with the questioned patent - the patent application describing the process does not refer to the use of hESCs, but where the implementation of the invention necessarily requires the destruction of human embryos. This CJEU decision binds all member states of the European Union and is unappealable. The court based its decision on art. 6 (1) and (2) of the European Biopatent Directive, which stipulate that patents may not be granted for inventions whose Commercial exploitation would be contrary to the European *ordre public*, and that, in particular, patents may not be granted for uses of human embryos for industrial or commercial purposes. The court reasoned that the purpose of the Biopatent Directive was to eliminate the possibility of patentability where respect for human dignity could thereby be affected. Finally, the court came to the result, that a patent is always connected with an industrial or commercial purposes since this is the nature of a patent. Therefore, the court reasoned, that the use of human embryos within the patent claims cannot be separated from the patent itself and the rights attaching to it. This project therefore brings forward the argument, that market approval for hESCs based therapies by European regulators must be linked to the CJEU's decision in the Brüstle case since both legal questions are governed by law of the same lawmaker, namely the European Union. Therefore, it should be expectable, that the same lawmaker expresses the same (moral) understanding in its different statues. Since stem cell based pharmaceuticals need market approval by a public agency to enter the market one must ask whether this public agency can have a different opinion on the European *ordre public* as an example of morality than the CJEU. By all means, the market approval of therapies based on hESCs is equivalent with the permission to use these cells for industrial or commercial purposes. Therefore, it must also be asked, if this correlates with an ineligible industrial or commercial use of those embryos which were needed to obtain the stem cells for therapies. If the use of hESCs for therapies is permissible by official market approval, there would be a discrepancy between the value of human embryos in patent law and pharmaceutical law. On the one hand, techniques using hESCs could not be patented, on the other hand, (same) techniques used to produce pharmaceuticals based on hESCs could get official market approval.

W-2244
EVALUATING THE OUTCOMES OF STEM CELL CLINICAL TRIALS

Fung, Moses E.[1], Atkins, Harold[2], Bubela, Tania[1]
[1]*School of Public Health, University of Alberta, Edmonton, AB, Canada,* [2]*Ottawa Hospital Research Institute, Ottawa, ON, Canada*

Stem cell therapy is a rapidly-expanding area of regenerative medicine. General optimism surrounds its potential for innovative treatment of organ failure and degenerative diseases. We have previously built an international dataset of 4,749 registered clinical trials up to 2013 from ClinicalTrials.gov and the World Health Organization's International Clinical Trials Registry Search Portal (Li et al., 2014). Of these trials, we defined 1,058 to be novel applications. Our current aim is to examine the quality and outcomes of novel clinical trials by assessing publications against objective guidelines for clinical trial conduct and against the corresponding registry reports. This analysis will enable a more accurate account of the progress stem cell therapies. We developed a coding frame to determine the quality of registry reports and their corresponding publications in accordance to guidelines from CONSORT and ICH E3. We found publications in PubMed

of Embase by searching for trial registration number, or failing that, primary author(s) and title keywords. This study investigates stem cell clinical trials from every disease category. We found 267 publications that matched novel registered trials. The majority of published trials indicate signs of safety but unproven patient benefit. This finding is typical of early phase trials and most authors advocate for further studies of efficacy. Nevertheless, more than 70% of the novel trials have not released their findings or are still underway, making it difficult to assess translational progress in the field.

W-2245

NATIONAL DETERMINANTS OF EMBRYONIC STEM CELL RESEARCH IN 50 COUNTRIES

Hughes, Virginia Clair
Political Science, Auburn University, Auburn, AL, USA

Many countries have enacted laws prohibiting or allowing research using embryonic stem cells (ESCs). This study investigates national factors such as religiosity, literacy rate, type and size of government, age of population, and number of biotechnology companies and their impact on ESC policy in fifty countries. The countries were selected based on a near equal proportion of unicameral or bicameral parliaments. These include Albania, Australia, Austria, Belgium, Brazil, Bulgaria, Canada, Chile, China, Costa Rica, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hong Kong, Hungary, Iceland, Ireland, Israel, Italy, Japan, Latvia, Lithuania, Lebanon, Malta, Mexico, The Netherlands, Norway, New Zealand, Morocco, Peru, Poland, Portugal, Singapore, Slovakia, Slovenia, South Africa, South Korea, Spain, Sweden, Switzerland, Trinidad and Tobago, Tunisia, Vietnam, United Kingdom, United States, and Uruguay. A binary logistic regression study was performed using restrictive or permissive policy for the dependent variable and factors listed above for the independent variables. The variable religiosity was significant yielding an odds ratio of 19.1 and P value of .007. The other variables were not significant. Catholicism was found to be most associated with restrictive laws on ESC research compared to other denominations. The ethics surrounding ESC research will continue to be opposed by Catholic leaders and pro life factions as research progresses. Future studies should focus on the scientific literacy rate and the impact of public and private funding on ESC research.

W-2246

CONSENTING FERTILITY PATIENTS FOR DONATION OF EXCESS FROZEN EMBRYOS FOR RESEARCH: REQUIRED ELEMENTS OF CONSENT AND WHAT PATIENTS WANT TO KNOW

Jonlin, Erica C.
Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA, USA

Federal regulations for human subjects protection, in addition to the NIH Stem Cell Guidelines, delineate what elements must be in a consent form used to consent fertility patients for donation of excess frozen embryos for research. Embryo donors have varying areas of concern, and ask a variety of questions during the consenting process. This paper will present topics that embryo donors ask about prior to deciding whether to donate their excess frozen embryos to research.

NEURAL CELLS

W-3001

DIRECT REPROGRAMMING OF FIBROBLASTS INTO NEURAL STEM CELLS BY SMALL MOLECULE COMPOUNDS

Zheng, Jie[1], Kang, Phil Jun[1], Yoon, Byung Sun[2], Moon, Jai-Hee[1], Suhyun, Kwon[1], Solji, Hyeon[1], Hong, Sunghoi[3], You, Seungkwon[1]
[1]Laboratory of Cell Function Regulation, Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Republic of Korea,, [2]Stem Lab,Venture Incubation Center Korea University, Seoul 136-701, Republic of Korea,, [3]Laboratory of Stem Cell Biology, Department of Biomedical Science, College of Health Science, Korea University, Jeongneung-dong, Sungbuk-gu, Seoul 136-701, Republic of Korea

The generation of induced neural stem cells (iNSCs) from somatic cells provides new avenues for basic research and cell therapies for various neurological diseases. Recent studies have suggested that iNSCs could provide an alternative to derivation from somatic cells or pluripotent cell by defined transcription factors. However, the transcription factors used in reprogramming process still have the potential of unexpected genetic modification that limit their potential application for cell therapies. Here, we show that a combination of four small molecules convert fibroblasts to NSC identity, which we term as chemically induced NSCs (ciNSCs). These ciNSCs express NSC markers (Pax6, PLZF, Nestin, Sox2 and Sox1) and resemble with neonatal NSCs in their morphology, self renewal, and gene expression profiles. These ciNSCs also possess tripotent differentiation potentials into glial and neuronal cell types, including several types of mature neurons (dopaminergic, GABAergic, cholinergic and serotonergic neurons) in vitro and in vivo. Importantly, the mature neurons derived from the ciNSCs exhibit physiological properties, such as sodium channel activity and generation of action potential spikes. Taken together, our results suggest that functional and stably expandable ciNSCs can be directly reprogrammed from mouse fibroblasts by small molecules without any genetic manipulations. These results provide alternative strategy to overcome transcription factor-derived direct reprogramming approaches and broaden application of cell therapy in the treatment of neurodegenerative diseases.

W-3003

UMBILICAL CORD BLOOD STEM CELLS TO MODEL HUMAN CORTICAL NEUROGENESIS: A FURTHER STEP TOWARD EFFECTIVE CLINICAL APPLICATIONS

Ali, Hamad
Department of Medical Laboratory Sciences (MLS), Kuwait University, Kuwait City, Kuwait

Umbilical cord blood stem cells have been proposed as a potential therapeutic tool for Central Nervous System diseases and disorders. Moving toward clinical applications requires extensive knowledge about cord blood stem cells differentiation potential and their ability to generate cells with similar developmental and functional characteristics to indigenous targeted cells, especially in proposed cellular replacement strategies. We have evaluated the ability of umbilical cord blood stem cells to model corticogensis by direct comparison between differentiating cord blood stem cells *in-vitro* and human neocortical neurogenesis. Our *in-vitro* system has recapitulated events occurring during neocortical development which are important in the differentiation of Glutamatergic and GABAergic cortical neurons which are commonly damaged in cerebral palsy as well as by stroke in adults. Such *in vitro* systems should allow us to better understand and

target potential brain repair strategies and provide *in vitro* models of neural diseases for toxicology and drug testing research.

W-3004
DEFINING SUBTYPE SPECIFICITY OF MOUSE INDUCED NEURONAL CELLS

Ang, Cheen Euong[1], Sadegh, Cameron[2], Macklis, Jeffrey D.[2], Wernig, Marius[3]
[1]*Institute of Stem Cell Biology and Regenerative Medicine, Stanford University, Palo Alto, CA, USA,* [2]*Harvard Medical School Department Neurology and Neuroscience, Boston, MA, USA,* [3]*Stanford University, Palo Alto, CA, USA*

Since our lab reported the first direct conversion of mouse and human fibroblasts to induced excitatory neuronal (iN) cells, several groups have since reported using the same four transcription factors (Brn2, Ascl1, Myt1l and NeuroD1) or in combination with other transcription factors or microRNAs to generate other neuronal subtypes. While the hierarchical mechanisms of reprogramming have recently been delineated in our lab's recent paper, the identity of the cells remains elusive. Herein, I will describe our efforts in defining the identity of those cells using bulk RNA-seq, single cell RNA-seq and immunofluorescence staining. I would also describe alternative sets of transcription factors which are capable of giving rise to iN cells endowed with more specific cortical identities Hopefully, all these efforts will culminate into generating a more defined final products which can be used for disease modeling and drug screening.

W-3005
NEURAL STEM CELLS-LADEN MICROFIBERS PROMOTE SURVIVAL OF TRANSPLANTS IN MOUSE TRANSECTED SPINAL CORD INJURY MODELS

Hori, Keiko[1], Nishimura, Soraya[1], Kato-Negishi, Midori[2], Onoe, Hiroaki[2], Kobayashi, Yoshiomi[1], Itakura, Go[1], Iwai, Hiroki[1], Takeuchi, Shoji[2], Okano, Hideyuki[3], Toyama, Yoshiaki[1], Nakamura, Masaya[1]
[1]*Department of Orthopedic Surgery, Keio University, School of Medicine, Tokyo, Japan,* [2]*Institute of Industrial Science, The University of Tokyo, Tokyo, Japan,* [3]*Department of Physiology, Keio University, School of Medicine, Tokyo, Japan*

[Purpose] Transplant of neural stem/progenitor cells (NS/PCs) is now considered to be a promising treatment for spinal cord injury (SCI). In most of the previous studies, however, NS/PCs transplantation was performed for incomplete SCI animal models. There still exist several problems to be resolved in treating complete SCI. To treat complete SCI patients, any scaffold is required to fill the cavity at the lesion epicenter in parallel with the NS/PCs transplantation. In the present study, we sought to determine the effectiveness of a novel artificial microfiber made of bio-absorbable material (collagen and alginate gel) as a candidate for the scaffold to treat complete SCI. [Method] NS/PCs derived from striatum of fetal transgenic mice encoding CAG-ffLuc (Venus fused to firefly luciferase) were used in this study. These NS/PCs were loaded into the artificial microfiber, and their viability and differentiation potential were evaluated in vitro. In addition, a bundle of these NS/PCs-laden microfibers was transplanted into the injured spinal cord with 3 mm defect in Th9-10 level, immediately after the transection. Survival of the grafted cells was evaluated using bioluminescence-imaging system. Motor function of the lower limbs was assessed in each mouse using BMS (Basso Mouse Scale) score. Histological evaluation was performed 6 weeks after the transplant. [Result] In vitro, the loaded NS/PCs within the microfibers proliferated well in normal NS/PCs medium and differentiated into the neural and glial lineages in the differentiation medium. In vivo, the grafted NS/PCs within the microfibers showed good survival rate at least for 6 weeks after the transplantation. Survival of the transplants was confirmed in histology as well. At the rostral and caudal area of transplanted microfiber, transplanted cells migrated into the host spinal cord, and differentiated into the neural and glial cells. [Conclusion] We achieved to make connection between stumps of transected spinal cord with novel NS/PCs-laden microfibers, in treating mouse transected SCI model. Cellular survival was promoted compared to the traditional injection method. This material is possible tool for NS/PC transplantation therapy for complete SCI.

W-3006
TRANSCRIPTIONAL/EPIGENETIC CIRCUITRY TO MAINTAIN THE ADULT NEURAL STEM CELL POOL

Hsieh, Jenny
UT Southwestern Medical Center, Dallas, TX, USA

Defining the transcriptional and epigenetic circuitry controlling neural stem cells is critical for harnessing the potential of adult hippocampal neurogenesis in regenerative medicine and science. We have systematically dissected the function and mechanisms of individual transcriptional factors that function in the stepwise progression of quiescent neural stem cells to mature dentate granule neurons in the adult mammalian brain. Using a combination of conditional knockout mice to perform genetic loss-of-function and neural stem cell cultures to perform molecular and biochemical analysis, we have characterized a negative regulator of quiescent and activated stem/progenitor cells (NRSF/REST) and its co-repressors (e.g., class I and class II histone deacetylases) to be important in maintaining the stem cell pool and preventing precocious activation of the neuronal lineage program. Furthermore, we have taken advantage of a reversible in vitro model of quiescence to identify the direct downstream targets of NRSF/REST involved in maintaining quiescent and proliferating stem cell populations. Our data suggest that NRSF/REST crosstalks with various signaling pathways to regulate the transition from quiescent to activated progenitors, including the Wnt, TGFβ, and IGF pathways. Our results also provide mechanistic insight for strategies to promote the adult neural stem cell pool towards preventing age-related cognitive decline.

W-3007
THE MOOD STABILIZER VALPROATE ACTIVATES HUMAN FGF1 GENE PROMOTER THROUGH INHIBITING HDAC AND GSK3 ACTIVITIES

Hsu, Yi-Chao
Mackay Medical College, New Taipei City, Taiwan

Valproic acid (VPA) is the primary mood-stabilizing drug to exert neuroprotective effects and to treat bipolar disorder in clinic. Fibroblast growth factor 1 (FGF1) has been shown to regulate cell proliferation, cell division and neurogenesis. Human *FGF1* gene 1B promoter (-540 to +31)-driven green fluorescence (F1BGFP) has been shown to recapitulate endogenous *FGF1* gene expression and facilitates the isolation of neural stem/progenitor cells (NSPCs) from developing and adult mouse brains. In this study, we provide several lines of evidence to demonstrate the underlying mechanisms of VPA in activating *FGF-1B* promoter activity: (i) VPA significantly increased the *FGF-1B* mRNA expression and the percentage of F1BGFP(+) cells; (ii) the increase of F1BGFP expression by VPA involves changes of RFX1-3 transcriptional complexes on the 18-bp *cis*-element of *FGF-1B* promoter; (iii) treatments of other HDAC inhibitors, sodium butyrate and trichostatin A, significantly increased the expression levels of *FGF-1B*, *RFX2* and *RFX3* transcripts; (iv) treatments of GSK-3 inhibitor, lithium, or GSK-3 siRNAs also significantly activated *FGF-*

1B promoter; (v) VPA specifically enhanced neuronal differentiation in F1BGFP(+) NSPCs rather than GFP(-) cells. This study suggested, for the first time, that VPA activates human *FGF1* gene promoter through inhibiting HDAC and GSK3 activities.

W-3008
CLINICAL TRANSLATION OF NEURAL STEM CELL TRANSPLANTATION: AN OVERVIEW AND UPDATE OF THE TRIAL EXPERIENCE WITH HUMAN CENTRAL NERVOUS SYSTEM STEM CELLS (HuCNS-SC)

Huhn, Stephen, Capela, Alexandra, Uchida, Nobuko, Tsukamoto, Ann
StemCells, Inc., Newark, CA, USA

The HuCNS-SC cell product is a purified and expanded composition of normal human neural stem cells that is currently in clinical development for a broad range of CNS diseases and disorders. Published studies have reported on the safety of transplanting these cells into the brain of pediatric patients with rare and fatal diseases (clinicaltrials.gov: NCT01005004 and NCT00337636). Additional clinical studies have been initiated to test the safety and preliminary efficacy of these cells in a multi-center Phase I/II trial in spinal cord injury and a multi-center Phase I/II trial in age-related macular degeneration. To date, HuCNS-SC cells have undergone clinical testing in 4 separate human trials targeting a spectrum of conditions and anatomic regions of the central nervous system (CNS). Each study was supported by published preclinical research with relevant animal models and authorized by the U.S. FDA, in addition to other regulatory agencies. The initial Phase I trial represented the first-in-human study and enrolled 6 subjects with Neuronal Ceroid Lipofuscinosis (NCL), a fatal lysosomal storage disorder. The second Phase I trial enrolled 4 subjects with Pelizaeus-Merzbacher disease, a severe X-linked hypomyelination disease. Both studies, performed in pediatric populations, demonstrated safety, feasibility, and tolerability of transplantation into the brain with total CNS doses ranging from 300 million to 1 billion cells. The results of the first two Phase I studies have also revealed intriguing clinical observations consistent with preclinical studies[1, 2] which have further supported clinical development. In addition to congenital disorders of the CNS, preclinical studies have provided the rationale to investigate the therapeutic potential of HuCNS-SC in spinal cord injury (SCI) and age-related macular degeneration (AMD). A multi-center international Phase I/II trial for thoracic SCI is currently underway (clinicaltrials.gov: NCT01321333) involving HuCNS-SC transplantation in 12 subjects in the chronic stage after SCI and testing a total spinal cord dose of 20 million cells. Comprehensive and sophisticated clinical and electrophysiological dermatomal assessments have been incorporated into the study protocol, and interim analysis of the Phase I/II data has revealed signs of multi-segmental gains in sensory function which are considered unexpected in this patient population. A multi-center dose-escalation Phase I/II trial (clinicaltrials.gov: NCT01632527) for patients with the dry form of AMD, also referred to as Geographic Atrophy, is investigating the therapeutic potential of HuCNS-SC cells transplanted into the subretinal space of the eye. The study is testing the safety and preliminary efficacy of 200,000 and 1 million cell doses administered to the subretinal space of the study eye. Post-transplant assessments include tests of visual acuity, fluorescein angiography, spectral domain ocular coherence tomography (OCT), microperimetry, multifocal electroretinography, and contrast sensitivity. Interim result from the SCI and AMD studies will be presented, along with an overview of the translational progress to date.

W-3009
SYNAPTIC PATHOPHYSIOLOGY IN TUBEROUS SCLEROSIS USING HUMAN PLURIPOTENT STEM CELL-DERIVED NEURONS

Jagasia, Ravi[1], Aigner, Stefan[2], Costa, Veronica[1], Patsch, Christoph[3], Mirko, Vukcevic[4], Bischofberger, Josef[4], Graf, Martin[2]
[1]F. Hoffmann-La Roche, Basel, CNS Res, Basel, Switzerland, [2]F. Hoffmann - La Roche Ltd., Basel, Switzerland, [3]Roche Pharma, Basel, Switzerland, [4]Department of Biomedicine, University of Basel, Basel, Switzerland

Autism spectrum disorder Several monogenic forms of autism spectrum disorder (ASD) are caused by mutations in genes functioning in the mTOR pathway, leading to translational dysregulation and altered synaptic signaling. *TSC1* and *TSC2* code for the negative mTOR regulators hamartin and tuberin, respectively, and mutation of either gene causes tuberous sclerosis, a multi-system disorder with high prevalence of ASD. Using zinc finger nuclease-mediated genome editing, we have generated human embryonic stem cell (hESC) lines with heterozygous and homozygous ablation of *TSC2*. Upon neuralization, cortical rosettes from *TSC2*$^{-/-}$ hESCs show changes in higher-order structural organization, suggesting early cellular specification defects. In order to identify synaptic signaling defects on the network level, we have developed a protocol to differentiate rosette-derived neuroepithelial precursor cells towards neuronal cultures with functional inhibitory and excitatory synapses. These neurons exhibit several synaptic properties relevant to ASD pathophysiology, including synchronous network activity, synaptic scaling and mGluR5-dependent long-term depression (LTD). We are currently investigating alterations in synaptic signaling properties of *TSC2*$^{+/-}$ and *TSC2*$^{-/-}$ neurons. We envision that our human cellular models of neuronal networks will be instrumental in elucidating the mechanisms underlying synaptic dysfunction in ASD and other neurodevelopmental disorders.

W-3010
TRANSCRIPTION FACTOR DIRECTED GENERATION OF OLIGODENDROCYTE PROGENITOR CELLS USING SYNTHETIC RNA

Karl, Robert T., Wanta, Jonathon, Tesar, Paul J.
Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH, USA

Myelin-related disorders present a unique target for cell replacement therapies. Such therapeutics will require large numbers of oligodendrocyte progenitor cells, the sole myelinating cell of the central nervous system. Currently, there is a lack of access to these cells types. Our lab has recently shown the capability to reprogram somatic cells to induced oligodendrocyte progenitor cells (iOPCs) using defined sets of transcription factors delivered with integrating lentiviral vectors. However, the ability to directly reprogram somatic cells to iOPCs without compromising the genome will be imperative for future cell replacement therapies. Here we describe a method for converting mouse embryonic fibroblasts to induced oligodendrocyte progenitor cells using a non-integrating RNA based method to express lineage specific transcription factors. Administration of these transcription factors resulted in generation of iOPCs with the attributes of bona fide OPCs, including global gene expression profiles and the ability to differentiate into multiprocessed oligodendrocytes. These results provide a basis for future autologous remyelinating cell therapies as well as the study of the maintenance of the oligodendrocyte lineage, and modeling of complex neurological diseases.

W-3011
DOWNREGULATING PTEN BY NDFIP1 TREATMENT PROMOTES THE SURVIVAL AND DIFFERENTIATION OF NEURAL PROGENITOR CELLS TO OLIGODENDROCYTE

Khazaei, Mohamad R., Fehlings, Michael G.
Toronto Western Research Institute and Krembil Neuroscience Centre, University Health Network,, Toronto, ON, Canada

Transplantation of neural progenitor cells (NPC) following spinal cord injury (SCI) leads to improved recovery. This recovery is largely associated with the ability of the exogenous NPCs to survive, differentiate to oligodendrocytes and remyelinate damaged axons. Despite the great promise of these cells, poor survival due to the harsh SCI environment could limit their effectiveness. Quantitative comparison of astrocytic, neuronal, and oligodendroglial gene expression profile demonstrate that PTEN expression is significantly higher in NPC cells differentiated to neurons and astrocytes compared to oligodendrocytes. On the other hand, PTEN down-regulation increases the population of oligodendrocytes. Here we show that treatment of NPC cells with Ndfip1, an adaptor protein which enhances the ubiquitination and degradation of PTEN, increases the population of Olig2 positive oligodendrocytes in differentiation medium. Additionally, treating NPCs with Ndfip1 induces the activation of pro-survival PI3K/Akt pathway, as assessed by phosphorylation of Akt target, BAD. Ndfip1 pre-treatment also increases NPC survival after treatment with injured spinal cord lysate, which mimics the harsh inflammatory milieu after injury, and results in reduction of cleavage of caspase-3. In summary, our results show that pre-treatment of NPCs with Ndfip1 down-regulates PTEN, increases their survival rate, and direct them towards the oligodendroglial lineage. This could significantly improve stem cell survival and function after transplantation into spinal cord leading to improved functional recovery following injury.

W-3012
CLONAL INDUCED NEURAL STEM CELL LINES EXHIBIT DIFFERENT REPROGRAMMING STATUS

Kim, Sungmin
Konkuk University, Seoul, Republic of Korea

Terminally differentiated cells can be directly converted into different types of somatic cells using defined factors, thus circumventing the pluripotent state. However, low reprogramming efficiency, along with the absence of proliferation of some somatic cell types, challenges the generation of sufficient material for clinical applications. Here we describe a protocol to directly convert mouse fibroblasts into self-renewing induced neural stem cells (iNSCs) that can be expanded in vitro, overcoming the limitations associated with low reprogramming efficiency. The four transcription factors required for direct conversion into iNSCs (Sox2, Klf4, c-Myc, and Brn4/Pou3f4) do not generate a pluripotent cell state, thus the risk for tumor formation after transplantation is reduced. Following the current protocol, iNSCs are observed between 4-5 weeks after transduction and two additional months are required to establish clonal iNSC cell lines. Here we describe a detailed protocol for the generation of clonal iNSC lines from MEFs by single cell sorting. Each clonal iNSC lines exhibit different levels of NSC marker genes, transgene expression and in vitro differentiation potentials into neurons, astrocytes and oligodendrocytes.

W-3013
COMMON PATHWAYS DISRUPTED IN HUMAN ALS MOTOR NEURONS IDENTIFIED THROUGH GENETIC CORRECTION OF MUTANT SOD1

Kiskinis, Evangelos[1], Sandoe, Jackson[1], Wainger, Brian[2], Williams, Luis[1], Boulting, Gabriella[3], Woolf, Clifford[4], Eggan, Kevin Carl[1]
[1]Harvard University, Cambridge, MA, USA, [2]Children's Hospital, Boston, MA, USA, [3]Harvard Medical School, Boston, MA, USA, [4]HMS, Chldren's Hospital, Boston, MA, USA

Although many distinct mutations in a variety of genes are known to cause Amyotrophic Lateral Sclerosis (ALS), it remains poorly understood how they selectively impact motor neuron biology and whether they converge on common pathways to cause neural degeneration. We have combined reprogramming and stem cell differentiation approaches with genome engineering and RNA sequencing to define the transcriptional and functional changes that are induced in human motor neurons by mutant SOD1. Mutant SOD1 protein induced a transcriptional signature indicative of increased oxidative stress, reduced mitochondrial function, altered sub-cellular transport, electro-physiological excitability as well as activation of the ER stress and unfolded protein response pathways. Functional studies demonstrated that perturbations in these pathways were indeed the source of altered transcript levels. Importantly, we used a genetic targeting approach to demonstrate that these phenotypes are reversed by genetic correction of the SOD1 mutation. Utilizing this patient-specific induced pluripotent stem cell (iPSC) approach we next addressed two important, outstanding questions in the field. Why are motor neurons selectively lost in ALS? And are there common molecular pathways between distinct ALS-causing mutations? We found that motor neurons exhibit inherent ER stress that is related to their physiological properties and renders them vulnerable to disease. Finally, interrogation of stem cell-derived motor neurons produced from ALS patients harboring a repeat expansion in C9ORF72 and FUS mutations identified electro-physiological excitability as a major feature of distinct types of ALS. These results provide an insight to the common functional defects that physiological levels of mutant proteins may lead to, in patient motor neurons. More broadly our study demonstrates that iPSC technology can be used to probe an adult-onset neurological disease such as ALS.

W-3014
DEFINED CELL-RECOGNIZABLE N-CAD-FC MATRIX FOR FACILITATED PRODUCTION AND PURIFICATION OF NEURAL CELLS FROM IPSCS

Kutsuzawa, Koichi
Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, Japan

Quick and efficient generation and purification of neuronal cells are extremely critical for successful tissue engineering and regenerative medicine protocols, particularly for recovering neural damage; however, suitable technologies for facilitated growth and efficient purification of neuronal cells are very limited. E-Cadherin (E-Cad) and N-Cadherin (N-Cad) switching occurs during the transformation of stem cells to neuronal cells, and these proteins can form dimers by homophilic interaction. We have exploited these classical cell biology phenomena and designed the chimeric protein N-Cad-Fc as a defined matrix, which is composed of the extracellular domain of N-Cad and the Fc domain of IgG, for enhanced differentiation and growth of neural cells from stem cells. Our recent work revealed that mouse embryonic carcinoma cell P19 and neural stem cell MEB5 were superiorly differentiated to neural lineage, as evident by higher level of *Neurog1 and MAP2* expression,

on this defined matrix compare with other reported substratum such as gelatin, fibronectin, and laminin. Using mouse embryonic stem cell and iPS lines, and in combination with regulation of Wnt- and Nodal-signaling pathways by using Dkk-1 and LeftyA, respectively, and by analyzing stage-specific expression of different neuronal markers we have revealed that N-Cad-Fc is a strong enhancer for generation of neural progenitor cells and the growth of neural cells. Our preliminary results with human iPSCs further potentiated similar beneficial roles of N-Cad-Fc matrix in facilitated differentiation and growth of neural cells. These findings are significantly advantageous for advancing this unique cell-recognizable defined matrix towards practical application in neuronal cell-related regenerative and tissue engineering protocols.

W-3015
WNT7A IN NEUROGENESIS AND POST-STROKE RECOVERY

Lacaria, Melanie[1], Faulkes, Sharlene[1], Lagace, Diane[2], Corbett, Dale[2], Rudnicki, Michael A.[1]
[1]Regenerative Medicine, Ottawa Hospital Research Institute, Ottawa, ON, Canada, [2]Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada

Previous studies in our lab determined that the secreted signalling protein Wnt7a and its receptor, Frizzled7 (Fzd7), are upregulated in satellite stem cells during muscle regeneration and that the overexpression of Wnt7a enhances muscle regeneration through symmetric expansion of the stem cell pool. More recently, Wnt signalling has also been shown to regulate the symmetry of NSC division - a process that is up-regulated following induced stroke in mice. Furthermore, Wnt7a is a known regulator of synaptic differentiation and axonal remodeling in the adult brain, and it has been shown to stimulate NSC proliferation and self-renewal, resulting in an increase in the number and function of excitatory neurons and synapses. Taken together, these previous studies suggest a role for Wnt7a as a key regulator of NSC division and neuroplasticity. We investigated the potential of Wnt7a as a therapeutic treatment for post-stroke recovery in vivo in a mouse model of stroke; following the induction of stroke by photothrombosis, delipidated Wnt7a protein was intracranially injected into the ventricle of the affected hemisphere of the brain. Functional recovery was monitored by neurobehavioral testing of motor function, and NSC expansion and neurogenesis were evaluated through immunostaining for markers of NSCs and cell division. Preliminary results indicate a role for Wnt7a in NSC regulation, leading to enhanced post-stroke recovery.

W-3016
CHARACTERIZATION OF ALPHA7 NICOTINIC ACETYLCHOLINE RECEPTOR PHARMACOLOGY USING HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS

Larsen, Hjalte M.[1], Rasmussen, Mikkel[1], Frederiksen, Kristen[2], Bastlund, Jesper F.[2], Mikkelsen, Jens D.[3], Hyttel, Poul[1]
[1]University of Copenhagen, Faculty of Health and Medical Sciences, Frederiksberg C, Denmark, [2]H. Lundbeck A/S, Valby, Denmark, [3]Neurobiology Research Unit, Rigshospitalet, Copenhagen, Denmark

The α7 nicotinic acetylcholine receptor (α7 nAChR) has been identified as a promising new drug target for treatment of cognitive dysfunction observed in Alzheimer's disease (AD) and schizophrenia patients. Thus, several positive allosteric modulators (PAMs) of the α7 receptor have been developed, such as PNU 120596 and NS 1738, both showing in vivo activity. Although, histological and electrophysiological studies have demonstrated the presence of α7 receptors on pyramidal neurons and GABAergic interneurons throughout layer II-VI of the cerebral cortex, the mechanistic involvement of the α7 receptor in cognition and pathological processes still remains fundamentally unexplored. In the present study, we aim at constructing an α7 disease model based on human induced pluripotent cells (hiPSCs) from AD patients and healthy control individuals. Using a healthy hiPSC cell line we are establishing an in-house protocol for derivation of neural progenitor cells (NPCs) expressing the markers LHX8 and NKX2.1, which correspond to the lateral ganglionic eminence (LGE) subventricular zone (SVZ). Subsequently, we will mature these NPCs to basal forebrain neurons (NKX2.1, MAP2, and TUBB3 positive) and test for α7 functionality using known agonists and antagonists including nicotine, PNU282987, MLA, and α-Bungarotoxin in a calcium-imaging assay. Next, neural differentiation and comparison with hiPSC derived from healthy control individuals will show whether there is an abnormal expression and/or altered mechanism of the a7 receptor in AD patients. Together these experiments will provide valuable insight into AD modeling, with a particular focus on the α7 receptor, and establish a framework for future personalized AD therapy.

W-3017
MIGRATION OF EMBRYONIC STEM CELL-DERIVED NEURAL PROGENITORS ON THE HOST VASCULATURE IN THE BRAIN

Lassiter, Chelsea, Becker, Sandy, Gal, Julian, Grabel, Laura
Wesleyan University, Middletown, CT, USA

Before we can safely use embryonic stem cell (ESC)-based cell replacement we must characterize the behavior of these cells following transplant. Embryonic stem-cell derived neural progenitors (ESNPs) transplanted to the dentate gyrus region of the hippocampus can differentiate into granule neurons, repopulating the upper blade lesioned by the injection. These transplants are also richly vascularized, and surprisingly, the ESNPs appear to migrate great distances from the original site of injection. We observe that doublecortin (DCX)+ migrating ESNPs are found in close proximity to endogenous blood vessels, outside of the transplant area. Our preliminary data suggests that blood vessels and their associated astrocytes provide a source of the chemokine CXCL12, which promotes ESNP migration in the brain. To test this model, we use organotypic hippocampal slice culture and find that ESNPs are found closely associated with blood vessels. To directly study the interaction between ESNPs and endothelial cells and identify a molecular mechanism, we are using a selective adhesion assay with brain endothelial cells, as an in vitro model. These data raise a concern for many therapeutic transplantation approaches that cells may migrate away from the original transplant, and be disruptive at a distant site.

W-3018
GENOME-WIDE CHANGES IN THE AGING NEURAL STEM CELL NICHE

Leeman, Dena Simona[1], Pollina, Elizabeth A.[2], Webb, Ashley E.[2], Brunet, Anne[3]
[1]Department of Genetics and Cancer Biology Graduate Program, Stanford University, Stanford, CA, USA, [2]Genetics, Stanford University, Stanford, CA, USA, [3]Stanford University School of Medicine, Stanford, CA, USA

Neural stem cell niches in the adult brain are the locations where neural stem cells produce new neurons necessary for the maintenance and plasticity of brain tissue and function. With age, neural stem cell niches deteriorate, with a decline in neural stem cell proliferation and production of new neurons. However, the exact nature of the molecular changes in the aging neural stem cell niche is largely

unknown, primarily due to the inability to freshly purify these rare cell types and perform ultra high-throughput molecular analysis on this limited material. Understanding these changes will be a key step toward delaying and even reverting age-dependent decline in many tissues, including the brain. We adapted a recently developed FACS protocol to simultaneously purify five populations of cells from the neural stem cell niche of the same individuals (astrocytes, quiescent neural stem cells, activated neural stem cells, neural progenitors and endothelial cells) over the course of aging, and performed whole-genome RNA-sequencing and microRNA profiling. Our preliminary unbiased integrative analysis of age-dependent gene expression changes in these populations has revealed tantalizing changes in inflammatory, biosynthetic and metabolic pathways. We are working toward manipulating these pathways in the neural stem cell niche, with the goal of generating more youthful levels of functional neurons in the aged brain.

W-3019
IDENTIFICATION OF CELLULAR SIGNAL PATHWAY CHANGES IN NERVES GROWTH FACTOR INDUCED NEURONAL DIFFERENTIATION BY CHEMICAL GENOMICS

Ling, Qing-dong[1], Lin, Jack Yu-Shih[2]
[1]Department of Medical Research, Cathay General Hospital, New Taipei, Taiwan, [2]Department of Ophthalmology, Taipei Medical University Municipal Wan-Fang Hospital, Taipei City, Taiwan

Nerve growth factor (NGF) plays an important role in the nervous system by supporting the survival and growth of neural cells, regulating cell growth, promoting differentiation into neuron, and neuron migration. Recently, the roles of NGF in the treatment of various neurodegeneration diseases by mesenchymal stem cells have been widely investigated both in vitro and in vivo, with growing evidence of associated NGF-pathway alterations. These preliminary reports led us to investigate, through microarray experiments and chemical genomics analysis, the genes and pathways associated with NGF induced neuronal differentiation using PC-12 as models. In our study, after cluster analysis and heat map generation, we identified 2020 NGF-induced genes with altered expression over time. Cross-matching with the KEGG database revealed 830 genes; among which, 395 altered genes were found to have 2-fold increase in gene expression over a two hour period. We then identified 191 associated biologic pathways in the KEGG database; the top 15 pathways showed correlation with neural differentiation. These include the neurotrophin pathways, MAPK pathways, genes associated with axonal guidance and the Wnt pathways. The activation of these pathways maybe involved in how NGF administration protects neurons from degenerative events. In conclusion, we have established a model system that allows one to systematically characterize the functional pathway changes in a group of neuronal population after an external stimulus.

W-3020
DETERMINISTIC PATTERNING OF HOX EXPRESSION PROFILES DURING NEURAL DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

Lippmann, Ethan[1], Estevez-Silva, Maria C.[2], Ashton, Randolph S.[3]
[1]Wisconsin Institute for Discovery, University of Wisconsin, Madison, WI, USA, [2]Biomedical Engineering, University of Wisconsin-Madison, Wisconsin Institutes for Discovery, Madison, WI, USA, [3]Biomedical Engineering and Wisconsin Institute for Discovery, University of Wisconsin-Madison, Wisconsin Institutes for Discovery, Madison, WI, USA

Positional identity is specified during posterior neural development in vivo by the combinatorial expression of Hox transcription factors, which are important determinants of neural circuit organization in the hindbrain and spinal cord. Many studies have indicated the Hox code is regulated by combinations of Wnt/β-catenin, fibroblast growth factor (FGF), growth differentiation factor (GDF), and retinoic acid (RA) signaling, but little is known about how these pathways regulate Hox behavior in human cells. Here, we used a chemically defined culture system to determine how these signaling pathways regulate Hox expression during posterior neural differentiation of human pluripotent stem cells (hPSCs). Wnt/β-catenin and FGF signaling are both required to coordinate hPSCs to a highly pure neuromesodermal progenitor state marked by co-expression of T/brachyury and Sox2, which mimics the phenotype found in axial progenitors that form both the posterior neural tissue and its flanking mesoderm in vivo. Within this neuromesodermal state, Wnt/β-catenin and FGF signaling also facilitate colinear HOX activation to paralogs that match the hindbrain (HOX1-5) and cervical/thoracic spinal regions (HOX4-9), and GDF11 is required to activate HOX10-12 paralogs found in the lumbar/sacral regions. At any point during HOX propagation, the addition of RA is sufficient to convert the neuromesodermal progenitors to definitive neural identity. Moreover, RA acts as a 'stop' signal for HOX progression, thus yielding a fixed positional location along the posterior rostral/caudal axis defined by Hox protein expression profiles. The scalability and fully defined nature of this differentiation process, combined with its ability to predictably generate neural progenitors with defined Hox profiles, should make it attractive for basic biological studies and potential regenerative medicine applications.

W-3021
NEUROPROTECTIVE EFFECTS ON IGF-1 FUSION PROTEIN ON RETINAL GANGLION CELL SURVIVAL

Ma, Jie, Guo, Chenying, Teague, Giann, Chen, Dong Feng, Lashkari, Kameran
Schepens Eye Research Institute, Boston, MA, USA

We have previously found that the neural progenitor cells isolated from human persistent fetal vasculature (hNPPFVs) can differentiate into retinal ganglion-like cells (RGCs) both in vitro and after transplantation into the mouse vitreous. Interestingly, hNPPFV cells were observed to spontaneously penetrate the inner retinal layers and integrate with the host RGCs. These observations led us to investigate whether hNPPFVs would make good candidates for local delivery of neuroprotective factors for treatment of retinal and optic nerve degeneration. As a proof of concept, we examined the neuroprotective effects of IGF-1 delivered as a fusion protein with a fluorescent reporter in the mouse model of stress-induced RGC loss. To this end, IGF-1 cDNA spliced into a double-red fluorescent reporter, tdTomato or tdTomato alone were cloned into pJ603-neo vector and transfected into hNPPFVs to create hNPPFVIGF-1/tdTomato and hNPPFVtdTomato cells, respectively. IGF-1-tdTomato fusion protein secreted from these cells was biologically active in in vitro models. An established bead model of murine glaucoma was created by injecting polystyrene microbeads into the anterior chambers of adult C57BL/6J mice. Animals were intravitrealy injected with 2 µl (~50,000 cells) of either hNPPFVIGF-1/tdTomato, hNPPFVtdTomato or hNPPFVnull cells. Intraocular pressures (IOP) were measured every 3 days for 4 weeks after cell transplantation. Whole mount retina and eyecup cross sections were prepared to quantify RGC density and alterations in nerve fiber architecture. Expression of β-III-tubulin, HLA Class I antigen and tdTomato were examined in retinal flatmounts and sections. Semi-thin optic nerve cross sections were used for axon quantification. Bead injection induced an elevation of IOP from baseline of 10 to high of 25 mmHg in 7 days with a sustained effect up to 4 weeks. At 4 weeks post

injection, IOPs began to decrease but remained above baseline levels. PBS injection did not induce a sustained IOP elevation and served as control. Baseline RGC density in control eyes was (~4100 RGCs/mm2). RGC density decreased in bead-injected eyes transplanted with hNPPFVtdTomato or hNPPFVnull cells (~2500 RGCs/mm2), but remained relatively unchanged in bead-injected mice that received hNPPFVIGF-1/tdTomato (~4100 RGCs/mm2). Immunohistochemical analysis of serial sections confirmed that significantly more RGC nuclei were lost in hNPPFVtdTomato or hNPPFVnul injected eyes vs. hNPPFVIGF-1/tdTomato injected eyes. Examination of optic nerve cross sections further demonstrated decreased number of axons in hNPPFVtdTomato and hNPPFVnull vs. hNPPFVIGF-1/tdTomato injected eyes. Transplantation of hNPPFVIGF-1/tdTomato cells secreting biologically active IGF-1 in a form of fusion protein, IGF-1-tdTomato, improves the survival of RGCs and optic nerve axons in the bead model of stress-induced RGC loss. These findings support the concept of using hNPPFV cells as for targeted treatment of retinal degeneration as exemplified by the neuroprotective effects of IGF-1 fusion protein against stress-induced RGC loss.

W-3022
ENHANCING SELF-REPAIR BY ACTIVATING ENDOGENOUS PRECURSORS IN A MODEL OF NEONATAL BRAIN INJURY

Mahmud, Neemat[1], Dadwal, Parvati[1], Sinai, Laleh[1], Azimi, Ashkan[1], Miller, Freda D.[2], Morshead, Cindi M.[1]
[1]University of Toronto, Toronto, ON, Canada, [2]SickKids, The Hospital for Sick Children Research Institute, Toronto, ON, Canada

Hypoxia/Ischemia (H/I) of the immature brain is thought to play a major role in the development of cerebral palsy. It results in white matter injury and a loss of neurons leading to motor and/or cognitive deficits. Our goal is to design therapeutically relevant methodologies to enhance the activation of endogenous subependymal (SE) neural precursor cells (NPCs) to promote tissue repair and functional recovery. We have previously demonstrated tissue regeneration and functional recovery following stroke in adult rodent models using biologics to promote activation of endogenous SE NPCs. Metformin (met), a widely used drug to treat type II diabetes, has been shown to increase neurogenesis and oligogenesis from embryonic cortical precursors *in vitro* and promote neurogenesis *in vivo*. Hence, we asked whether met would promote brain repair following neonatal H/I. We examined the effects of met on early postnatal NPCs using the neural stem cell colony forming assay (neurosphere assay). We found that exposure to met *in vitro* led to a 2.2 fold increase in the numbers of neurospheres from postnatal day 8 (PND8) mouse pups. Moreover, met treated neurospheres that are differentiated in the presence of met resulted in a 2.6 fold increase in both neurons and oligodendrocytes. Based on these findings we hypothesized that met treatment following H/I would lead to an expansion in the size of the NPC pool, the formation of new neurons and oligodendrocytes, and functional recovery in sensory-motor tasks. To test our hypothesis we used the Vanucci model of H/I on PND8 mice followed by met treatment 24 hours post injury and for 7 days. Pups received met or vehicle through the mother's milk. We found that H/I alone caused a 4 fold increase in the size of the neural stem cell pool, similar to what is observed following stroke in adult mice. Met treatment alone (no injury) resulted in a 2.0 fold increase in the numbers of neurospheres, identical to what was observed following met treatment *in vitro*. We examined the fate of SE NPCs using Nestin-creERT2/Rosa YFP mice that received TAM via the mother's milk from PND1-5. We observed a significant 15 fold increase in the numbers of SE derived YFP+ cells within the brain parenchyma of H/I + met treated mice at 2 weeks post-injury. Strikingly, we observed a >20 fold increase in SE derived NPCs that differentiated into oligodendrocytes in H/I + met versus H/I only brains. Most important, the H/I + met treated mice displayed significant sensory/motor recovery following treatment. Our results show that metformin treatment for just one week following neonatal brain injury leads to expansion of the SE derived NPC pool, cell migration and differentiation leading to tissue repair and functional recovery.

W-3023
MODELING NEUROGENESIS AND SYNAPTOGENESIS USING HUMAN PLURIPOTENT STEM CELLS

Maroof, Asif M.[1], Suzuki, Naoki[2], McNeish, John D.[3], Chuang, Tsu Tshen[4], Eggan, Kevin Carl[2]
[1]Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA, [2]Harvard University, Cambridge, MA, USA, [3]Glaxo Smith Kline, Acton, MA, USA, [4]GSK, GlaxoSmithKline, King of Prussia, PA, USA

Despite synaptic remodeling and neurogenic events initiated in the repair process following injury in the mammalian central nervous system, new neurons capable of synaptic integration into existing adult circuitry have yet to be defined. While the adult stem cell niche produces new neurons, the glial environment is capable of exerting either beneficial or deleterious effects on the remodeling of neuronal circuitry. In order to study the specification of neural progenitor cells (NPCs) into neurons and the subsequent establishment of synaptic connections, we developed an in vitro co-culture system using human pluripotent stem cell-derived NPCs. In addition, the astroglial environment will be characterized to determine whether they exhibit inflammation or synapse-forming processes. This in vitro cell-based model will be a highly useful tool for high throughput screening of compounds that enhance the production of neurons capable of forming functional synaptic connections.

W-3024
TRANSPLANTATION OF ADULT MONKEY NEURAL STEM CELLS INTO CONTUSION SPINAL CORD INJURY MODEL IN RHESUS MACAQUES MONKEY

Moghiminasr, Reza[1], Nemati, Shiva[2], Baharvand, Hossein[3], Kiani, Sahar[4]
[1]Department of Stem Cells and Developmental Biology, Cell Science Research Center, ACECR, Royan Institute, Tehran, Iran, [2]Department of Stem Cells and Developmental Biology, Cell Science Research Center, ACECR, Royan Institute, Tehran, Iran, [3]Department of Stem Cells and Developmental Biology, Cell Science Research Center, ACECR, Royan Institute, Teheran, Iran, [4]Department of Stem Cells and Developmental Biology, Cell Science Research Center, ACECR, Royan Stem Cell and Dev Biology, Tehran, Iran

Background: Currently, cellular transplantation for spinal cord injuries (SCI) is the subject of numerous preclinical studies. Among the many cell types in the adult brain, there is a unique subpopulation of neural stem cells (NSC) that can self-renew and differentiate into neurons. The study aims, therefore, to explore the efficacy of adult monkey NSC (mNSC) in a primate SCI model. Methods: Isolated mNSCs were analyzed by flowcytometry, immunocytochemistry, and RT-PCR. Next, BrdU-labeled cells were transplanted into a SCI model. The SCI animal model was confirmed by magnetic resonance imaging (MRI) and histological analysis. Animals were clinically observed for 6 months. Results: Analysis confirmed homing of mNSCs into the injury site. Transplanted cells expressed neuronal markers (TubIII). Hind limb performance improved in transplanted animals based on Tarlov's scale.

Conclusion: Our findings indicate that mNSCs could facilitate recovery in SCI. Additional studies are necessary to determine the improvement mechanisms after cell transplantation.

W-3025
PREDICTION OF CENTRAL TRANSCRIPTION FACTORS IN GENE REGULATORY NETWORK DURING IPS DIFFERENTIATION TO PRIMARY NEUROSPHERE IN MOUSE

Mohammadnia, Abdulshakour[1], Yaqubi, Moein[2], Fallahi, Hossein[3], Massumi, Mohammad[4]

[1]Nanobiomaterial and Tissue Engineering, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran, [2]Nanobiomaterial and Tissue Engineering, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran, [3]Department of Biology, Razi University, Kermanshah, Iran, [4]National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

Introduction: Neurosphere is one of the crucial steps in derivation of neuronal cells form stem cells. The generation of neuroshperes is precisely tuned by Transcription factors (TFs). This bioinformatic study is aimed to predict some new TFs involved in neurosphere formation from induced Pluripotent Stem (iPS) cells by scrutinizing the high throughput gene expression profile of iPS-derived neurosphere. Method: Data were obtained from GEO server using GSE31598 accession number. Robust Multiple Averaging (RMA) algorithm was used for normalization of raw data and differentially expressed genes detected using fold change algorithm. Fold changes at 3 set as threshold for differential gene expression analyses. Functional clustering of differentially expressed genes performed using DAVID 6.7 and Enrichment scores more than 1.3 assumed as significant clusters. Network construction and visualization were conducted using Cytoscape 3.0.2.Topological and centrality parameters analyses of constructed network were performed using Cytoscpe's CentiScaPe v2.0 plugin. Eccentricity, Closeness and Degree, Betweenness, Stress and Centroid applied to find central genes in the network. ClueGO v1.8 and CluePedia v1.3 used for biological process and KEGG pathway analysis. MCODE plug-in was used to find high score module in protein-protein interaction network and JActveModules was used to find core activate modules in the network. Result: Differentiation of iPS to Primary Neurosphere (PNS) revealed 2521 differentially expressed genes from whose 1413 up- and 1108 were down-regulated. From these regulated genes, fifty six TFs regulate expression of 2249 differentially expressed genes in differentiation of iPS to PNS based on ChIP enrichment analysis and 223 valid protein-protein interactions were found for 50 of these TFs. POU5F1, NANOG, TRP53, KLF4, REST, STAT3, TET1, BMI1, OLIG2, SUZ12 and SALL4 ranked as 10 top central TFs in constructed gene regulatory network (SUZ12 and SALL4 have the same rank). Nervous system development was first Go term in top module of gene regulatory network ontology with 66 differentially expressed genes and lowest p-value. TFs include POU5F1, NANOG, TRP53, KLF4, REST, STAT3, BMI1, OLIG2, SUZ12 and SALL4 were found to present in 5 top active module of gene regulatory network. Finding of 56 TFs specially these 10 TFs may be helpful to increase efficiency of iPS to PNS differentiation.

W-3026
FUSARIC ACID UPREGULATING A SET OF DOPAMINE ASSOCIATED GENES THROUGH A TEAD1 TRANSCRIPTIONAL FACTOR, EFFICIENTLY DIFFERENTIATES FETAL HUMAN MESENCEPHALIC NEURAL PROGENITOR CELLS INTO DOPAMINE NEURONS.

Moon, Jisook[1], Schwarz, Sigrid[2], Schwarz, Johannes[3], Kim, Kwang-Soo[4], Cha, Kwang Yul[5], Chung, Sang-sup[1]

[1]CHA University, Seoul, Republic of Korea, [2]German Center for Neurodegenerative Diseases (DZNE), Technical University, Munich, Germany, [3]German Center for Neurodegenerative Diseases, Technical University, Munich, Germany, [4]Department of Psychiatry, Program in Neuroscience, McLean Hospital/Harvard Medical School, Belmont, MA, USA, [5]CHA University, Seoul, Republic of Korea

Current clinical applications of cell therapy in Parkinson's disease are limited due to lack of sufficient quality control, tissue availability, limited efficacy and safety concerns. Human midbrain neural progenitor cells (hmNPCs) derived from fetal tissue are a promising source to generate human dopamine (DA) neurons. Here, we extensively characterize hmNPCs of various weeks of gestation (GW) and develop a novel factor, Fusaric Acid (FA) for an efficient DA differentiation. We demonstrate that prolonged expansion of hmNPC retain floorplate markers and stemness without genetic modication. The potential to differentiate into large quantities of A9 specific DA neurons is elevated with FA which upregulated a set of DA associated genes such as Nurr1, TH, SLCO1C1, DCT, VAT1L, and KIAA1598 through a novel transcriptional factor, TEAD1. Furthermore, biological process of gene ontology (GO) and KEGG pathway results as well as in vitro study demonstrated the neuroprotection and differentiation effect of FA. FA treated hmNPCs improve impaired motor function in rodents, survive well in marmoset monkeys and do not exhibit tumor formation in immunodeficient nude mice short- (8w) and long-term (30w). We conclude, that hmNPCs are a promising source for GMP compliant long-term expansion, thus, hmNPC may provide an auspicious and safe alternative to generate sufficient dopaminergic neurons for clinical applications.

W-3027
THE ROLE OF NRF2 AND OXIDATIVE STRESS REGULATION IN ADULT NEUROGENESIS

Morante-Redolat, Jose Manuel[1], Pérez-Villalba, Ana[1], Belenguer Sánchez, Germán[1], Pérez-Sánchez, Francisco[1], Cuadrado, Antonio[2], Farinas, Isabel[1]

[1]Departamento de Biología Celular, Facultad de Biología, Universidad de Valencia/CIBERNED, Valencia, Spain, [2]Departamento de Bioquímica. Facultad de Medicina, Universidad Autónoma de Madrid, Madrid, Spain

Stem cells are found at specific locations within adult tissues and their behavior and lifelong maintenance is regulated by both cell intrinsic factors and signals from the microenvironment or niche in which they reside. However, stem cell niches in mammalian tissues are still poorly characterized likely owing to the dynamic changes required for the continuous production of new cells and to the complexity of the interactions between stem cells and their neighbors. In the subependymal zone (SEZ) of the murine adult brain, relatively quiescent multipotent neural stem cells (NSCs), bearing typical features of astroglial/radial glial cells, continually generate neuroblasts, via mammalian achete-schute homologue 1 (Mash1)+/ distal-less homeobox 2 (Dlx2)+ transit-amplifying progenitor (TAP) cells. Subsequently, these progenitor cells give rise to immature neuroblasts that migrate towards the olfactory bulbs where they mature into different types of olfactory interneurons.

Additionally, subependymal NSCs produce oligodendroblasts that migrate and generate mature oligodendrocytes in the white matter tracts of corpus callosum, fimbria fornix and striatum. Several studies have found that stem cells niches are hypoxic microenvironments and that high levels of reactive oxidative species are associated with stem cell dysfunction. For example, disruption of oxidative stress pathways leads to premature senescence of hematopoietic stem cells due to loss of their quiescence and to a decline in the capacity for self-renewal in mouse models. Ubiquitously expressed nuclear factor erythroid 2-related factor 2 (NRF2) is a major transcriptional regulator of the global cell response to oxidative stress. Interestingly, NRF2 response to oxidative stress declines with age, suggesting that a deficient redox balance could indeed underlie the decline in neurogenesis associated to aging. However, although it has been reported that NRF2 plays a regulatory role in several aspects of the homeostasis in hematopoietic stem cell pools, functional studies in NSCs are missing. We have investigated how NRF2 regulates the behavior of subependymal NSCs, by analyzing the SEZ and neurosphere cultures of NRF2-deficient mice. The results suggest that lack of NRF2 leads to alterations in the proliferation of NSCs and the production of differentiated progeny, both in vivo and in vitro, that cannot be attributed exclusively to oxidative stress caused by a defective antioxidant response. In fact, NRF2-null NSCs require the presence of antioxidants in the medium in order to expand and generate neurosphere cultures in vitro, but still show alterations in proliferation and differentiation under reduced oxidative conditions.

W-3028
REWIRING OF INHIBITORY INTERNEURONS BY IN VIVO REPROGRAMMING OF CORTICAL PROJECTION NEURONS

Mostajo Radji, Mohammed[1], Ye, Zhanlei[2], Rouaux, Caroline[1], Srubek Tomassy, Giulio[1], Brown, Juliana[1], Hensch, Takao[2], Arlotta, Paola[1]
[1]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA, [2]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA

We have previously demonstrated that direct lineage reprogramming of post-mitotic excitatory projection neurons can be induced in the cerebral cortex within a defined window of time. Upon overexpression of the master transcription factor Fezf2, post-mitotic upper layer callosal projection neurons (CPN) acquire molecular properties of deep layer corticofugal projection neurons (CFuPN) and change their axonal connectivity from interhemispheric, intracortical projections to corticofugal projections directed below the cortex. Here, we sought to determine whether reprogramming of upper layer CPN into deep layer CFuPN is sufficient to instruct the remodeling of their surrounding local inhibitory circuits. We first investigated the extent of projection neuron reprogramming by performing single-cell gene expression analysis of reprogrammed neurons. Our data shows that a subset of cells that received Fezf2 significantly upregulated multiple molecular markers of CFuPNs compared to control CPNs. In agreement, we find that reprogrammed CPNs acquire electrophysiological properties that are typical of CFuPNs. Notably, recordings of miniature inhibitory post-synaptic currents (mIPSCs) demonstrated that reprogrammed neurons receive increased inhibitory inputs, which resembled those of deep layer CFuPNs. In order to gain insight into this electrophysiological adaptation, we studied more specifically the contribution of Parvalbumin- (PV-) positive interneurons (INs). We found that PV-positive INs form more perisomatic synapses onto reprogrammed neurons than onto CPNs, with levels indistinguishable from endogenous CFuPNs. Optogenetic recordings and immunohistochemical analysis further confirmed an increased number of synapses by PV INs onto reprogrammed neurons. Further, we demonstrate that reprogrammed projection neurons are not intrinsically more sensitive to inhibition and therefore the observed increase of inhibitory input likely reflects rewiring of the cortical circuit, rather than the expression of more GABA receptors. Altogether, our results indicate that reprogramming the identity of projection neurons is sufficient to reshape the local inhibitory circuitry and highlights the importance of projection neuron diversity in controlling this process.

W-3029
DEVELOPMENT OF CORTICAL NEURON DIFFERENTIATION FROM PLURIPOTENT STEM CELLS USING SMALL MOLECULES

Motono, Makoto
Clinical Application, Laboratory of Neuronal Regeneration, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

The mammalian neocortex is a complex six-layered structure that contains various types of neurons and glia. It has the regions of the brain responsible for cognitive function, sensory perception, motor function and so on. Methods to model human cortical development in a controlled, defined manner from pluripotent stem cells (PSCs) have considerable potential to enable functional studies of human cortical development, circuit formation and function, and for constructing in vitro models of cortical diseases. Furthermore, the cells differentiated from PSCs using such methods are the promise source to compensate neurons lost with diseases or injury. The use of human PSCs as a donor cell source for transplantation therapy requires defined and controlled differentiation conditions. Previously, some laboratories showed that cortical neurons can be generated from mouse and human PSCs by monolayer culture and serum-free floating embryoid body-like quick aggregates differentiation methods. However, these culture methods require the addition of recombinant proteins such as Dkk1 (Wnt inhibitor), Lefty-1 (TGF-b/activin/nodal inhibitor) or BMPRIA-Fc (BMP inhibitor), which are produced in animal cells or E.coli, raising the possibility of infection or immune rejection due to cross-species contamination. By contrast, using chemical compounds to induce differentiation offers several advantages compared with using recombinant proteins for human clinical application. For examples, they are non-biological products, show stable activity, and have small differences between production lots. Thus, establishment of chemical compound-based culture systems will be necessary for human pluripotent cell-based transplantation therapies. In this study, we examined chemical compounds, especially Wnt inhibitors, and found that there was a difference in differentiation according to the inhibitors.

W-3030
A STEM CELL-BASED PLATFORM FOR DISCOVERY OF REMYELINATING THERAPEUTICS

Najm, Fadi[1], Zaremba, Anita[2], Madhavan, Mayur[1], Shick, Elizabeth[1], Karl, Robert[1], Sargent, Alex[2], Factor, Daniel C.[1], Miller, Tyler[3], Quick, Kevin[4], Tang, Hong[5], Papoian, Ruben[5], Miller, Robert[2], Tesar, Paul J.[1]
[1]Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH, USA, [2]Neuroscience, Case Western Reserve University, Cleveland, OH, USA, [3]Pathology, Case Western Reserve University, Cleveland, OH, USA, [4]Perkin Elmer, Waltham, MA, USA, [5]University of Cincinnati, Cincinnati, OH, USA

Millions of patients worldwide suffer from neurological disorders, diseases, or injuries involving central nervous system (CNS) demyelination. However no approved therapeutics currently exist which can promote repair of damaged myelin. We have developed a platform for the rapid production of pure, highly expandable

populations of rodent oligodendrocyte progenitor cells (OPCs), the premyelinating cells in the CNS, from pluripotent stem cells. This provides a system for high throughput phenotypic screening for small molecules that enhance the generation of mature oligodendrocytes from OPCs. We performed a primary phenotypic screen using both drug repurposing libraries and diversity collections of novel chemical entities. Hits were identified with activity 5 standard deviations above vehicle and validated across an 8-point dose curve. Validated primary hits were then subjected to functional assays to assess their ability to promote precocious myelination in ex vivo rodent brain slice cultures and in vivo in early postnatal mouse pups. Functional assay hits were then tested in the MOG35-55 chronic EAE rodent model of multiple sclerosis where 3 compounds showed significant enhancement of remyelination and reduction of clinical severity. When tested on human OPCs in vitro, these compounds also enhanced the generation of mature human oligodendrocytes providing direct relevance to human OPC biology. Our screening pipeline provides a powerful platform to discover candidate small molecule therapeutics that enhance remyelination in the CNS.

W-3032
EFFECTS OF ALTERED GTF2I AND GTF2IRD1 EXPRESSION ON THE GROWTH OF NEURAL PROGENITORS AND ORGANIZATION OF THE MOUSE CORTEX

Oh, Hyemin Amy
Institute Of Medical Sciences, University of Toronto, Toronto, ON, Canada

Williams-Beuren syndrome (WBS) is a rare neurodevelopmental disorder caused by the deletion of 26 genes on human chromosome 7q11.23. People with WBS exhibit an array of cognitive and behavioral features including intellectual disability, social disinhibition, anxiety and specific phobias, deficits in visuospatial construction and attention deficit hyperactivity disorder, but the neurobiological basis for these symptoms remains unknown. Clinical studies and analysis of WBS mouse models suggest that deletion of distal genes in WBS region contribute to the development of neurocognitive features of the disorder. Our lab has generated mouse models with altered gene dosage of two candidate genes from the WBS commonly deleted region, General Transcription Factor 2 I (Gtf2i) and GTF2I Repeat Domain containing protein 1 (Gtf2ird1). These models include mice with hemizygous deletion of Gtf2i (Gtf2i+/-) or of both genes (Gtf2i/Gtf2ird1+/-) and mice with duplication of Gtf2i (Gtf2i+/Dup). We have used these mice to investigate effects of altered copy number of Gtf2i and/or Gtf2ird1 on neural stem cell growth and neurogenesis, using neural precursor cells (NPCs). NNPCs from the brain cortex of embryonic day 12.5 mice were dissected, dissociated, seeded at equal densities and cultured for 3 days in vitro (d.i.v.). We then used cell-specific markers to analyze cell proliferation, apoptosis and differentiation. Our results showed that the changes in gene copy number of both Gtf2i and Gtf2ird1 affected neural precursor physiology, maintenance, proliferation and differentiation in a dose dependent manner in the midgestation developing mouse cortex. Hemizygous deletion of Gtf2i and Gtfi2rd1 had an overall reduction in the number of neuronal precursors compared to wild type littermates with no changes in proliferation and apoptosis. In contrast, duplication of Gtf2i had an increase in the number of precursors with increased proliferation. Similarly, deletion mice had reduced neurogenesis whereas duplication mice had enhanced neurogenesis. These disturbances incurred during early stages of brain development resulted in aberrant postnatal brain formation. Increased cell-packing density in cortical layer 5 was observed in deletion mice. There was an increase in layer 2-3 cortical thickness in duplication mice with no changes in overall cell density. The growth and differentiation of the neural precursors into neurons and the specification of specific neuronal subtype identities are crucial in the proper development of the cortex, and we hypothesize that some of the neurological features of WBS may stem from impairments in these early stages of neuronal development.

W-3033
INDUCTION OF GABAERGIC NEURON FROM ADULT RAT OLFACTORY SPHERE CELLS

Ohnishi, Yu-ichiro[1], Iwatsuki, Koichi[1], Shinzawa, Koei[2], Yoshimine, Toshiki[1]
[1]Department of Neurosurgery, Osaka University Medical School, Suita, Osaka, Japan, [2]Department of Medical Genetics, Osaka University Medical School, Suita, Osaka, Japan

Olfactory spheres (OSs) are clusters of cells generated by culturing the olfactory mucosa. We used a serum-free culture method to generate OSs from adult rat olfactory mucosa. We previously reported that OS cells expressed oligodendrocyte precursor markers, and underwent oligodendrocyte and Schwann cell differentiation in vitro and in vivo. In this study, we have demonstrated that valproic acid (VPA), the histone deacetylase inhibitor and clinical use as an anticonvulsant drug for epilepsy, induced the gabaergic neuronal differentiation of adult rat OS cells in vitro in concentration-dependent manner. VPA increased the number of neuronal marker-positive cells, and decreased oligodendrocyte marker-positive cells as measured by immunofluorescence staining. Western blotting and immunofluorescence analyses showed that OS cells treated with VPA had increased levels of acetylated histones H3 and H4. VPA also increased the number of GAD67 and GABA-positive cells. Western blotting analyses presented that VPA treatment increased the levels of GAD67. RT-PCR analysis showed that OS cells expressed Dlx1/2 gene, and VPA increased the expression of neuronal marker genes of OS cells.

W-3034
HYPERPOLARIZATION-ACTIVATED CYCLIC-NUCLEOTIDE GATED CATION CHANNELS REGULATE MURINE NEURAL PROGENITOR CELL PROLIFERATION

Omelyanenko, Anna[1], Johard, Helena[1], Gao, Fei[1], Zilberter, Misha[2], Harkany, Tibor[2], Blomgren, Klas[3], Andäng, Michael[1]
[1]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden, [2]Department of Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden, [3]Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden

The role of calcium in cell proliferation has long been established. However, it is still unknown what role, if any, other ions may play in this process. We have previously reported that GABA A channels, conducting a chloride current, regulate the proliferation of murine neural progenitor cells (NPC) isolated from the subventricular zone (SVZ). In the present work we looked at the possible contribution of cation channels to determining the proliferative rate of NPCs. We found that channels belonging to the hyperpolarization-activated cyclic nucleotide-gated channel (HCN) family, namely HCN2 and HCN3, are expressed in NPCs isolated from the SVZ of adult mice and are electrically active. On a protein level, channel abundance varies between NPCs, but is significantly higher in cells in the S and G2/M cell cycle phases. Correspondingly, the HCN current could only be recorded in cell from S and G2/M cell cycle fractions after FACS according to DNA content, suggesting a cell cycle phase specific role for the HCNs. To test for possible functional importance of these channels, we blocked their function by shRNA knockdown and small molecule inhibition.

Both treatments resulted in a significant reduction in NPC numbers by reducing their proliferative rate. NPCs with perturbed HCN function accumulated in G1, with little induction of apoptosis or differentiation. In the case of small molecule inhibition, the effect was fully reversible and upon washout, normal proliferation of the NPCs was restored. The same effect was observed on NPC proliferation in vivo upon injection of the specific inhibitor. We are currently investigating the role of HCNs in cell cycle progression and the mechanism mediating the observed reversible accumulation of NPCs in the G0/G1 phase and reduction in proliferation upon attenuation of HCN function.

W-3035
CONVERSION OF PRIMARY AND PLURIPOTENT STEM CELL-DERIVED NEUROEPITHELIAL STEM CELLS INTO REGION-SPECIFIC RADIAL GLIA

Ostermann, Laura[1], Ladewig, Julia[1], Müller, Franz-Josef[2], Tailor, Jignesh[3], Smith, Austin[3], Koch, Philipp[1], Oliver, Brüstle[1]
[1]Institute of Reconstructive Neurobiology, University of Bonn, Bonn, Germany, [2]Department of Psychiatry and Psychotherapy, Centre for Integrative Psychiatry, Kiel, Germany, [3]Wellcome Trust Centre for Stem Cell Research, Cambridge, United Kingdom

In recent years it has become possible to isolate and expand neural stem cells (NSCs) from different sources using growth factor-based protocols. A still open question is to what extent these diverse stem cell systems reflect physiological stem cell states observed in vivo. During nervous system development, early neuroepithelial stem (NES) cells with a highly polarized morphology and responsiveness to regionalizing morphogens give rise to radial glia (RG) cells, which generate region-specific neurons. Recently, stable neural cell populations reminiscent of NES cells have been obtained from pluripotent stem cells (lt-NES cells) and the fetal human hindbrain (hbNES cells). Here, we explore whether these cell populations, similar to their in vivo counterparts, can give rise to regionally specified RG-like cells. To that end we propagated lt-NES and hbNES cells temporarily in differentiating conditions. Upon re-initiation of growth factor treatment, these cells were found to enter a developmental stage reflecting major characteristics of RG cells. These RG-like NSCs could be expanded for at least 25 passages and expressed markers typically associated with RG cells, while NES cell markers were down-regulated. RG-like cells exhibited stable region-specific transcription factor expression with anterior, hindbrain- or spinal cord-derived RG-like cells maintaining their positional identity during multiple passages of in vitro proliferation and upon in vivo transplantation. Preservation of positional identity was robust and could not be overcome even by strong regionalizing factors such as retinoid acid. Along this line, RG-like cells generated region-specific neurons appropriate for their positional identity. Importantly, RG-like cells obtained from PSC-derived lt-NES cells and hbNES cells as well as primary human RG cells showed similar properties, indicating that conversion of NES cells into RG-like cells recapitulates the developmental progression of early NES cells into radial glia cells observed in vivo.

W-3036
COMPARATIVE STUDiES OF PRIMATE CEREBRAL CORTEX DEVELOPMENT USING PLURIPOTENT STEM CELL MODELS

Otani, Tomoki, Livesey, Frederick J.
Wellcome Trust /CRUK Gurdon Institute, Cambridge, United Kingdom

The cerebral cortex is the region of the central nervous system responsible for many higher brain functions, including sensory perception, executive functions, consciousness and creativity. Compared to other primate species, the human cortex contains an increased number of neurons, as well as a relatively enlarged surface area and expanded volume. These evolutionary differences are thought to contribute to humans' increased cognitive ability. Using methods to replay cerebral cortex development from human pluripotent stem cells, we have compared in vitro cortical development between humans and non-human primates, focusing initially on macaques. Many aspects of human cortical development are similar in macaques, including the diversity of neural progenitor cell populations, the developmental progression from lower to upper cortical neurogenesis, and eventual emergence of neuronal networks with functional synapses. However, the timings of these events in macaque development are notably abbreviated compared with humans: macaque cortical progenitor cells cycle faster, and the temporal order of neurogenesis is compressed relative to humans. Individual neurons also undergo electrophysiological maturation over a shorter period of time. Clonal analysis of cortical progenitor cells found that macaque cells switch their mode of cell division from symmetric expansion to asymmetric neurogenesis much earlier in development, resulting in smaller clonal output and reduced clone sizes. Initial results to investigate the relative roles of cell autonomous mechanisms and intercellular signaling/environment in species-specific development, using human/macaque cortical stem cell co-cultures, suggest that these differences are driven by cell autonomous, species-specific mechanisms.

W-3037
HUMAN FETAL BRAIN-DERIVED NEURAL STEM/PROGENITOR CELLS GRAFTED INTO THE ADULT EPILEPTIC BRAIN RESTRAIN SEIZURES IN RAT MODELS OF TEMPORAL LOBE EPILEPSY

Park, Kook In[1], Lee, Haejin[2], Yun, Seokhwan[2], Kim, Il-Sun[3], Lee, Il-Shin[3], Shin, Jeong Eun[3], Park, Soo Chul[4], Kim, Won-Joo[4]
[1]Severance Children's Hospital, Department of Pediatrics, Brain Korea 21 Plus Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea, [2]Brain Korea 21 Plus Project for Medical Science, Yonsei University College of Medicine, Seoul, Republic of Korea, [3]Severance Children's Hospital, Department of Pediatrics, Yonsei University College of Medicine, Seoul, Republic of Korea, [4]Department of Neurology, Yonsei University College of Medicine, Seoul, Republic of Korea

Temporal lobe epilepsy (TLE)_the most prevalent type of partial epilepsy_is typically resistant to antiepileptic drug therapy. Cell transplantation has been suggested as an alternative therapy because this can suppress epileptogenesis and spontaneous recurrent motor seizures in animal models. To evaluate the therapeutic potential of human neural stem/progenitor cells (NSPCs) for treating TLE, we transplanted human NSPCs, derived from an aborted fetal telencephalon at 13 weeks of gestation and expanded in culture as neurospheres over a long time period, into the epileptic hippocampus of fully kindled and pilocarpine-treated adult rats exhibiting TLE. NSPC grafting reduced behavioral seizure duration, afterdischarge duration on electroencephalograms, and seizure stage in the kindling model, as well as the frequency of spontaneous recurrent motor seizures in pilocarpine-induced animals. However, NSPC grafting neither improved spatial learning or memory function in pilocarpine-treated animals. Human NSPCs not only gave rise to all three central nervous system neural cell types in vitro, but also differentiated into ganglionic eminences-derived γ-aminobutyric acid (GABA)-ergic interneurons and released GABA in response to the depolarization induced by a high K^+ medium. Following transplantation, grafted cells showed extensive migration around the injection site, robust engraftment, and long-term survival, along with differentiation into

βtubulin III+ neurons (~34%), APC-CC1+ oligodendrocytes (~28%), and GFAP+ astrocytes (~8%). Furthermore, among donor-derived cells, ~24% produced GABA. Additionally, NSPC grafting restores the anticonvulsant glial cell-derived neurotrophic factor levels in host hippocampal astrocytes of the epileptic brain. These results suggest that human fetal brain-derived NSPCs possess some therapeutic effect for TLE treatments although further studies to both increase the yield of NSPC grafts-derived functionally integrated GABAergic neurons and improve cognitive deficits are still needed.

EYE OR RETINAL CELLS

W-3038
AUTOPHAGY MEDIATES THE TISSUE REGENERATION OF CORNEAL STEM CELLS FOR ULTRAVIOLET A-INDUCED CORNEAL DAMAGE

Chen, Ying-Ting, Pollreisz, Andreas, Schmidt-Erfurth, Ursula
Ophthalmology, Medical University of Vienna, Vienna, Austria

Autophagy is a cellular program for the lysosomal degradation of damaged organelles, protein aggregates and bulk cytoplasm. Critical roles of autophagy have been reported for various epithelia exposed to cellular stress caused by UV radiation and implicated in stem cell-mediated tissue regeneration. In current study we aim to establish an in vivo model for determining the function(s) of autophagy in the limbal stem cells (LSC). Mice carrying floxed (loxP sites containing) alleles of the essential autophagy-related gene Atg7 were crossed with mice expressing the Cre recombinase under the control of the basal progenitor's Krt14 promoter. This led to efficient deletion of Atg7 in the basal progenitors at the limbus. UVA at the dose of 100J/cm-2 was applied to 3 Atg7f/f Krt14-Cre mice and 3 Atg7f/f controls. RNA and protein lysate of enzymatic isolated corneal epithelia were collected for qPCR and WB analysis. Rapid adhesion with fibronectin was used to enrich Krt14-expressing basal progenitors for determining the differential roles of autophagy for LSCs during tissue repair. Before UVA exposure, Atg7f/f controls in WB expressed basal expression of autophagy-associated LC3-I and LC3-II proteins, while absent in Cre mice. Six hours after UVA exposure, WB analysis revealed accumulation of autophagy-associated LC3-II protein in the corneas of Atg7f/f controls with a LC3 I to LC3 II shift, in contrast to negligible signals of LC3 II in Atg7f/f Krt14-Cre mice. Corneal progenitor marker p63 was activated in WT post-UVA irradiation (+4.88 fold vs. control) while inactivated in Atg7-KO (+0.65 fold vs. control) at transcriptional level ($p<0.05$). The central corneal thickness in WT vs. Atg7-KO is 49.4 vs. 36.8 um ($p <0.05$). The current study established Atg7f/f Krt14-Cre mice as a model suitable for characterizing the roles of autophagy in the LSC. Autophagy might play a critical role in promoting LSCs in post-UVA corneal tissue regeneration.

W-3039
P-CADHERIN IS NECESSARY FOR MOUSE RETINAL STEM CELL CLONAL COLONY FORMATION IN VITRO, BUT IS DISPENSIBLE FOR THE DEVELOPMENT OF RETINAL STEM CELLS IN VIVO.

Coles-Takabe, Brenda, van der Kooy, Derek J.
Molecular Genetics, University of Toronto, Toronto, ON, Canada

Adult retinal stem cells (RSCs) are rare quiescent cells within the bi-layered ciliary epithelium (CE) of the eye, which is made up of non-pigmented inner (NPE) and pigmented outer cell layers (PCE). The pigmented CE expresses the cell surface marker P-Cadherin (P-Cad) and the non-pigmented layer expresses N-Cadherin (N-Cad). Through FACs and single cell analyses, we previously have shown that RSCs arise from the pigmented CE and express P-Cad. However, the clonal RSC colonies that arise from the CE are made up of both pigmented and non-pigmented retinal precursor cells, and the spheres express both P-Cad and N-Cad. In order to test whether these cell surface cadherins were important to the formation of RSC sphere colonies, function-blocking cadherin antibodies were used. Mouse CE cells were dissociated into single cells and function-blocking antibodies were employed to generate dose response effects on clonal sphere formation. Both P- and N-Cad antibody treated cells revealed significant decreases in sphere formation compared to controls (there were no effects of a control E-Cad antibody). These results suggest that P- and N-Cad may facilitate the adherence of the cells in the formation of the clonally derived spheres. P-Cad-/- mice were employed to test whether P-Cad is required for RSC development and maintenance in vivo. The P-Cad-/- mice had 2.5 times more clonal RSC spheres than control mice; however the extent of the PCE in vivo and the frequencies of clonal sphere formation were similar in the knockout and wild-type mice, suggesting that fewer RSCs and other CE cells were damaged during their dissociation from P-Cad-/- mice. This suggests that we may be underestimating the numbers of RSCs present in vivo. The ubiquitous P-Cad-/- mice may up-regulate compensatory adhesion molecules that keep the ciliary epithelium together, and may mask any effect that loss of the P-Cad may have on the retinal stem cell. Experiments that selectively knock out P-Cad in an adult eye may elucidate any effects that P-Cad may have on the RSC in vivo. These experiments demonstrate that the RSCs reside in the PCE layer and that they express P-Cad, which may be important to the formation of adherent sphere colonies in vitro, but is not essential for the development of RSCs in vivo.

W-3040
DERIVATION OF TRACEABLE AND TRANSPLANTABLE PHOTORECEPTORS FROM MOUSE EMBRYONIC STEM CELLS

Decembrini, Sarah[1], Koch, Ute[2], Radtke, Freddy[2], Moulin, Alexandre[3], Arsenijevic, Yvan[1]
[1]Unit of Gene Therapy and Stem Cell Biology, Jules-Gonin Eye Hospital, Lausanne, Switzerland, [2]Institut Suisse de Recherche Expérimentale sur le Cancer, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [3]Eye Pathology Laboratory, Jules-Gonin Eye Hospital, University of Lausanne, Lausanne, Switzerland

Retinal degenerative diseases resulting in the loss of photoreceptors are a major cause of blindness. Recently, different groups validated the possibility to reactivate dormant retinal circuits of degenerating retinas using retinal prosthesis, gene therapy and 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 cells (ESC) for their capacity to generate retinal progenitors and photoreceptor cells *in vitro*. In this study, we derived a new transgenic ESC line in which the reporter gene, the *Crx*-GFP transgene, is expressed in both post-mitotic immature and mature photoreceptors, and assessed the extent to which this protocol recapitulates photoreceptor development *in vitro*. Various oxygen concentrations were tested at different development stages to improve photoreceptor production. As observed during retinogenesis, the optimized 3D-retina induction protocol allows the production of GFP-positive photoreceptors between 12 and 14 days of culture reaching the peak of birth between day 18 and 20 of culture. Similarly the intensity of the GFP signal and their alignment increased over time. We observed

that hyperoxic condition improved photoreceptor survival only when present from photoreceptor differentiation onset. Up to 10 layers of photoreceptors can be formed in each *in vitro*-generated retina. In addition we proved that transplantation of ESC-derived photoreceptors is feasible. No appearance of tumour formation was detected after transplantation of sorted photoreceptor cells. Many *Crx*-GFP-positive cells show the presence of outer-segments, ribbon synapses, and light signal transduction pathway proteins. These experiments show the feasibility to reliably generate a large quantity of integration-competent photoreceptors from ESC. A further characterization of the transplanted photoreceptors to reveal their capacity to mediate light stimuli is underway.

W-3041
BMPS AND SFRP2 MAINTAIN ADULT RETINAL STEM CELL QUIESCENCE IN VITRO AND THEIR INHIBITION IN VIVO ELICITS PROLIFERATION IN THE MOUSE EYE

Grise, Kenneth Neil, Balenci, Laurent, van der Kooy, Derek J.
Molecular Genetics, University of Toronto, Toronto, ON, Canada

Adult retinal stem cells (RSCs) are a rare subset of cells that reside in the pigmented ciliary epithelium (CE) of the mammalian eye. Once dissociated from the CE, RSCs readily proliferate to form clonal, free floating spheres after 7 days. These spheres have the capacity to self-renew as well as differentiate into retinal pigmented epithelium (RPE) and all of the cell types of the neural retina (NR). Despite having the capacity to proliferate *in vitro*, RSCs do not proliferate or generate new retinal cells in adult mammals *in vivo*. Yet, the proliferative capacity of RSCs outside of the eye suggests that quiescence is not an intrinsic property of RSCs, but rather, resultant of interactions with inhibitory factors in the RSC niche. Thus, we aimed to identify the factors responsible for maintaining RSC quiescence and determine if those factors can be targeted *in vivo* to disinhibit RSC proliferation and induce retinal neurogenesis. To investigate if discrete tissue types in the eye contribute to the inhibitory RSC niche, we generated tissue-specific conditioned media (CM) from postnatal day 2 (PND2) and adult mouse eyes and added it during the primary RSC clonal sphere formation. The CM from PND2 eye tissues had no effect on sphere growth, whereas the CM from the lens and cornea of adult eyes showed dose-dependent reductions in sphere number. Furthermore, sphere number was rescued upon CM washout, suggesting the presence of secreted factors unique to the adult lens and cornea that could reversibly suppress RSC proliferation. Due to previous evidence that the TGFβ and Wnt pathways regulate retinal stem cell proliferation, we considered two proteins known to be expressed in the adult eye as potentially mediating the lens and cornea CM inhibition: bone morphogenic proteins (BMPs) and secreted frizzled related protein 2 (sFRP2). We found that BMP proteins can dose-dependently impede sphere formation and that the BMP inhibitor, noggin, was able to restore sphere formation to control levels. Similarly, recombinant sFRP2 suppressed sphere number and this was reversible upon the addition of a function blocking antibody against sFRP2 (α-sFRP2). When noggin and α-sFRP2 were added in combination to lens and cornea CM, sphere number returned to control levels. Thus, inhibiting BMP and sFRP2 signaling eliminated lens and cornea CM-induced quiescence of adult RSCs *in vitro*. Next, we investigated whether BMP and sFRP2 inhibition could disinhibit RSC quiescence *in vivo*. We injected noggin or α-sFRP2 intravitreally 3 times at 24 hour intervals into the right eye. The left eye was injected with an equivalent volume of PBS as a control. All injections also included 0.5ug/uL of EdU. Seven days after the last injection, EdU-positive cells were detected in both noggin and α-sFRP2 treated eyes at a much higher frequency than control at all doses. Also, the number of EdU-positive cells in both conditions appeared to be dose-dependent. These results establish that proliferation can be induced through inhibition of BMP and sFRP2 signaling within the adult mouse eye. This is a promising, yet very preliminary, result. Experiments are underway to better characterize the cell types being induced to proliferate, the combinatorial effects of noggin and α-sFRP2 together and with mitogens, as well as whether any retinal neurogenesis ensues.

W-3042
COMBINED CELL AND GENE THERAPY TOWARDS THE TREATMENT OF AGE-RELATED MACULAR DEGENERATION AND DIABETIC RETINOPATHY

Hacibekiroglu, Sabiha[1], Michael, Iacovos[2], Westenskow, Peter[3], Ballios, Brian George[4], Mitrousis, Nikolaos[4], Jingsheng, Tuo[5], Chan, Chi Chao[5], Boyd, Shelley R.[6], van der Kooy, Derek J.[4], Shoichet, Molly S.[4], Friedlander, Martin[3], Nagy, Andras[7]
[1]LTRI/Mount Sinai Hospital, Toronto, ON, Canada, [2]Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland, [3]The Scripps Research Institute, La Jolla, CA, USA, [4]Donnelly Centre for Cellular and Biomolecular Research, Toronto, ON, Canada, [5]National Eye Institute, National Institutes of Health, Bethesda, MD, USA, [6]Li Ka Shing Knowledge Institute, Toronto, ON, Canada, [7]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

Age-related macular degeneration (AMD) and diabetic retinopathy (DR) are the most common blindness-causing eye diseases affecting millions of people worldwide. Both are associated with abnormal blood vessel growth (neovascularization) and properties caused by the up-regulation of vessel endothelial growth factor A (VEGF-A). This leads to vascular leakage, vessel dilatation, tortuosity, haemorrhage and ultimately to cell death, severe vision loss or even blindness. Current treatments include injection of anti-angiogenics such as VEGF-A antibody (e.g. Ranibizumab). Such treatments improve visual function through regression of abnormal blood vessels and haemorrhage that blocks vision. However, the repair is temporary, thus the patients require monthly injections. In addition, these treatments could have side effects such as stroke, gastrointestinal perforations and bleeding. To overcome the problems of current therapies, we hypothesized that replacement of damaged cells that produce doxycycline inducible novel, local acting anti-VEGF mini-bodies can inhibit abnormal blood vessel growth in AMD and DR in a controlled and long-term manner, thereby improving vision and diminishing any potential side effects. In previous studies, we have generated a bi-functional VEGF sticky-trap (by modifying the original VEGF trap) that is able to trap VEGF as well as bind (i.e. "stick") to the extracellular matrix (ECM) through binding to heparan-sulphate proteoglycans. This consequently allows inhibition of neovascularization only at the site of expression or administration. We have shown that VEGF sticky-trap, upon intravitreal and subretinal injection, binds to the ECM components of the eye. In contrast to the original VEGF trap, VEGF sticky-trap was undetectable in circulation 6 hrs post eye injection. These results indicate that VEGF Sticky-trap remains locally at the site of administration and once it enters the circulation it is degraded within a short amount of time (<6 hrs) limiting systemic side effects. Furthermore, we have shown that VEGF sticky-trap is able to inhibit neovascularization in a murine model of DR demonstrating the effectiveness of this biologic in an in vivo environment. Functional retinal pigment epithelium (RPE) cells and neuronal progenitor cells expressing VEGF sticky-traps in a doxycycline (DOX)-inducible manner have been generated from human and mouse embryonic stem cells. We have shown that VEGF sticky-trap, expressed by these cells in vitro, is able to bind to cell ECM and trap soluble VEGF only upon DOX induction. These

transgenic RPE cells were able to incorporate into the eye, and we are currently evaluating the ability of these cells to express VEGF sticky-trap in vivo, to inhibit NV and improve vision in animal models of AMD and DR, such as the laser-induced chorodial NV mouse, the Ccl2;Cx3cr1 double KO mouse and the OIR mouse. The injection of these cells into the diseased eye will allow for long-term treatment of AMD and DR by replacement of atrophic cells, doxycycline controlled inhibition of neovascularization and reduction of abnormal blood vessel. Ultimately, this novel combination of stem cell and gene therapy approach may translate into improved treatment options for patients with AMD and DR and contribute to currently on-going clinical trials using RPE cells transplantation into the eye of patients with AMD.

W-3043
DIRECTED DIFFERENTIATION OF SKIN-DERIVED PRECURSORS INTO FUNCTIONAL CORNEAL ENDOTHELIUM

Inagaki, Emi[1], Hatou, Shin[1], Yoshida, Satoru[1], Kawakita, Tetsuya[1], Okano, Hideyuki[2], Tsubota, Kazuo[1], Shimmura, Shigeto[1]
[1]Keio University School of Medicine, Tokyo, Japan, [2]Keio University, School of Medicine, Tokyo, Japan

Purpose: Corneal endothelial dysfunction remains a major indication for corneal transplantation. However, corneal transplantation has problems such as shortage of donors and graft rejection. Skin-derived precursors (SKPs) are postnatal stem cells with neural crest derivation same as corneal endothelium. These cells can be easily isolated from skin segments and have the capacity to differentiate into multiple cell types. In this study, we show that upon exposure to some growth factors, murine SKPs acquire sufficient corneal endothelial features that could make these cells suitable in future autologous stem cell therapy for corneal endothelium dysfunction patients. Methods: SKPs were isolated from neonate murine skin and cultured in SKPs proliferation medium. SKPs-TECE (SKPs derived corneal endothelium) was engineered in a medium containing retinoic acid and glycogen synthase kinase (GSK) 3b inhibitor (activator of Wnt/b-catenin signaling). RT-PCR, Immunohistochemistry and in vitro measurement of NaK-ATPase pump function was measured by using chamber. To assess function of TECE in vivo, we used rabbit penetrated keratoplasty model. Normal rabbit cornea was stripped endothelium with D's membrane. TECE with collagen carrier was transplanted by using this cornea, whereas control group was transplanted only collagen carrier. Results: SKPs-TECE express major corneal endothelial cell markers (Atp1a1, Slc4a4, Col4a2, Col8a2, and Cdh2). Immunohistochemistry staining revealed major tight junction marker, ZO1 staining in SKPs-TECE. Na,K-ATPase pump activity of SKPs-TECE was 300.3±37.9μA/cm2, which was significantly higher than control 3T3 cells, and over 2.5-fold higher than cultured mouse corneal endothelial cells (116.3±34.4μA/cm2). Furthermore, SKPs-TECE transplanted into rabbit corneas (n=4) maintained transparency and corneal thickness (544.0±85.0μM), whereas control corneas without SKPs-TECE (n=6) showed marked edema and increased corneal thickness (1105.8±165.9μM). Conclusion: We successfully induced functionally differentiated corneal endothelium from murine postnatal stem cells derived from skin. This may lead to a novel autologous stem cell therapy for corneal endothelial function.

MUSCLE CELLS

W-3049
PERICYTES INDUCE BOTH SATELLITE CELL QUIESCENCE AND DIFFERENTIATION DURING POST-NATAL MUSCLE GROWTH

Kostallari, Enis, Baba-Amer, Yasmine, Lafuste, Peggy, Gherardi, Romain
INSERM U955 / Team 10, Créteil, France

Muscle microvasculature is often considered solely as a source of nutrients and oxygen for growing muscle cells. However, muscle microvascular cells are also important cell players in muscle satellite cells (mSCs) niche, which represents a major aspect of muscle stem cell biology and just starts to be explored. The present study aims at understanding in depth the functional relationships between microvascular cells and mSCs during post-natal muscle growth. The project is in line with previous findings of our lab indicating that (1) similarly to rodents, muscle microvasculature is stereotypically organized into microvascular units of 6-to-8 capillaries in humans; (2) capillaries are closely associated with, and functionally interact with mSCs in adult humans and mice; 3) both autocrine and paracrine Angiopoietin1 (Angpt-1/Tie2) signalling promote mSC quiescence and self-renewal. Capillaries host both endothelial cells (ECs) and peri-endothelial cells called pericytes that exert ECs stabilizing properties, and represent one important source of Angpt-1. In vitro, we performed indirect co-cultures, muscle myofibers culture in conditioned media and long term culture in conditioned media to study the effects of pericytes and ECs on the behavior of mpc (mSC in culture). We showed that ECs stimulated mSC proliferation through PDGF-BB and Angpt-2, whereas pericytes had dual effects, promoting myogenic cell differentiation through IGF-1 and inducing mSC quiescence through Angpt-1. In vivo, we used C57Bl/6 mice to study the post-natal development. We show that in adult muscle almost all capillary sections show pericyte coverage, and ~75% of quiescent mSCs are closely associated with a pericyte. During post-natal mouse muscle development, NG2+ pericytes are initially remote from cycling mSCs, and progressively move towards mSCs, entering in their vicinity as myofibers increase in size and mSCs become quiescent. The phenomenon is associated with myofiber growth suggesting coordinated angio-myogenesis. We used also two transgenic mouse strains: Tg:NG2-Cre:iDTR mice and Tg:TNAP-CreERT2:Angpt-1 mice to study the effect of pericyte ablation or pericyte-derived Angpt-1 depletion on the behavior of mSC during post-natal development and regeneration. Consistently, DT-induced ablation of muscle pericytes in adult muscle of Tg:NG2-Cre:iDTR mice and early conditional inhibition of pericyte Angpt-1 production in Tg:TNAP-CreERT2:Angpt1 mice, induced the release of mSCs from homeostatic quiescence. This basic research study will allow a better understanding of cell interplays and molecular pathways supporting muscle growth and regeneration. It may lead to novel therapeutic strategies aimed at stimulating the regenerative angio-myogenic program in injured and diseased muscle, in line with recent evidence that muscle regeneration can be improved by combined delivery of angiogenic and myogenic factors.

W-3050
SELF-RENEWAL AND DIFFERENTIATION OF SKELETAL MUSCLE SATELLITE CELLS ARE REGULATED BY C/EBP BETA

Lala-Tabbert, Neena, Marchildon, Francois, Fu, Dechen, Wiper-Bergeron, Nadine
Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada

Post-natal growth and repair of skeletal muscle relies upon a population of quiescent muscle precursor cells, called satellite cells (SCs). These cells are capable of being activated to proliferate and differentiate into new myofibres, as well as self-renew to re-populate the SC niche. Our lab is interested in examining the role of CCAAT/Enhancer Binding Protein beta (C/EBPβ), a bZIP transcription factor, in myogenesis. We found that overexpression of C/EBPβ in C2C12 myoblasts and primary myoblasts reduced MyoD and myogenic protein levels during differentiation, in addition to reducing fusogenic ability. Furthermore, C/EBPβ increased Pax7 protein expression, in vitro, and was localized to Pax7+ SCs, in vivo. C2C12 myoblasts overexpressing C/EBPβ were phenotypically similar to slowly proliferating satellite cells as demonstrated by their decreased BrdU incorporation and increased expression of the quiescent satellite cell marker, Caveolin-1. Using genetic tools to conditionally abrogate C/EBPβ expression in SCs, we found that loss of C/EBPβ led to increased differentiation and decreased self-renewal of SCs in myofiber cultures. Furthermore, loss of C/EBPβ in satellite cells led to a decrease in the number of Pax7+ cells in the satellite cell niche. Our results suggest that C/EBPβ is a novel regulator of satellite cell self-renewal and differentiation.

W-3051
ANTI-APOPTOTIC ROLE FOR CCAAT/ENHANCER BINDING PROTEIN BETA (C/EBPBETA) IN MUSCLE SATELLITE CELLS

Marchildon, Francois, Wiper-Bergeron, Nadine
University of Ottawa, Ottawa, ON, Canada

Muscle satellite cells are the main source of regenerative capacity in skeletal muscle. CCAAT/Enhancer Binding Protein beta (C/EBPβ) a bzip transcription factor whose expression is high in muscle satellite cells, acts as a repressor of the myogenic program at least in part by inhibiting MyoD expression and function, and is downregulated upon stimulation to differentiate. Given that C/EBPβ expression is regulated by a number of inflammation-related molecules, we hypothesized that expression of C/EBPβ in satellite cells in the context of muscle injury would protect satellite cells from apoptosis, though also inhibit repair. Using a conditional knockout mouse model in which C/EBPβ expression was abolished in Pax7+ cells, we found that loss of C/EBPβ increased myoblasts apoptosis via treatment with thapsigargin or with recombinant TNFα. Following an acute muscle injury by cardiotoxin, conditional knockout animals failed to repair muscle damage as efficiently as littermate controls, with a concomitant increase in satellite cell apoptosis. Our findings support the notion that C/EBPβ is a pro-survival factor in adult muscle stem cells and that failure to stimulate or maintain its expression coupled with an apoptotic stimulus will decrease cell survival and cripple muscle regeneration.

W-3052
DISCOVERY OF SMALL MOLECULES TO TREAT SARCOPENIA

Gee, Amanda, Schneider, Joel, **Price, Feodor**, Buchanan, Sean, Castiglioni, Alessandra, Tabebordbar, Mohammadsharif, Wagers, Amy, Rubin, Lee
Harvard University Department of Stem Cell and Regenerative Biology, Cambridge, MA, USA

The functional and structural decline of skeletal muscle is one of the first hallmarks of aging in many organisms. The growth, maintenance, and regeneration of skeletal muscle is attributed to the satellite cell: a mitotically quiescent stem cell that resides between the basal lamina and sarcolemma of the muscle fiber. Intriguingly, as an organism ages, a decrease in satellite cell numbers accompanies and partially accounts for deteriorating skeletal muscle function. To explore a potentially new method for slowing the decline in skeletal muscle function, we established a screen capable of identifying small molecules or biologicals that promote the proliferation of satellite cells. We discovered multiple compounds that display a cell autonomous effect in increasing satellite cell numbers and do so in the nM concentration range. Following subcutaneous injection of some of these compounds, mice following cardiotoxin-induced muscle injury displayed an increase in the number of total satellite cells, an increase in expression of the satellite cell marker Pax7 and the cross sectional area of regenerating fibers. Furthermore, we confirmed the ability of our compounds to promote proliferation of satellite cells from aged mouse muscle and from human skeletal muscle, while having no effect on fibroblast proliferation. Taken together, our results provide compelling evidence that small molecule screens provide a viable method to identify biologically relevant compounds with the potential to treat a variety of skeletal muscle disorders.

W-3053
DEVELOPMENT OF FUNCTIONAL NEUROMUSCULAR JUNCTIONS IN NEURAL DIFFERENTIATION CULTURES OF HUMAN PLURIPOTENT STEM CELLS

Puttonen, Katja Annina[1], Ruponen, Marika[2], Naumenko, Nikolay[3], Kauppinen, Riitta[1], Hovatta, Outi[4], Tavi, Pasi[3], Koistinaho, Jari[1]
[1]Department of Neurobiology, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland, [2]Department of Neurobiology, A.I. Virtanen Institute for Molecular Sciences and School of Pharmacy, University of Eastern Finland, Kuopio, Finland, [3]Department of Biotechnology and Molecular Medicine, A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Kuopio, Finland, [4]Karolinska Intitutet, Stockholm, Sweden

Disorders of the neuromuscular junctions (NMJs), the cholinergic synapses between lower motor neurons and skeletal muscle fibers, include myasthenia gravis (MG) and Lambert-Eaton myasthenic syndrome (LEMS). In addition, recent studies indicate that progressive paralyzing diseases, amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), involve early pathological changes at NMJ. NMJ is also the most studied model of synapse research. While research on NMJ development and function is of utmost importance, there is an apparent lack of a completely human based NMJ model system. We recently reported that the human pluripotent stem cells (hPSCs) differentiated into neural progenitors in a suspension without addition of mesendodermal inhibitors produce a heterogenous cell population containing both small spindle-shaped neural cells and big flat cells. The latter cell type was negative for a variety of neuronal and glial markers. Here we show that a portion of these non-neural cells differentiate into multinucleated myotubes in the conditions designed

for neural induction, proliferation and maturation. The differentiation switch towards myotubes was associated with increased expression of myogenic markers MyoD1, Myogenin and type I Ryanodine receptor, but with compromised expression of neuroectodermal (Pax-6) and neuronal (Map-2) genes. Close examination of these cultures revealed that MHC-immunoreactive myotubes with myo-oriented actin filaments were surrounded by Tuj1-positive neuronal cells, forming intimate connections with them. Next, the cultures were labeled with a fluorogenic α-bungarotoxin, a marker of nicotinic acetylcholine receptors (nAChR) highly expressed in the postsynaptic membrane of NMJs. We detected highly packed nAChR areas on the surface of myotubes, particularly at sites of close neuronal contacts. Patch clamp recordings revealed that both striated and unstriated myotubes generated spontaneous and evoked action potentials (APs) with variable waveform and duration, indicating the presence of myotubes with various degrees of maturation. To test the functionality of the NMJ and nAChRs on myotubes, a cholinomimetic carbacholine and NMDA, an agonist of excitatory glutamate receptors known to be present in motoneurons, were administered and the responses analysed with confocal calcium imaging microscopy and patch clamp recordings. Carbacholine was found to produce a fast and strong depolarization of myotube membrane and Ca2+ release from its intracellular stores. Importantly, stimulation of motoneurons with NMDA resulted in reproducible APs in myotubes, indicating synaptically active NMJs. Our data indicates that functional NMJs are co-produced in the neurally-induced suspension cultures of hPSCs. This novel method may be useful for analyzing normal human NMJ development and pathology in NMJ diseases.

W-3054
ENGINEERING A 3D IN VITRO MODEL OF HUMAN SKELETAL MUSCLE

Serena, Elena[1], Zatti, Susi[2], Vetralla, Massimo[1], Giulitti, Stefano[3], Vitiello, Libero[4], Elvassore, Nicola[1]

[1]Department of Industrial Engineering, University of Padova, Padova, Italy, [2]University of Padova, Padova, Italy, [3]University of Padova, Padova, Italy, [4]Department of Biology, University of Padova, Padova, Italy

Myofibers, the basic structural elements of skeletal muscle tissue, are formed and regenerated after injury in a unique series of events that include myoblasts adhesion, fusion and differentiation. In this process a key role is played by morphological, mechanical and biochemical stimuli provided by the extracellular environment in vivo. Traditional in vitro two-dimensional (2D) cell culture systems have been very useful to elucidate early steps of myogenesis. However, cells cultured on flat substrates differ considerably in their morphology, cell-cell/cell-matrix interaction, and differentiation from those in the physiological three-dimensional (3D) environments. The aim of this work was to engineer three-dimensional (3D) human skeletal myofibers in vitro for: i) studying human myogenesis in an in vivo-like physiological microenvironment, ii) developing 3D implantable myofibers for repairing muscle defects. To achieve myoblasts spatial organization and alignment, we designed a soft hydrogel (HY) scaffold with 3D parallel micro-channels (80-160 μm in diameter, 10-15 mm long) functionalized with Matrigel. The HY ensures mass transport of metabolite and cytokines required for the proper myoblasts growth and differentiation. HY chemical composition was optimized in order to obtain a soft scaffold surrounding myobasts and myotubes, with mechanical properties (elastic modulus, E) similar to those of the physiological microenvironment of muscle in vivo (E≈12±4kPa). Human myoblasts ($1\div3x10^4$ cells/channel) were injected into the micro-channels and cultured for up to 10 days. The composition of HY was optimized based on the final application: poly-acrylamide was used for in vitro studies, while hyaluronic acid for in vivo experiments. The developed HY were biocompatible and maintained the expression of myogenic markers such as desmin. After 10 days of culture, tightly packed human myotubes bundles have been obtained, expressing the differentiation markers myosin heavy chain, α-actinin and dystrophin. It is worth to underline that we observed spontaneous contractions of human myotubes bundles. Further to be three-dimensional, thanks to their relevant dimensions (up to 15 mm in length) and their compact and elastic nature, myotubes bundles could be easily manipulated for surgical implantation. GFP+ve muscle precursors cells were cultured into the channels and implanted in the tibialis anterioris of syngenic wilde type mice. After two weeks, the HY scaffold was completed degraded, without forming fibrous tissue, and implanted cells migrated from the implantation site and gave rise to newly formed myofibers: GFP+ve with central nuclei. Taken together, the obtained results showed that the 3D HY scaffold surrounding myoblasts simulates in vitro the mechanical and biochemical properties of the physiological cell microenvironment, allowing the formation of human differentiated and contracting myotubes bundles. On the other hand, in vivo studies showed an optimal degradability of the scaffold and the formation of new myofibers integrated within the host tissue.

W-3055
SIRT1 DEACETYLASE INTERACTS WITH PAX7 IN MUSCLE SATELLITE CELLS AND INTERFERES WITH PAX7-MEDIATED TRANSCRIPTIONAL ACTIVITY

Sincennes, Marie-Claude[1], Pasut, Alessandra[1], Kawabe, Yoichi[1], Rudnicki, Michael A.[2]

[1]Regenerative Medicine, Ottawa Hospital Research Institute, Ottawa, ON, Canada, [2]Ottawa Hospital Research Institute, Ottawa, ON, Canada

Satellite cells are adult stem cells responsible for skeletal muscle regeneration following injury or in degenerative diseases, such as muscular dystrophy. Satellite cells are interesting candidates for stem cell-based therapies for the treatment of muscular dystrophy. Therefore, the understanding of satellite cell biology is crucial for the development of such therapies. The transcription factor PAX7 is a critical regulator of satellite cell activity. In *Pax7*$^{-/-}$ mice, satellite cells are totally absent, resulting in severe muscle weakness, and ultimately leading to death. We previously determined that PAX7 methylation is important for its transcriptional activity and its function in satellite cells. In order to further determine how PAX7 function is regulated, we asked whether PAX7 displays other post-translational modifications important for its function. By mass spectrometry using immunoprecipitated FLAG-PAX7, we identified two lysine residues (K105 and K193) within the PAX7 protein that are acetylated; both residues are conserved between species. In order to determine if acetylation is important for PAX7 transcriptional activity, we performed luciferase assays using the PAX7-responsive *Myf5 -111kb* reporter. We show that treatment with trichostatin A, a deacetylase inhibitor, significantly increases the transcriptional activity of PAX7. We also mutated the individual lysine residues into arginine, to abolish PAX7 acetylation on these residues, and observed a decrease in *Myf5* reporter activity. Importantly, mutation of both residues further decreases PAX7 transcriptional activity. In an attempt to identify the deactetylase responsible for PAX7 deacetylation, we used a candidate gene approach; SIRT1 is expressed in muscle satellite cells and is known to deacetylate the myogenic transcription factor MyoD. We detected an interaction between PAX7 and SIRT1 by co-immunoprecipitation, and we also demonstrate by proximity ligation assay that this interaction takes place specifically in satellite cells. Moreover, expression of SIRT1 significantly reduces *Luc-Myf5* reporter activity, suggesting that SIRT1 regulates PAX7 transcriptional activity. The effect of SIRT1 on the expression of

PAX7 target genes and on PAX7 function in satellite cells remains to be determined. Taken together, these results suggest that PAX7 acetylation, and possibly PAX7 deacetylation by SIRT1, are important for PAX7-mediated transcriptional regulation in satellite cells.

W-3056
UNRAVELLING PERICYTE SELF-RENEWAL AND SATELLITE CELL FATE PLASTICITY IN ADULT MOUSE SKELETAL MUSCLE REGENERATION

Tedesco, Francesco Saverio[1], Gerli, Mattia FM[1], Antonini, Stefania[2], Ragazzi, Martina[1], Cossu, Giulio[3]
[1]Department of Cell and Developmental Biology, University College London, London, United Kingdom, [2]Department of BioSciences, University of Milan, Milan, Italy, [3]University of Manchester, Manchester, United Kingdom

Skeletal muscle regeneration depends on satellite cells, which upon activation generate transient amplifying and committed progenitors called myoblasts. Despite the promising results obtained in murine models, clinical trials with myoblast transplantation in patients with muscular dystrophy provided very limited evidence of efficacy. Moreover, myoblasts are considered not to be suitable for systemic delivery, adding another layer of complexity in protocols aiming to treat patients with systemic muscle disorders. However, other myogenic stem/progenitor cells can be isolated from skeletal muscle. Among them, pericyte-derived mesoangioblasts are one of the most promising populations. The ability of skeletal muscle stem cells to self-renew after transplantation guarantees their long lasting therapeutic effect: this is best studied by serial transplantation assay. Although this is well established for hematopoietic and epithelial stem cells, there is very limited evidence of self-renewal by serial transplantation of skeletal muscle cells different from satellite cells. Moreover, a first-in-man phase I/II clinical trial based upon transplantation of HLA-identical mesoangioblasts for Duchenne muscular dystrophy is currently approaching completion. Hence, investigating mesoangioblast self-renewal upon transplantation is of key importance for future efficacy trials. Here we show that it is possible to serially transplant wild type and dystrophic pericyte-derived mesoangioblasts in dystrophic mice. Transplanted cells were able to generate both skeletal myofibers and undifferentiated muscle stem cells. This property was maintained at the clonal level and after genetic correction using a human artificial chromosome containing the entire dystrophin locus. Preliminary results show also in vivo self-renewal of freshly-isolated skeletal muscle pericytes transgenically labelled with an inducible Alkaline Phosphatase CreERT2. Although we have focused our transplants mainly on pericyte-derived satellite cells, we have been able to consistently purify populations with and without satellite cell markers after every serial transplant, with evidence of generation in vivo of satellite-like cells from pericytes and vice versa. Notably, iPS cell-derived mesoangioblast-like cells showed similar self-renewal dynamics upon transplantation. To better investigate this lineage promiscuity between satellite cells and pericytes, primary satellite cells were exposed to Notch Delta Ligand 4 (DLL4) and PDGF-BB to assess whether these pathways might be responsible of lineage cross-talk in the adult muscle, similarly to what has been recently reported to happen in embryonic myoblasts. Upon treatment with DLL4 and PDGF-BB, satellite cell-derived myoblasts acquired pericyte properties in vitro, showing expression of alkaline phosphatase, stabilization of human endothelial networks and reversible inhibition of their myogenic potential. Importantly, treated cells showed also increased ability to transmigrate across an endothelial layer, suggesting acquisition of properties similar to those of mesoangioblasts. These findings provide insights into skeletal muscle stem cells regenerative dynamics. Moreover, this bi-directional fate plasticity could be exploited to give the cells beneficial properties for cell therapies, such as the ability to cross the vessel wall upon systemic delivery while retaining a remarkable myogenic potential.

W-3057
THE ROLE OF EUCHROMATIC HISTONE-LYSINE N-METHYLTRANSFERASE 2 (EHMT2)/G9A IN MYOGENESIS

Zhang, Regan-Heng, Rossi, Fabio M.V.
The Biomedical Research Centre, The University of British Columbia, Vancouver, BC, Canada

EHMT2 (euchromatic histone-lysine N-methyltransferase 2), also known as G9a, has been characterized as an epigenetic regulator, as it is responsible for the production of the unique histone modification H3K9me2. In addition, EHMT2 also functions via protein complex interactions and its transcriptional coactivator activity. EHMT2 has been implicated in cell fate decisions in development, the immune system, the brain, and overexpressed in many different types of cancers. Recent reports employing in vitro cell lines indicate that EHMT2 knockdown promotes myogenic differentiation. To characterize the role of EHMT2 in vivo, we have bred a floxed allele of the gene to mouse strains expressing Cre recombinase under the control of muscle-specific and related promoters. We established an inducible conditional deletion of EHMT2 in adult satellite cells, the main stem cells of adult muscle regeneration, using PAX7-CreERT2. Here we report that, complicating published cell line data, EHMT2 knockout in satellite cells in vivo do not present an observable effect on muscle fiber regeneration by histology. We further deleted EHMT2 in the entire myogenic lineage in development and neonatal growth with MYOD1-Cre, an early myogenic regulatory factor. We confirmed that the knockout in vivo does not pose significant growth disadvantages, as knockout mice were born at expected frequencies and weights, with normal skeletal muscle histology. Primary satellite cells from knockout mice show normal capacity to proliferate and differentiate into mature myoblasts. Similar observations were also made when we deleted EHMT2 using PRRX1-Cre, which is expressed in the entire developing limb bud. However, when EHMT2 is conditionally deleted using PDGFRA-Cre, which is expressed in many mesoderm-derived tissues but not in adult satellite cells, we observed a significant developmental disadvantage. PDGFRA-Cre EHMT2 conditional knockouts have significant lower rates of birth than expected and most embryos do not reach birth. We discuss EHMT2's varying degrees of regulatory influence in different mesoderm-derived tissues, and hypothesize that EHMT2 is not crucial in myogenesis in vivo, but important in other mesodermal lineages.

CARDIAC CELLS

W-3058
QUANTITATIVE PROFILE OF CARDIAC STEM CELLS FROM SPONTANEOUSLY HYPERTENSIVE RATS

Sun, Jiyuan[1], Ribeiro, Patricia de Carvalho[2], Ribeiro Machado, Marcus Paulo[2], Oliveira, Lucas Felipe[2], Almeida, Thalles Ramos[2], Silva, Marcus Vinicius[2], **Dias da Silva, Valdo Jose**[2]
[1]Institute of Materia Medica, The Fourth Military Medical University, Xi'an, China, [2]Triangulo Mineiro Federal University, Uberaba, Brazil

In the last decade, cardiac stem cells (CSCs) such as c-kit+ cells, sca-1+ cells or cardiac mesenchymal stem cells were discovered into the mammalian hearts, which could contribute to heart regeneration in both physiological and pathophysiological states like myocardial infarction, aortic banding, etc. The major aim of the present study

was to evaluate the number counting of c-Kit+ and Sca-1+ cardiac stem cells, by means of flow cytometry, and of cardiac mesenchymal stem cells, by means of colony forming units-fibroblasts (CFU-F) assay, into the heart from spontaneously hypertensive rats (SHR), compared to normotensive Wistar-Kyoto (WKY) rats. Briefly, four-, 16-22- or 25-30-old SHR and WKY rats were studied, having their hearts excised after euthanasia in order to proceed the harvesting of stem cells via mechanical tissue fragmentation, enzymatic digestion (with collagenase type I at 1%) and differential filtration (at 40μm). The resulting cardiac cell suspension was then analyzed by means of flow cytometry, in order to quantify the number of c-Kit+ and Sca-1+ cardiac stem cells or it was seed in low density in culture dishes, in order to quantify the number of CFU-Fs, which are clonally derived from individual cardiac mesenchymal stem cells. The SHRs presented with hypertension and cardiac hypertrophy after 16th week of age. The amount of c-Kit+ cardiac stem cells into the hearts of SHRs was higher at the 4th week of age (0,74±0,22% versus 0,46±0,21% in WKY rats, p<0,05) and lower at the 16-22th and 25-30th week of age (16-22 weeks: 0,45±0,19% versus 0,78±0,23% in WKY rats p<0,05 and 25-30 weeks: 0,41±0,19% versus 0,77±0,26% in WKY rats, p<0,05). The number of Sca-1+ cardiac stem cells did not differ between to rat strains in any studied age. On the other hand, the counting of CFU-F was markedly higher into the hearts of SHR at 4th and 16-22th or 25-30th weeks of age, when compared with same aged control WKY rats. The reduced number of c-Kit+ cardiac stem cells into the heart of SHR observed at the evolving phase (16-22 weeks) and at the established phase (25-30 weeks) of hypertension, with ongoing cardiac hypertrophy, suggests that this numeric reduction could be a consequence of hypertensive and cardiac hypertrophic process. On the contrary, the higher number of CFU-Fs, precociously detected in pre-hypertensive phase (4th week) seems to suggest that this increase could participate in some way of the pathogenesis of cardiac hypertrophy associated with systemic arterial hypertension. Further studies are necessary to elucidate the real role played by these stem cells in the context of systemic arterial hypertension and cardiac hypertrophy.

W-3059
AUTOMATED MICROSCOPY-BASED CHARACTERIZATION OF STEM-CELL DERIVED CARDIOMYOCYTES

Maddah, Mahnaz, Burkhardt, Mathew F., Shoukat-Mumtaz, Uzma, Loewke, Kevin
Cellogy Inc., Menlo Park, CA, USA

Recent studies have shown that iPSC-derived cardiomyocytes hold tremendous potential for drug development and safety testing related to cardiovascular health. The characterization of iPSC-derived cardiomyocytes and their response to drugs is most commonly analyzed using electrophysiological potentials captured by a patch clamp or micro-electrode array. While these systems are considered gold-standards for characterization, they can be expensive and difficult to use, and due to the direct contact between cells and foreign objects, may induce undesirable cellular response. Here, we present a new microscopy-based method for non-invasive characterization of iPSC-derived cardiomyocytes using video microscopy and computer vision analysis. Our algorithms can reliably quantify beating signals in a fully automated manner based on contraction and relaxation motion patterns of the cells. Our approach has several advantages compared to more traditional beating assays: first, it follows standard cell-culture practices using multi-well plates; second, it accommodates cell cultures with varied ranges of confluency, from single-cell to monolayer plating, without requiring any user interaction; and third, it is noninvasive to cells, enabling repeated and direct measurements of the same cell culture as often as desired. Our input data consists of low-light phase-contrast microscopy images of cardiomyocytes in culture, captured at a high frame rate (e.g. 24 fps). Images are first segmented into regions that consist of cells that are expected to exhibit a cyclic motion (beating cells) and regions that consist of cells that do not show a cyclic motion (non-beating cells) and background. The result is a set of segmented beating regions, where each region includes a cell or a group of cells that are spaced close to each other and beat with approximate synchrony. Next, a beating signal is calculated for each region from the image sequence by capturing the intensity variation of successive images. Subsequently, features that describe the beating signal, such as beating frequency, irregularity, and duration, are extracted. Finally, a clustering algorithm is performed to identify regions with unique beating characteristics and merge regions that have similar beating characteristics. We show that our non-invasive beating assay can successfully measure the beating signals of healthy and diseased iPSC-derived cardiomyocytes in both low and high-confluency plating under typical culture conditions. The accuracy of beating frequency and irregularity were confirmed by comparison with the manually derived values from the video of the sequence as well as correlation with more traditional assays. In addition, we showed that our assay can characterize the cellular response from the addition of Norepinephrine (Ne) and Cisapride (Cp) applied to high-confluency cell cultures. We measured an average of 40% increase in beating frequency of Ne-treated cells compared with the controls, which lasted for 2 days, and an average 3-fold increase of beating irregularity with Cp-treated cells compared with the controls, which lasted for 6 days. To our knowledge, our presented microscopy-based approach is the first fully-automated and non-invasive beating assay for characterization of iPSC-derived cardiomyocytes that accommodates varied cell culture confluencies and beating arrhythmia.

W-3060
LINEAGE MAPPING OF NKX2-5 EXPRESSING CELLS IN THE DEVELOPING CARDIOVASCULAR SYSTEM

Anderson, David, Skelton, Rhys, Stanley, Ed, Elefanty, Andrew George, Elliott, David
Murdoch Childrens Research Institute, Melbourne, Australia

NKX2-5 marks early multipotent progenitor cells and fully differentiated cardiomyocytes in the developing cardiovascular system. We have examined the potential of NKX2-5 expressing cells to contribute to various cardiovascular lineages by targeting GFP to the NKX2-5 locus in human pluripotent stem cells. In early stages of mesodermal differentiation, NKX2-5low CD34pos cells can be identified that further differentiate primarily to an NKX2-5neg CD34pos population. At the same early stage of differentiation, NKX2-5low CD34neg cells can also be identified and further differentiated to generate an NKX2-5neg CD34pos population. The CD34pos cells derived from both NKX2-5low populations are proliferative, predominantly KDRpos, and express the endothelial markers TEK, CDH5 and PECAM1. The NKX2-5low CD34neg cells can also give rise to an NKX2-5high population that is contractile in culture and expresses the cardiac markers MYH6, TNNT2 and MYL2. This indicates the potency of early NKX2-5pos cells to give rise to both endothelial and cardiac lineages in vitro. At later stages of differentiation NKX2-5low cells lose their ability to transition to a CD34pos or NKX2-5high fate and progressively become NKX2-5neg. Later stage NKX2-5high cells retain contractility and cardiac gene expression, with few cells transitioning away from this phenotype. This indicates a loss of potency in NKX2-5pos cells during in vitro differentiation. We are currently investigating the potential of later stage NKX2-5neg cells to differentiate to smooth muscle and fibroblast lineages, and also further multipotency of early NKX2-5low cells. These studies demonstrate restricted potency of NKX2-5

expressing cells during the course of mesodermal differentiation in vitro.

W-3061
DEVELOPMENT OF A SCALABLE SUSPENSION CULTURE FOR CARDIAC DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS AND CELL CHARACTERIZATION

Chen, Chang-Yi Vincent[1], Ye, Jingjing[1], Hua, Giau[1], Liu, Jian-chang[1], Chen, Danlin[1], Liu, Ziguang[1], Chai, Jin[1], Shukla, Praveen[2], Wu, Joseph C.[2], Hsu, David[1], Couture, Larry[1]
[1]CATD, Beckman Research Institute, City of Hope, Duarte, CA, USA, [2]Stanford University School of Medicine, Stanford, CA, USA

Myocardial infarction and heart failure are leading causes of death worldwide. As myocardium has very limited regenerative capacity, endogenous cell regeneration can not sufficiently compensate heart damage. The concept of cell replacement is an appealing therapy to treat such cardiac diseases. Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSCs), are attractive sources for the cell replacement therapy since they can be enormously expanded in culture and differentiated into specific cell lineages under proper inductions. To meet the need for a large quantity of hPSC-derived cardiomyocytes for pre-clinical and clinical studies, a robust, scalable, and manageable differentiation system for cardiomyocyte production is essential. Previously we have established a scalable hPSC suspension culture system to expand undifferentiated hPSCs in the form of aggregates with defined media conditions. With H7 cell aggregates generated from the suspension, we have developed a strategy to directly induce hESCs into cadiomyocytes in suspension with small molecules modulating Wnt signaling. Concentrations and induction timing of small molecules and agitation rates were optimized for cardiac differentiation scaled up from 6-well plates to 125, 500, and 1000 ml spinner flasks. We have investigated the correlation between sizes of cell aggregates and effective concentrations of small molecules, and influences of agitation on cardiac differentiation. As the differentiation process was scaled up from static 6-well plates to different sizes of stirring spinner flasks, we noticed the optimal differentiation conditions could be changed, suggesting geometries of culture vessels and shear stress may affect cardiac differentiation. With those critical parameters we identified, we optimized cardiac differentiation for other hESC and iPSC lines. The results of process optimization showed that the sensitivity to small molecules and shear stress are cell line dependent, and thus individual cell lines may show different optimal conditions for the differentiation. With optimized differentiation conditions, we were able to consistently obtain 70-90% purity of cardiomyocytes from scales up to 1000 ml spinner flasks. Analysis of gene expression showed that the differentiation of mesoderm, cardiac mesoderm, cardiac progenitors, and cardiomyocytes induced by the small molecules occurred in a timely manner. The cardiomyocytes from the suspension culture displayed typical striated structures. As analyzed by electrophysiology, ventricular-, atrial-, and nodal-like action potentials were detected. In addition, the size of cardiac population exhibiting ventricular-like action potential increased over time. In summary, we have developed a robust process for scalable manufacturing of hPSC-derived cardiomyocytes in suspension culture. More importantly, we present a strategy to optimize differentiation process in suspension for individual cell lines. The suspension culture system from expansion of undifferentiated hPSCs to cardiomyocyte differentiation provides a bioreactor prototype for automation of cell manufacturing, which will accelerate the advance of hPSCs in pre-clinical and clinical applications.

W-3062
NUCLEAR RECEPTOR INTERACTION PROTEIN HAS FUNCTIONS FOR CALCIUM HOMEOSTASIS AND MAINTENANCE OF SARCOMERE INTEGRITY

Chen, Show Li
Microbiology, National Taiwan University, Taipei, Taiwan

Previously, we demonstrate a gene, nuclear receptor interaction protein (NRIP, also named DCAF6 or IQWD1) as a Ca2+- dependent calmodulin binding protein that can activate calcineurin phosphatase activity and calmodulin kinase II. Here, we extensively found that α-actinin-2 (ACTN2), is one of NRIP-interacting proteins from the yeast two-hybrid system using NRIP as a prey. Due to ACTN2 is a biomarker of muscular Z-disc complex; we found that NRIP is a novel ACTN2-interacting protein. We then used conventional NRIP knock out (NRIP-/-) mice to investigate insights into in vivo function of NRIP. Electronomic microscopy revealed that the reduction of I-band width, extension length of Z-disc and shortened sarcomere length in sarcomeric structure of NRIP-/- cardiomyocytes compared to wild type mice; indicating that NRIP locates in Z-disc and loss of NRIP would impair the integrity structure of sarcomere. The echocardiography of NRIP-/- mice showed the diminished fractional shortening [FS] and the ration of end-systolic diameter to end-diastolic diameter [ESD/EDD]). Besides, Ca2+ plays important role for muscle contraction. Therefore, we isolated primary cardiomyocytes from embryonic heart and measured the calcium transient by infection with adenovirus encoding shNRIP. The results revealed that the deficiency of NRIP decreased the amplitude of calcium transient and sarcomere length that is consistent with the reduction sarcomere length of heart tissures of NRIP-/- mice through EM assay at adult mice (12 wks). In a conclusion, the loss of NRIP impairs the structure of sarcomere, the amplitude of calcium transient during muscle contraction and the function of muscle contraction resulting in cardiomyopathy.

W-3063
IDENTIFICATION OF NOVEL CHEMICALS POTENTLY INDUCING CARDIOMYOCYTE DIFFERENTIATION FROM MOUSE AND HUMAN PLURIPOTENT STEM CELLS

Fukushima, Hiroyuki[1], Yamashita, Jun[2]
[1]Kyoto University Center for IPS Cell Research and Application, Kyoto, Japan, [2]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Mammalian cardiomyocytes largely lack the capacity of proliferation and regeneration in the adult heart. And this deficiency leads heart failure such as after myocardial infarction. Recently, we have been investigating potentials of chemicals in cardiac regenerative medicine. One of goals is application of chemicals as a cardiac regenerative drug for direct in vivo regeneration through effects on endogenous stem or progenitor cells to induce proliferation and differentiation of cardiomyocytes. To realize this novel therapy, we attempted to discover novel chemicals that induced cardiomyocyte differentiation with pluripotent stem cell differentiation systems. Previously, we established a 2-dimentional culture-based stepwise cardiovascular differentiation system from mouse embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). In our ESC cardiac differentiation system, cardiomyocytes were efficiently induced from ESC-derived Flk1+ mesodermal cells by co-culturing with OP9 cells. Recently, we established a high-throughput screening system for chemicals promoting cardiomyocyte differentiation (CDCs) from Flk1+ cells on OP9 cells. Here we report that we successfully identified several CDCs from natural chemical library derived from marine invertebrates. Particularly, natural CDC1 (nCDC1) increased

cardiomyocyte percentage and cell number that appeared from Flk1+ cells approximately 20 times more than control. Even in the absence of OP9 cells, nCDC1 directly and drastically induced cardiomyocytes from ES cell-derived Flk1+ mesodermal cells and a cardiac progenitor population, Flk1+/CXCR4+/VE-cadherin- (FCV) cells. nCDC1 also directly enhanced cardiomyocyte differentiation from human iPS cell-derived KDR+ mesoderm cells. Furthermore, nCDC1 promoted cariomyocyte differentiation of a somatic cardiac progenitor population, rat neonate cardiac side population (CSP) cells, in vitro. To investigate whether nCDC1 promotes cardiac regeneration in vivo and ameliorate cardiac function, we are examining in vivo administration of nCDC1 to subacute rat myocardial infarction model. These findings would provide a clue for cardiomyocyte differentiation mechanisms and offer novel cardiac regenerative strategies including cardiac regenerative drugs.

W-3064
GENERATION AND CHARACTERIZATION OF A HUMAN EMBRYONIC STEM CELL REPORTER LINE WITH A VENTRICULAR CARDIOMYOCYTE-SPECIFIC PROMOTER

Futakuchi-Tsuchida, Akiko[1], Pabon, Lil[1], Reinecke, Hans[1], Murry, Charles[2]

[1]Pathology, University of Washinton, Seattle, WA, USA, [2]Pathology, University of Washington - Center for Cardiovascular Biology, Seattle, WA, USA

Embryonic stem cell (ESC)-derived cardiomyocytes are a potential source for future cell replacement therapies of cardiac failure. Since significant loss of ventricular cardiomyocytes (VCMs) occurs during myocardial infarction, a large number of ventricular cardiomyocytes are needed for such therapy. Despite the progress in increasing the efficiency of cardiac differentiation from ESCs, obtaining pure populations of VCMs under current differentiation protocol is still challenging. In order to determine and optimize the protocol which results in high yield of VCMs, a simple and high sensitive evaluation system to detect VCMs is necessary. We have established RUES2 human ESC reporter lines that express secreted Metridia luciferase (MetLuc) under the control of the ventricular myosin light chain 2 (MLC2v) promoter. A 560 bp fragment of MLC2v untranslated region, -513 to +47 related to the transcription start site, was used for the promoter region. Upon cardiac differentiation, cells start to secrete luciferase as they differentiate into beating cardiomyocytes. Time course analysis of luciferase activity and MLC2v mRNA expression pattern showed good correlation between these two parameters. On the other hand, expression of MLC2a mRNA, found in both atrial and ventricular cardiomyocytes, did not correlate with the luciferase activity. This system allows us to monitor the efficiency of cardiac differentiation towards ventricular subtype by simply collecting the culture supernatant and measuring the luciferase activity. Also, this system will be useful in discovering small molecules that could be combined with current differentiation protocol and provide promising tools for future clinical application.

W-3065
COMPARATIVE DEVELOPMENTAL GENOMICS IN PRIMATES USING IPSC-DERIVED CARDIOMYOCYTES

Gallego Romero, Irene[1], Pavlovic, Bryan J.[1], Burnett, Jonathan E.[1], Huang, Constance H.[1], Banovich, Nicholas Eli[1], Kagan, Courtney L.[1], Friedrich Ben-Nun, Inbar[2], Laurent, Louise[3], Loring, Jeanne F.[2], Gilad, Yoav[1]

[1]Human Genetics, University of Chicago, Chicago, IL, USA, [2]The Scripps Research Institute, La Jolla, CA, USA, [3]University of California, San Diego, La Jolla, CA, USA

Comparative studies in humans and non-human apes have the potential to provide insights into human-specific traits and diseases. Such studies are currently extremely restricted because we only have access to a few types of cell lines and to a limited collection of frozen tissues. To circumvent this, we have established panels of matched induced pluripotent stem cells (iPSCs) from 8 humans and 8 chimpanzees as a new transformative model system for comparative studies in primates. Motivated by differences in the types and prevalence of cardiac disease between the two species, we performed stepwise differentiation of these iPSC lines into functional cardiomyocytes, and identified changes in gene expression between sequential stages of differentiation and between the differentiated iPSCs and cardiac tissue samples, both within and across species. First, we have demonstrated that iPSC-derived cardiomyocytes are more similar to cardiac tissue from humans and non-human primates than they are to either the source iPSCs or other tissues, validating our approach. Next, we identified genes that are differentially expressed between species at each stage of differentiation. Further analysis of the genome-wide data we have collected during cardiac differentiation of the iPSC lines will allow us to make important contributions to our understanding of the regulatory changes that underlie human-specific developmental adaptations in the heart. In addition, we hope that the human and chimp iPSC lines developed during the course of this project will serve as the nucleus for a larger resource, consisting of larger numbers of iPSC lines derived from a wider collection of primate species, that will ultimately facilitate a broader research program into numerous outstanding questions in human evolution, including the characterization of adaptations that are associated with increased risks for diseases and sensitivities to certain drugs.

W-3066
ROLE FOR BETA ARRESTINS IN STEM/PRECURSOR CELL FUNCTION AND CARDIAC REGENERATION

Gumpert, Anna M.[1], Chen, Mai[1], Peppel, Karsten[2], Gao, Erhe[1], Koch, Walter[1]

[1]Center for Translational Medicine, Temple University School of Medicine, Philadelphia, PA, USA, [2]Center for Translational Medicine, Thomas Jeeferson University, Philadelphia, PA, USA

Development of chronic heart failure (HF) syndrome following myocardial injury is characterized by an extensive loss of myocytes due to considerable apoptosis and necrosis. As bone marrow derived stem cells (BMSCs) are capable of transdifferentiating, they also show potential for regenerating the myocardium after infarction. Stem cell mobilization, egress from the bone marrow and recruitment to the site of injury can be regulated by signals through G protein-coupled receptors (GPCRs). βArrestins are known for their signalling and scaffolding functions and as downstream regulators of GPCR desensitization and endocytosis in particular. In the present study we explored the potential role for β-Arrestins in cardiac precursor cell function, concentrating on the properties of BMSCs. Using knockout (KO) mice, we investigated the role β-arrestin1 (βArr1) and β-arrestin2

(βArr2) with respect to modulation of regenerative competence of BMSCs and their contribution to cardiac repair following ischemic injury. In the *in vitro* system we observed that BM-derived cells devoid of either βArr1 or βArr2 proliferate, colonize and migrate in a significantly deficient manner compared to BM cells isolated from wild-type (WT) mice. Furthermore, we observed significantly elevated cell death in βArr2 deficient cells as compared to WT or βArr1-KO cells following oxidative stress. In addition, the number of cKit positive stem cells present, thought to be potential cardiac precursor cells, were significantly lower in the BM and blood of βArr KO mice compared to WT. Similarly, BM and blood of the chimeras contained fewer and less viable cardiac stem/precursor cells pre- and post myocardial infarction (MI), compared to WT transplanted controls. In our *in vivo* study, we carried out BM transplants to determine whether the βArrs may be involved in cardiac repair. WT mice were irradiated and then received BM transplants from either WT donors as a control or BM from βArr1 or βArr2 KO mice. Subsequent to BM reconstitution, mice underwent MI and their recovery and progress were followed. Interestingly, chimeric mice with βArr1 and βArr2 KO BM had significantly inferior outcomes than mice receiving WT BM. This included significantly decreased post-MI survival with βArr2 KO BM and both βArr chimeras had significantly lower cardiac function post-MI compared to mice receiving WT BM. Histological investigation revealed that both chimeras developed larger infarcts and hypertrophy at an accelerated rate. We conclude that βArrs play a novel role downstream of GPCR desensitization in cardiac progenitor cells in BM and accordingly, appear to be critically involved in the heart's response to ischemic injury via cardiac repair and regeneration.

W-3067
SPATIOTEMPORAL ANALYSIS OF CARDIAC TROPONIN T GENE EXPRESSION IN CARDIAC CELL DIFFERENTIATION OF STEM CELLS

Hayashi, Taro, Hatta-Ohashi, Yoko, Sakane, Isao, Suzuki, Hirobumi
Corporate R and D Center, Olympus Corporation, Tokyo, Japan

The heart is one of the organs attracted in regenerative medicine. In the typical studies in this field, differentiation processes of pluripotent stem cells (ES or iPS cells) into specific organs are evaluated by the status of the cells such as organ-specific phenotypes, gene expression profiles, protein accumulation or epigenetic modifications. However, information available for determining the differentiation processes and underlying mechanisms is still limited due to technical difficulties. In this study, we demonstrated a spatiotemporal monitoring and analysis method effective in revealing the cardiac cell differentiation process. Gene expression levels of cardiac troponin T (cTnT) in the differentiating iPS cells were monitored for five days by bioluminescence microscopy. The promoter region of mouse cTnT was inserted into a luciferase reporter vector, and stably transfected mouse iPS cells were differentiated into embryoid bodies (EBs) by a standard EB-based differentiation protocol. Then, EBs were plated on 0.1% gelatin-coated dishes for further differentiation in IMDM containing 20% FBS for 24 h, and EBs differentiated into cardiac cells were identified by cTnT expression and beating property of the cells. As a result, cTnT expression appeared in some parts of early-differentiated EBs (0 h after plating on gelatin-coated dishes), and the expression increase and spreading were observed. Then, spontaneous beating appeared in the cTnT expressing regions (71 h), and the beating areas spread spatiotemporally. After that, the spontaneous beating turned to autonomous (81h). Thus, we confirmed that cTnT expressing cells in EBs differentiated into cardiac-like cells that beat autonomously. Furthermore, we analyzed the motion of beating cells by spatiotemporal image correlation spectroscopy to visualize beating property quantitatively. Beating directions and periods among different parts of single EBs expressing cTnT were found to synchronize autonomously during the time course. Thus, we confirmed that cTnT expressing cells in EBs differentiated into functionally beating cardiac cells during cardiac cell differentiation and also demonstrated the technical validity of spatiotemporal promoter assay by bioluminescence microscopy in the study of cellular differentiation.

W-3068
A MASSIVE FLOATING CULTURE SYSTEM WITH METABOLIC PURIFICATION FOR HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Hemmi, Natsuko, Tohyama, Shugo, Nakajima, Kazuaki, Hirano, Akinori, Kanazawa, Hideaki, Seki, Tomohisa, Kishino, Yoshikazu, Okada, Marina, Tabei, Ryota, Ohno, Rei, Fujita, Chihana, Yamaguchi, Miho, Hattori, Fumiyuki, Yuasa, Shinsuke, Sano, Motoaki, Fujita, Jun, Fukuda, Keiichi
Keio University School of Medicine, Tokyo, Japan

Cardiac regenerative therapy for severe heart failure using human embryonic stem cells and induced pluripotent stem cells (hiPSCs) is hampered by a lack of efficient strategies for generating clinically relevant numbers of functional cardiomyocytes (CMs) to treat patients. The development of massive floating culture systems (MFCS) with spinner flasks or bioreactors has highlighted this critical issue for a decade, although it remained unclear how efficiently MFCS could provide purified functional CMs from hiPSCs. The proliferative rate of differentiating hiPSCs in MFCS tended to be higher than in floating cultures in dishes, wherein the embryoid bodies (EBs) grew larger by self-aggregation. Notably MFCS provided more homogeneous and fine EBs at every time point, eventually preventing apoptosis, and PCR analysis revealed chronological expression of mesodermal markers (T, brachyury and MESP-1) and cardiac lineage markers (NKX2-5 and troponin T). In addition, the differentiation rate of CMs from hiPSCs in MFCS was approximately 30%. Surprisingly, the pluripotent marker, OCT3/4, was still highly expressed at 2 weeks after differentiation in EBs cultured in MFCS, and immunohistochemistry revealed TRA-1-60-positive cells. However, any remaining undifferentiated stem cells have the potential to induce teratoma formation, posing the worst risk for clinical applications of hiPSCs-derived CMs. To overcome this issue, a metabolic purification method was invented based on the different metabolic characteristics between pluripotent stem cells and CMs (Tohyama, et al. Cell Stem Cell 2013 12:127-37). This purification strategy using glucose-depleted and lactate-enriched medium successfully eliminates residual undifferentiated stem cells, resulting in a refined hiPSCs-derived CM population. The next step was therefore to investigate if this metabolic purification system could be successfully applied to generate large volumes of CMs. In colony formation assays, non-purified and purified dispersed hiPSC-derived cells were cultured under hiPSC maintenance conditions. Non-purified hiPSC-derived cells formed a large number of TRA-1-60 positive colonies, but the purified CMs formed no colonies. In addition, the undifferentiated hiPSCs generated large teratomas (average, 18 mm) in more than 90% of the cell populations. In MFCS, the non-purified CMs produced teratomas in approximately 60% of populations, although they were much smaller than those generated from the hiPSCs (average, 5 mm), and these teratomas showed complete differentiation into the three germ layers. On the other hand, purified CMs never induced teratomas. Quantitative PCR confirmed the enrichment of CMs and complete elimination of OCT3/4-positive undifferentiated stem cells, and the strong expression of cardiac genes indicated successful enrichment of the purified CMs. The purified CMs also expressed the gap junction marker, connexin 43, and showed proper electrophysiological properties

in a multiple-electrode system and active calcium signaling with the fluo-4 calcium indicator. In this MFCS and metabolic purification system, 1 x 10^8 of enriched and purified CMs were generated from approximately 5 x 10^8 iPSCs. The combination of MFCS and metabolic selection is an effective and practical approach to purify and enrich a large number of functional CMs, and could therefore be an essential technique for cardiac regenerative therapy with hiPSCs-derived CMs.

W-3069
NON-INVASIVE IMAGE-BASED ASSESSMENT OF THE ELECTROPHYSIOLOGY AND METABOLIC STATE OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Heylman, Christopher[1], Rupsa, Datta[1], Kurokawa, Yosuke[1], Tran, David D.[1], Conklin, Bruce R.[2], Gratton, Enrico[1], George, Steven[1]
[1]University of California, Irvine, Irvine, CA, USA, [2]J. David Gladstone Institutes, San Francisco, CA, USA

The objective of this study is to develop and validate non-invasive methods for assessing the electrophysiology and metabolic state of human induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs). Many drugs showing promise in preclinical trials fail during clinical development due to the emergence of cardiac side effects. Hence, there exists a great need for developing novel *in vitro* platforms that more accurately mimic the biology of human organs, and thus provide a reliable model for high-throughput drug screening. The emergence of human induced pluripotent stem (iPS) cell technology has expanded the possibilities for sourcing human cardiomyocytes for this purpose. We use a defined small molecule protocol that modulates Wnt signaling to differentiate large quantities of spontaneous and rhythmically beating human cardiomyocytes from human iPS cells (cell line WTC-11). The cells may then be used to engineer cardiac tissues *in vitro* to screen for potential cardiac side effects of new drug candidates. To assess the electrophysiological phenotype and drug-response of these engineered tissues, we developed image-based methods using the membrane voltage sensitive dye (VSD), di-4-ANE(F)PPTEA. This dye exhibits a proportional increase in fluorescence intensity to an increase in membrane voltage allowing characterization of the dynamic electrophysiology (i.e., the action potential) of cardiomyocytes in a non-invasive manner. To assess the metabolic phenotype of these engineered tissues, we developed methods using fluorescence lifetime imaging microscopy (FLIM). We take advantage of the unique autofluorescent properties of nicotinamide adenine dinucleotide (NADH), a central metabolite. When excited at 740nm using two-photon excitation, protein-bound NADH (indicative of oxidative phosphorylation) fluoresces and exhibits a fluorescence decay (i.e. lifetime) that is markedly different than that produced by NADH in a free or unbound state (indicative of glycolysis). Thus, by measuring the fluorescence decay at 740nm, we can determine the ratio of glycolysis to oxidative phosphorylation in human iPS-CM with subcellular resolution. The results obtained from this study demonstrate the ability of our system to capture subcellular resolution data of human iPS-CM membrane depolarization events and shifts in metabolism. The temporal resolution of the voltage-dependent fluorescent signal is sufficient to capture the distinct features of a single cell's membrane action potential. Non-selective beta blocker propranolol (10^{-5} M) and the beta adrenergic agonist isoproterenol (10^{-7} M) elicited a 56% decrease and a 19% increase in beat frequency, respectively. Significant shifts in cellular metabolism were also demonstrated using FLIM. 24 hours exposure to hypoxia generated a shift of 17% of the pixels analyzed from oxidative phosphorylation to glycolysis (p=0.015). From these results, we conclude that the use of VSDs and FLIM of NADH allows for non-invasive image-based assessment of human iPS-CM electrophysiology and metabolism. Using these unique electrophysiological and metabolic endpoints to assess human cardiac tissues grown *in vitro*, we can develop a drug screening platform that better mimics the *in vivo* biology of cardiac tissue and provides early identification of potential cardiac side effects.

W-3070
DOXORUBICIN-INDUCED TOXICITY IN CARDIOMYOCYTES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Holmgren, Gustav[1], Synnergren, Jane Marie[1], Bogestål, Yalda[1], Améen, Caroline[2], Åkesson, Karolina[2], Holmgren, Sandra[2], Lindahl, Anders[3], Sartipy, Peter[2]
[1]Systems Biology Research Center, School of Bioscience, University of Skövde, Skövde, Sweden, [2]Cellectis AB, Gothenburg, Sweden, [3]Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden

Doxorubicin is an efficient chemotherapeutic agent for a variety of cancers, including leukemia, lymphomas, and many solid tumors. Doxorubicin treatment is however associated with severe cardiotoxicity, often resulting in early discontinuation of the treatment. The doxorubicin-induced cardiotoxicity is dose-dependent and there is also an age and gender difference in the sensitivity to doxorubicin. Importantly, the onset of the toxicity can occur several years after the termination of the doxorubicin treatment. The exact mechanisms involved in doxorubicin-induced cardiomyopathy are not known, but the formation of reactive oxygen species and cellular iron accumulation has been suggested to be causative effects. The variation in time-to-onset of toxicity, gender- and age differences suggest that several mechanisms may be involved. In this study, the toxic effects of doxorubicin exposure have been investigated in pure cardiomyocyte cultures derived from human embryonic stem cells (hESC). The cardiomyocytes (Pure hES-CM™, Cellectis AB) were grown in a homogenous monolayer and the cells were exposed to a low- (50nM), medium- (150nM), and high (450 nM) dose of doxorubicin for 48h, followed by a 9 days wash-out period. The cell morphology and contractile ability was monitored during the exposure and recovery period. Cells were harvested for RNA extraction after 24h and 48h of doxorubicin exposure as well as after 5 and 9 days recovery, post exposure. In addition, Lactate Dehydrogenase (LDH) and Troponin T leakage from the cells were measured in the culture medium. There was an evident effect of the doxorubicin exposure even after the wash-out period. The cell morphology was altered and the cells showed a reduced contractile ability, most prominent in the highest concentration at the later time points. A general cytotoxic response measured by LDH leakage was seen after 48h exposure compared to the vehicle control. Nonetheless, this response vanished during the wash-out period. A similar pattern was observed for the cardiac specific Troponin T response and after 48h exposure, Troponin T release was increased in a dose-dependent manner. Compared to vehicle control, this doxorubicin effect vanished during the wash-out period. The global transcriptional profiles in the cells were analyzed using the WT Gene 2.0 ST arrays from Affymetrix Inc. and revealed clusters of genes that were altered during doxorubicin exposure, some of which remained even after the wash-out period. The results from this study show that Troponin T release can be a measurement of acute cardiotoxicity due to doxorubicin exposure. However, for the late-onset of doxorubicin-induced cardiomyopathy, Troponin T release might not be a relevant biomarker. As reported here, a defined list of genes altered after doxorubicin exposure could provide more relevant biomarkers. The differentially expressed genes identified in this study may also help to explain the cellular mechanisms behind the late onset apoptosis, associated with doxorubicin-induced cardiomyopathy.

W-3071

CREATING ENGINEERED, 3D CARDIAC TISSUES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS USING A ONE-STEP, SCALABLE DIFFERENTIATION SYSTEM

Kerscher, Petra[1], Hodge, Alexander J.[1], Kim, Joonyul[1], Turnbull, Irene C.[2], Bussie, Blakely S.[1], Seliktar, Dror[3], Easley, Christopher J.[1], Costa, Kevin D.[2], Lipke, Elizabeth Ann[1]

[1]Auburn University, Auburn, AL, USA, [2]Icahn School of Medicine at Mount Sinai, New York, NY, USA, [3]Technion-Israel Institute of Technology, Haifa, Israel

The high potential of human pluripotent stem cells (hPSCs) to provide cardiac tissues for use in high-throughput pharmaceutical drug testing and myocardial repair has been limited by the need to pre-differentiate hPSCs into cardiomyocytes (CMs) and then dissociate them prior to tissue assembly. Initial hPSC differentiation protocols relied on formation of 3D embryoid bodies, mimicking physiological development primarily in their structural nature; drawbacks, including variability in outcomes and insufficient CM production, resulted in state-of-the-art cardiac differentiation protocols transitioning to use of 2D sheets and a fully defined media including small molecules. In this study we draw from both approaches to directly differentiate hPSCs into mature, aligned, and synchronously contracting engineered cardiac tissues composed primarily of SC-CMs. By encapsulating hPSCs in hydrogel biomaterials, we create readily reproducible 3D environments for hPSC differentiation; using this approach, microenvironmental conditions experienced by the cells can be manipulated by providing both physical and chemical stimuli to drive differentiation and cardiac tissue formation. To create a highly reproducible 3D hydrogel differentiation system, hiPSCs were encapsulated into a liquid precursor poly (ethylene glycol) (PEG)-fibrinogen solution and photocrosslinked to form a 200 μm thick gel-like tissue. Encapsulated hiPSCs were then cultured in mTeSR-1 media for three days prior to initiation of differentiation (day 0). HiPSCs remained viable within PEG-fibrinogen and proliferated to form microsphere-like structures. Throughout differentiation, cells formed areas of highly connected tissue; isolated areas of contraction were first observed on day 7. The number and size of spontaneously contracting areas and force of contraction increased over time, resulting in a fully and synchronously contracting 3D cardiac tissue by day 10 which maintained its function for over three months. Temporal changes throughout cardiac differentiation were assessed by phase contrast microscopy, immunohistochemistry, gene expression, transmission electron microscopy (TEM), and electrophysiology. Cardiac localization, alignment, and maturation were assessed by immunocytochemistry staining of engineered cardiac tissues with sarcomeric α-actinin (cardiac marker) and connexin 43 (gap junction protein) on day 10, day 20, and day 130. Sarcomeres were visible at all three time points but developed a more defined and highly aligned structure within the engineered cardiac tissue over time. In addition to highly aligned CMs, connexin expression transitioned from the cell perimeter to longitude ends, suggesting mature cell-cell connections. Furthermore, to assess information on electrophysiological properties within the cardiac tissue, cells were dissociated on day 15 and calcium transients from spontaneously contracting and paced CMs were recorded. CMs showed 1:1 correspondence to electrical stimuli of up to 2 Hz. Action potential duration (APD) 50% to 80% ratio at 0.5 Hz and 1 Hz were 0.58±0.09 and 0.59±0.08, respectively. CMs produced by a conventional 2D sheet differentiation protocol were used as controls for all experiments. Finally, we demonstrate the successful implementation of this one-step scalable differentiation technique using multiple geometries, including printable microislands, macro-tissues and injectable microspheres.

W-3072

A THREE DIMENSIONAL, VASCULARIZED CARDIAC TISSUE DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS AS AN IN VITRO DRUG SCREENING PLATFORM

Kurokawa, Yosuke, Tran, David D., Sobrino, Agua, Moya, Monica L., Alonzo, Luis F., Tu, Christina, Lock, Leslie, Hughes, Christopher C.W., George, Steven C.

University of California, Irvine, Irvine, CA, USA

Cardiovascular diseases are the leading causes of morbidity and mortality in the world, resulting in significant health care costs in developed nations. Furthermore, drug discoveries targeted at cardiovascular diseases are inefficient as animal models fail to fully recapitulate cardiac drug responses in humans. In order to advance the drug screening process, there is a need for human cardiomyocytes, the contractile cells of the heart, which are normally inaccessible due to their postmitotic state *in vivo*. The advent of human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) has created a promising cell source that can potentially be utilized to screen for new cardioactive drugs, or for drugs that target other organs but have minimal negative impact on the heart. Here we propose to create a vascularized cardiac tissue using human iPSC-CMs that can meet this need. A key feature of our proposed system is the vascular network, which allows the platform to mimic the physiological delivery of drugs to the heart through the vascular system. We first create cardiomyocyte spheroids from human iPSC-CMs using AggreWell™ (STEMCELL Technologies). The cardiomyocyte spheroids are then combined with cord blood endothelial colony-forming cell-derived endothelial cells (ECFC-ECs) and normal human lung fibroblasts (NHLFs) in a fibrin gel. The mixture is injected into the central microchamber (volume 0.1 mm^3) of a microfluidic device constructed of optically clear polydimethysiloxane (PDMS) using soft lithography techniques. Nutrients are delivered through microfluidic channels that connect to the central tissue chamber. Over a period of 2 weeks, a 3D continuous vascular network formed in conjunction with the spontaneously contracting (30-40 beats/min) cardiomyocyte spheroids. The vascularized cardiomyocyte spheroids were then exposed to the β-agonist isoproterenol and the β-blocker propranolol, and the EC50 (0.79 nM) and IC50 (1.3 μM), respectively, were both within ½ log order of the *in vivo* value. In addition, we have produced a similar vascularized system using iPSC-derived endothelial cells (iPSC-ECs) instead of ECFC-ECs, as we progress towards a completely iPSC-derived platform. The cardiomyocyte spheroids in the iPSC-EC network show spontaneous contractions and a dose-dependent response to norepinephrine (EC50 41.8 nM). The microfabricated platform has the potential to facilitate high throughput designs, thus enabling rapid screening of drug candidates which are intentionally cardioactive or which minimize cardiac side effects. By advancing this technology, we hope to alleviate the cost of drug development and expedite the discovery of new drugs. Furthermore, by deriving all of the cells from a single iPS cell source, we hope to expand the utility of the platform for disease-specific and patient-specific drug screening applications.

W-3073

SB203580 ANALOGUES INHIBIT CK1 DELTA AND EPSILON IN THE WNT PATHWAY DURING CARDIOMYOCYTE DIFFERENTIATION OF PLURIPOTENT STEM CELLS

Laco, Filip[1], Low, Joo Leng[2], Woo, Tsung Liang[1], Zhong, Qixing[2], Elliott, David[3], Reuveny, Shaul[1], Chai, Christina Li Lin[2], Oh, Steve K.W.[1]
[1]Bioprocessing Technology Institute, Singapore, Singapore, [2]Institute of Chemical and Engineering Sciences, Singapore, Singapore, [3]Murdoch Childrens Research Institute, Parkville, Australia

Current highly efficient cardiomyocyte differentiation protocols use small molecule inhibitors as a strategy to increase the yield and purity of cardiomyocytes from human embryonic stem cells (hESC) for human cell-based therapy. A widely accepted mechanism to differentiate hESC into cardiomyocytes is through perturbation of the canonical Wnt/β-catenin pathway. Recently a small molecule p38α mitogen activated protein kinase (MAPK) inhibitor, SB203580, was discovered for its potential to induce cardiac differentiation in embryoid bodies with moderate efficiency of 10-15%. We studied the mechanism of cardiac differentiation with p38α MAPK inhibitors and then developed a protocol that increased the efficiency of cardiomyocytes differentiation with newly designed SB203580 analogues. We synthesized and screened 42 compounds that are 2,4,5-trisubstituted azole analogues of SB203580 for efficient cardiomyocyte differentiation. Our screen identified novel compounds that have similar cardiac differentiation activity as SB203580. Moreover a novel compound was identified which induced cardiomyocyte differentiation at a 5 times lower concentration than SB203580. However, the cardiac differentiation did not correlate with p38α MAPK activity. Surprisingly, we found out that p38α MAPK kinase downstream signaling pathways were not inhibited by our novel cardiogenic compounds, which indicated an alternative mechanism in cardiac differentiation. Upon profiling several 2,4,5-trisubstituted azole compounds against a panel of 97 kinases we identified several off targets, among them casein kinases 1 (CK1). The cardiomyogenic activities of SB203580 and 18 analogues showed a high correlation with post mesoderm Wnt/β-catenin pathway inhibition of CK1 epsilon and delta. Furthermore, cardiac differentiation and inhibition of the canonical Wnt signaling pathway were more efficient using 2,4,5-trisubstituted azoles containing the pyridin-4-yl/4-fluorophenyl moiety. Consequently an efficient cardiomyocyte protocol was developed with Wnt activator CHIR99021 and 2,4,5-trisubstituted azoles to give high yields of 50-70% cardiomyocytes and a 2 fold improvement in growth. In summary, our findings disprove the involvement of p38α MAPK inhibition in cardiomyocyte differentiation with SB203580 and 2,4,5-trisubstituted azole analogues. Instead, we have identified CK1 inhibition as a leading mechanism in cardiomyocyte differentiation with these analogues. These outcomes unite the mechanism of 2,4,5-trisubstituted azoles in cardiac differentiation with the current theory of Wnt/β-catenin pathway regulated cardiac differentiation, via the novel CK1 targets. We identified several preferred structural features of 2,4,5-trisubstituted azoles which would aid in future development of small molecules for CK1 inhibition and efficient cardiac differentiation. Finally, these novel compounds were shown to generate high yields of cardiomyocyte populations, which will be beneficial in future cell-based therapy.

MESENCHYMAL CELL LINEAGE ANALYSIS

W-3076

MICRORNA-336 TARGETING BNIP3 INHIBITS MITOCHONDRIA-MEDIATED APOPTOSIS OF MESENCHYMAL STEM CELLS IN CARDIAC HYPOXIC MICROENVIRONMENTS

Lee, Jiyun[1], Ham, Onju[1], Lee, Se-Yeon[1], Lee, Chang Youn[2], Park, Jun-Hee[2], Lee, Jiyun[1], Seo, Hyang-Hee[1], Seung, Minji[1], Yun, INa[1], Han, Sun M.[1], **Choi, Eunhyun**[1], Hwang, Ki Chul[3]
[1]Yonsei University College of Medicine, Brain Korea 21 Plus Project for Medical Science, Seoul, Republic of Korea, [2]Yonsei University, Department of Integrated Omics for Biomedical Sciences, Seoul, Republic of Korea, [3]Yonsei University College of Medicine, Severance Biomedical Science Institute, Seoul, Republic of Korea

The ischemic heart generates diverse apoptotic stimulators such as reactive oxygen species and Ca^{2+}; these factors influence MSC survival via mitochondria-mediated apoptosis after transplantation. A well-known Bcl-2 family protein located in the mitochondrial membrane with a role in the induction of mitochondrial-mediated apoptosis, BNIP3 is minimally expressed in normal conditions but highly expressed in hypoxic conditions. MicroRNAs have been utilized as regulators and suppressors of target proteins in cardiovascular diseases. Here, we hypothesized that microRNA-336 regulates BNIP3-induced mitochondria-mediated apoptosis of transplanted MSCs under hypoxic conditions. In ischemia/reperfusion (I/R) models, BNIP3 was over-expressed in transplanted MSCs. When the exposure times to hypoxic conditions were increased, the mortality of MSCs dependently escalated, and expression levels of apoptotic molecules such as Bax, Bcl-xL, Bak, cytochrome c, and caspase-3 changed. In MSCs transfected with miR-336 (miR-336-MSCs), BNIP3 and apoptotic signals were suppressed, by which survival rate was up-regulated compared to that of un-transfected MSCs. Accordingly, the I/R model indicated that the survival rate of miR-336-MSCs transplanted into the ischemic heart was higher than that of un-transfected MSCs. The number of Annexin V/PI and TUNEL-positive cells decreased in the heart transplanted with miR-336-MSCs. MiR-336-MSCs resisted reduction in cell numbers and enhanced recovery of cardiac function such as left ventricular ejection fraction, and also more effectively reduced infarct size, inflammation, and fibrosis area. These results demonstrate that miR-336 suppresses BNIP3 in hypoxic MSCs and subsequently offers beneficial effects to the ischemic heart by enhancing MSC survival.

W-3077

MULTIPLEX CELL-BIOMATERIAL INTERFACE CUES REGULATE STEMNESS IN HUMAN MESENCHYMAL STEM CELLS

Crowder, Spencer William, Balikov, Daniel A., Lewis, Holley N., Ambrose, Caitlyn M., Lee, Sue Hyun, Sung, Hak-Joon
Department of Biomedical Engineering, Vanderbilt University, Nashville, TN, USA

Statement of Purpose: Human mesenchymal stem cells (hMSCs) offer great therapeutic potential for clinical applications, but exhibit a decline in overall health when isolated from aging patients or serially expanded in vitro. Cell "stemness" refers to the ability to retain self-renewal capacity and differentiation potential, and is controlled by specific transcription factors such as Nanog and SOX21. hMSCs have been shown to originate from pericytes, an elusive extravascular cell type, but dissection and clarification of this relationship is absent in

the literature2. The expression of stemness proteins declines with in vitro expansion1, and this correlates with increasing "developmental" distance from the pericyte phenotype, suggesting that rejuvenation of hMSCs might result in phenotypic reversion to yield bona fide pericytes. Although matrix-derived physicochemical cues have been shown to regulate cell fate decisions, alterations in stemness in response to changing substrate properties remain unknown. Therefore, the aims of this study are to elucidate i) the influence of multiplex biomaterial matrix cues in modulating hMSC stemness, ii) how this modulation affects the hMSC/pericyte phenotype, and iii) the pathway(s) responsible. Methods:Spin-coated copolymers composed of three subunits were used in this study: poly(ε-caprolactone) (PCL), poly(ethylene glycol) (PEG), and carboxyl-PCL (cPCL). Copolymers are identified as x%PEG-y%PCL-z%cPCL (x, y, and z: molar ratio). Extensive material characterization was first performed, followed by in-depth biological analyses of cellular function, including flow cytometry, qRT-PCR, immunofluorescence staining, and functional assays. Results: The copolymer substrates exhibited significant differences in physicochemical properties, including protein adsorption, hydrophilicity, roughness, and stiffness. The cellular response was heavily influenced by copolymer composition, including gene- and protein-level expression of Nanog and SOX2. Specifically, the copolymer 4%PEG-96%PCL (referred to as 4%PEG) stimulated the most significant increase in the expression of both Nanog and SOX2. Interestingly, hMSCs cultured upon 4%PEG, but not TCPS, expressed proteins that mark pericytes, including neuron-glial antigen 2 (NG2p), platelet endothelial cell adhesion molecule (PECAM), and α-smooth muscle actin (αSMA), as well as several other pericyte-specific markers2, including CD146 and PDGFR-β, at the gene-level. The hypothesized mechanism driving this event includes integrin α2 and PECAM, two proteins heavily expressed in the vascular compartment that, according to gene- and protein-level analysis, are upregulated in response to altered presentation of adsorbed protein at the cell-biomaterial surface. Furthermore, paracrine signaling present in pericyte-endothelial interactions, such as the angiopoietin-1/Tie2 system, was upregulated on this polymer substrate, and in vivo implantation of three-dimensional, hMSC-loaded 4%PEG scaffolds improved vascularization over three weeks. Conclusions: This study is one of the first to investigate how multiple cues from synthetic culture substrates affect hMSC stemness, and aims to identify the biological mechanisms responsible for this outside-in signaling. The findings from this study will be exploited for maintaining/reinstating the therapeutic efficacy of aging hMSCs for basic science, engineering, and clinical applications.

W-3078
PROLIFERATION OF DENTAL PULP STEM CELLS SUBMITTED TO LOW LEVEL LASER IRRADIATION

Barboza, Carlos Augusto Galvao[1], Zaccara, Ivana M.[1], Ginani, Fernanda[1], Mota-Filho, Haroldo G.[1], Barreto, Mardem P. V[1], Henriques, Águida G.[2]

[1]Department of Morphology, Federal University of Rio Grande do Norte, Natal, Brazil, [2]School of Dentistry, Federal University of Bahia, Salvador, Brazil

A positive effect of low level laser irradiation (LLLI) on proliferation of some cell types has been observed, but little is known about its effectiveness on dental pulp stem cells (DPSCs). The aim of this study was to identify the lowest energy density capable of promoting proliferation of DPSCs and maintaining its viability. Human DPSCs were isolated from two healthy third molars extracted due to surgical indication. The multipotential nature of the cells was confirmed by expression of stem cell surface markers CD105, CD73, and CD90 by flow cytometry and conversion into osteogenic and adipogenic phenotypes after culture in differentiation medium. In the third subculture, the cells were irradiated or not (control), with a laser diode InGaAlP, power of 30 mW, wavelength of 660 nm, continuous action mode, with a tip diameter of 0,01 cm^2, and using two different energy densities (0.5 and 1.0 J/cm^2). The cells were irradiated at 0 and 48 h, with the laser probe fixed perpendicular to each plate at a distance of 0.5 cm from the cells. Cell proliferation and viability was evaluated by Trypan blue exclusion method and measuring mitochondrial activity using the MTT-based cytotoxicity assay at intervals of 24, 48, 72 and 96 h after the first laser application. Events related to cell death were evaluated by expression of Annexin V and propidium iodide and the cell cycle was also analyzed by flow cytometry. The analysis of the number of cells in the different groups by the Trypan blue exclusion method revealed an increase of cell proliferation over time in all groups. A higher proliferation rate was seen in the irradiated groups when compared to the control group, with statistically significant difference ($p<0.05$) at the interval of 72 h. At the 96 h interval, a significant difference was only observed with an energy density of 1.0 J/cm^2. There was no difference in cell viability analyzed by the Trypan blue exclusion method among the groups at all time points studied. Mitochondrial activity in the irradiated groups followed the pattern observed by the Trypan blue exclusion method. Irradiation with energy densities of 0.5 and 1.0 J/cm^2 promoted significantly higher number of cells when compared to the control group after the second irradiation at interval of 72 h ($p<0.05$). It was observed that the cells had low positive staining for Annexin V and propidium iodide, markers of cell death. No significant changes were observed in cell viability throughout the experiment, although a slight increase in the percentage of viability was observed in the two irradiated groups after the second application of LLLI. The analysis of the distribution of the cells in the cell cycle phases showed a higher percentage of cells in G0/G1 phase (over 50%) 24 h after plating, but no statistically significant difference was found among the groups. In the last two intervals examined (72 and 96 h), approximately 85% of the cells were distributed in phases S and G2/M, which was consistent with proliferating cells in all three of the groups. It can be confirmed that the patterns of LLLI used in the present study (power 30 mW, wavelength of 660 nm and energy density of 1,0 J/cm^2) promotes proliferation of DPSCs and maintaining its viability. These results have potential clinical relevance, because the use of low level laser irradiation associated with DPSCs can represent a therapeutic opportunity in dentistry and regenerative medicine.

W-3079
COMPREHENSIVE CHARACTERIZATION OF HUMAN EMBRYONIC STEM CELL-DERIVED MESENCHYMAL STEM CELLS

Billing, Anja M.[1], Dib, Shaima S.[1], Ben Hamidane, Hisham[1], Kumar, Pankaj[2], Hayat, Shahina[2], Halabi, Najeeb[3], Yousri, Noha[2], Pasquier, Jennifer[3], Suhre, Karsten[2], Rafii, Arash[3], Graumann, Johannes[1]

[1]Proteomics Core, Weill Cornell Medical College in Qatar, Doha, Qatar, [2]Physiology and Biophysics, Weill Cornell Medical College in Qatar, Doha, Qatar, [3]Genetic Medicine, Weill Cornell Medical College in Qatar, Doha, Qatar

Mesenchymal stem cells (MSC) are self-renewing multipotent cells which hold great potential in reconstructive medicine and tissue engineering. They have the ability to differentiate into cells of the mesoderm lineage and possibly to transdifferentiate. MSC can be derived from multiple adult tissues but have only limited expansion capacity in cell culture. We show here an adapted, easily reproducible protocol (Raynaud et al., 2013) to differentiate human embryonic stem cells (ESC) into highly-proliferative MSC. We characterized

ESC-derived MSC in-depth and compared them to adult tissue-derived MSC (bone marrow BM-MSC) as well as to their origin (ESC). We performed large-scale proteomic profiling on organelle level (cytosol, nucleus, chromatin) using quantitative high resolution mass spectrometric analysis based on stable isotope labeling with amino acids in cell culture (SILAC, Cox and Mann, 2008). Those results were complemented with a DNA aptamer-based array quantifying over 1100 proteins (SOMAScan™ assay). This assay, developed for plasma proteome profiling, includes proteins which are normally expressed at very low copy number such as cytokines and chemokines, as well as key proteins of common signaling pathways. In total, over 5000 proteins were quantified with 300 common in both techniques. In SILAC experiments, around 2000 proteins are differentially expressed when ESC and MSC (both ESC-MSC and BM-MSC) were compared. ESC-derived MSC differ from BM-MSC in 400 proteins. Similar results were obtained with the aptamer-based assay: approximately 800 proteins are differentially expressed between ESC and MSC, with 55 proteins differentiating between ESC-MSC and BM-MSC. To further enhance the analytical depth we analyzed the transcriptome using next-generation RNA sequencing enabling us to quantify the expression of over 14000 genes. ESC and MSC greatly differ in their gene expression (around 5000 genes), whereas ESC-MSC and BM-MSC are closely related (around 400 genes). Data integration of all three large-scale techniques was performed. Bioinformatic analysis revealed a high enrichment of transcription/translation-related functions for ESC, in particular transcription factors, regulators of cell cycle and proteins involved in RNA processing/splicing. For MSC highly significant were vesicle transport, angiogenesis and extracellular matrix generation. The study presented here is the first part of an in-depth characterization of the differentiation process of ESC into MSC currently ongoing in our lab.

W-3080
PROLIFERATION OF HUMAN ADIPOSE-DERIVED STEM CELLS TREATED WITH ESSENTIAL OIL EXTRACTED FROM AN AMAZONIC PLANT - LIPPIA ORIGANOIDES SCHAUER

Brito, Felipe Nunes[1], Vendramin, Fabiel Spani[1], Lopes, Cinthia Távora De Albuquerque[2], Costa, Mayra Pauline Ribeiro[2], Sá, Andre Luiz Alves De[2], Miranda, Carla Maria Figueiredo De Carvalho[3], Ohashi, Octavio Mitio[2], Silva, Joyce Kelly Do Rosário Da[2], Miranda, Moyses Dos Santos[2]
[1]School of Medicine, Federal University of Para, Belém, Brazil, [2]Institute of Biological Sciences, Federal University of Para, Belém, Brazil, [3]University of São Paulo, São Paulo, Brazil

Adipose tissue (subumbilical) was collected from one female patient submitted to liposuction cosmetic procedure. Human adipose-derived stem cells were isolated by collagenase II digestion for one hour, cultured in Iscove's Modified Dulbecco's Media (IMDM) at 98,6 °F in a 5% CO_2 incubator up to passage two and frozen in liquid nitrogen. Mesenchymal stem cells were thawed, cultured until reaching 80% confluency, trypsinized and utilized for the experiments at passage 4. It was tested for *in vitro* differentiation potential in adipocytes, chondrocytes and osteocytes followed by tissue-specific histological staining. Also, 3 x 10^3 cells were plated in 12 wells of a 96 well plate. After 24 hours, the culture medium was supplemented with three concentrations (12.5, 25 and 50 $mg.ml^{-1}$) of *Lippia origanoides* essential oil extracted by hydrodistillation and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). It was used a triplicate plate for each oil concentration tested and for the negative control. Treated and negative control cells were cultured for 1 day and evaluated by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. Differentiation results were evaluated qualitatively and proliferations results were evaluated by ANOVA at 5% significance level. This study was approved by the Brazilian Committee for Ethics in Human Research. Cells at passage 4 demonstrated adipogenic, osteogenic and chondrogenic differentiation potential *in vitro*. MTT assay results showed that treatment with 25 mg/mL of essential oil increased cellular proliferation in comparison to the others groups ($p<0.05$), while treatment with 50 mg/mL negatively affected mesenchymal stem cell proliferation. Culture medium supplementation with essential oil from *Lippia origanoides* plant can be an alternative for increasing *in vitro* mesenchymal stem cells proliferation. Assuming that Timol (60%) as a major component in this oil we could speculate about an antioxidant protecting effect occurring in cell culture over the culture time. Further experiments will address this hypothesis. On the other hand, attention must be given to the use of this essential oils at higher dosages once it can show a toxic effect on cell culture. Finally, essential oils from the Amazon plant can potentially become a cheap and efficient way to increase *in vitro* adult stem cell expansion for cell therapy.

W-3081
INTERCHANGEABLE FATES OF OSTEOGENIC AND CHONDROGENIC PROGENITORS REVEALED BY COMPREHENSIVE LINEAGE-MAPPING OF MULTIPOTENT SKELETAL STEM CELLS

Chan, Charles
Stem Cell Institute, Stanford University, Stanford, CA, USA

Bone, cartilage, and bone marrow stroma are the primary components of the skeletal system but the origins of these tissues remain undefined. Here we prospectively isolated eight distinct progenitors of bone, cartilage, and stromal tissue and delineated the lineage relationships between them to map bone and cartilage development from a population of highly pure, post-natal skeletal stem cells (SSC). The transcriptome of each individual subset of progenitors was investigated to identify unique developmental programs underlying the intrinsic and extrinsic regulation of SSC lineage commitment. This analyses revealed that several varieties of SSC-derived hematopoietic supportive-stroma express factors that directly regulate SSC expansion and differentiation, indicating that SSC generated-stroma plays dual roles in regulating both hematopoietic and skeletal niches. Such SSC niche-factors can be potent inducers of skeletal regeneration. We find several combinations of recombinant proteins that could activate SSC genetic programs in situ, even in non-skeletal tissues, resulting in de-novo formation of cartilage or bone and bone marrow stroma.

W-3082
HUMAN ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS IN VITRO DOUBLING TIME USING AUTOLOGOUS PLATELET RICH PLASMA COMPARED TO FETAL BOVINE SERUM

Degheidy, Tamer Abdel Razik[1], Abbas, Samir[2]
[1]Dr. Samir Abbas Medical Centers, Jeddah Saudi Arabia. Medical Experimental Research Center, Mansoura University, Egypt, Mansoura, Egypt, [2]Dr. Samir Abbas Medical Centers, Jeddah Saudi Arabia, Jeddah, Saudi Arabia

The increasing evidences for the efficacy of mesenchymal stem cells (MSCs) as an advanced cell therapy and their low frequency in adult tissues have triggered the need for their in vitro expansion. Most of the current protocols for expansion included fetal bovine serum (FBS) in culture media with the risk of immunogenicity and zoonosis during clinical application. The aim of the current work is to investigate the efficacy of autologous platelet rich plasma (PRP) with its high contents of autologous growth factors as an alternative to FBS in culture media. In vitro doubling time (DT) of human adipose tissue MSCs using

culture media containing autologous PRP was compared to that of media using FBS. After written consent, aspirated fat was obtained from ten cases during liposuction and was subjected to collagenase digestion for obtaining Stromal Vascular Fractions (SVF). Harvested plastic adherent MSCs in passage 0 were characterized and counted MSCs were cultured in 10% FBS containing media and 10% buffered autologous PRP containing media at density of $4x10^5$ mL^{-1} for 4 passages to compare cells expansion's DT using both media. Adherent MSCs showed statistical significant lower DT ($P<0.05$) when cultured in autologous PRP containing media compared to FBS containing media. In autologous PRP containing media, MSCs showed an average DT of (3.03, 3.24, 3.78, and 4.35) for P1, P2, P3, and P4, respectively, and average DT of (3.69, 4.15, 5.02, and 6.25) for P1, P2, P3, and P4, respectively in FBS containing media. Lower MSCs in vitro DT using autologous PRP containing media, is carrying a promising solution for lower MSCs expansion time and for the potential immunologic and zoonotic risks accompanied with the use of xeno-derived materials.

W-3083
IN VITRO COMPARATIVE ANALYSIS OF CRYOPRESERVATION OF STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

Ginani, Fernanda[1], Soares, Diego M.[1], Rabêlo, Luciana M.[2], Freire-Neto, Francisco P.[2], Rocha, Hugo A.[2], Barboza, Carlos A.[1]
[1]Department of Morphology, Federal University of Rio Grande do Norte, Natal, Brazil, [2]Department of Biochemistry, Federal University of Rio Grande do Norte, Natal, Brazil

Dental pulp stem cells have been widely investigated because of their vast potential for use in therapies involving pulp regeneration and tissue engineering. The technique of cell cryopreservation represents a viable alternative for the conservation of these cells for long periods, since it reversibly stops all of their biological functions at an ultra-low temperature and in a controlled manner. The present study aimed to evaluate, using *in vitro* experiments, the influence of a cryopreservation protocol on the proliferation and viability of stem cells from human exfoliated deciduous teeth (SHED). Cells obtained from the pulp of three deciduous teeth at end-stage exfoliation and with indicated extraction were expanded in α-MEM culture medium supplemented with antibiotics and 15% fetal bovine serum. The cells were characterized as stem cells by expression of cell surface markers and by differentiation into osteoblasts and adipocytes under induced conditions. At second passage (P2), part of the cells was maintained in 10% DMSO diluted in fetal bovine serum and submitted to the cryopreservation protocol: 2 h at 4°C, 18 h at −20°C, and then at −80°C for 30 (cryopreserved I) and 180 days (cryopreserved II), while a third group (non-cryopreserved cells) followed under normal conditions of cell culture. Cell proliferation was evaluated in the groups (cryopreserved I, cryopreserved II and non-cryopreserved) by Trypan blue stain essay at intervals of 24, 48 and 72 h after plating. Cell cycle analysis of SHEDs submitted or not submitted to the cryopreservation protocol were performed in the same intervals. Events related to cell death were analyzed by Annexin V and propidium iodide expression under flow cytometry at the interval of 72 h. It was observed that all the groups exhibited an upward cell proliferation curve without significant statistical differences compared to the cryopreserved and control (non-cryopreserved) groups ($p>0.05$). The distribution of cells in cell cycle phases was consistent with cell proliferation, indicating that in all groups the majority of the cells were in the S and G2/M phases at the 3 intervals analyzed. In relation to cell viability, all groups showed a percentage of over 99% of viable cells which proves that the viability throughout the experiment was not affected by cryopreservation protocol used. Therefore, it is concluded that the proposed cryopreservation protocol is effective for storing the studied cell type for long periods, allowing for their use in future experimental studies.

W-3084
DEREGULATED HOX EXPRESSION IN MESENCHYMAL STROMAL/STEM CELLS DERIVED FROM G-CSF PRIMED BONE MARROWS

Gunel-Ozcan, Aysen[1], Cagnan, Ilgin[1], Aerts-Kaya, Fatima[1], Kuskonmaz, Baris[2], Uckan, Duygu[1]
[1]Department of Stem Cell Sciences, Hacettepe University, Ankara, Turkey, [2]Department of Pediatrics, Hacettepe University, Ankara, Turkey

HOX and TALE transcription factors play an important role in the proliferation and commitment of hematopoietic cells as well as the development of malignancies like leukemia. Bone marrow transplantation (BMT) is a commonly used therapy for patients with certain malignant or non-malignant hematopoietic diseases. Donors in pediatric age are sometimes treated with granulocyte colony-stimulating factor (G-CSF) because of their low body weight compared to patients. Here we aimed to assess the effects of in-vivo exposure to G-CSF on gene expression of HOX and their co-factors TALE in bone marrow stromal/stem cells. Human bone marrow mesenchymal stromal/stem cells (MSCs) were isolated from 3 day G-CSF (10 μg/kg/d) treated (n=8) and untreated (n=9) healthy donors. Mean age of G-CSF treated and untreared donors were 7.4 and 16.4 years, respectively. MSCs were expanded in vitro and characterized for their cell surface markers and for their differentiation capacity to adipocytes and osteoblasts. Thirty-nine HOX (HOXA/B/C/D) and eight TALE (MEIS, PBX, PREP) gene expression levels were determined by real-time qPCR using UPL probes and LightCycler 480-II machine (Roche). BM-MSCs exhibited similar cell surface marker expression as well as capacity to differentiate to adipogenic and osteogenic lineages. In BM-MSCs from G-CSF treated donors, HOXA1 expression levels were significantly increased whereas HOXA7 and HOXB7 expression levels were significantly decreased ($p\leq0.05$). These results suggest that G-CSF treatment changes HOX code of bone-marrow mesenchymal stromal/stem cells.

W-3085
RB MAINTAINS QUIESCENCE AND PREVENTS PREMATURE SENESCENCE THROUGH UP-REGULATION OF DNMT1 IN MESENCHYMAL STEM CELLS

Hung, Shih-Chieh
Department of Medical Research, Taipei Veterans General Hospital, Taipei City, Taiwan

Mesenchymal stem cells (MSCs) are now applied in lots of cell therapies. However, MSCs start entering the senescence state upon proliferation long-term in vitro. The role of retinoblastoma (Rb) protein in regulating MSC properties is not well studied. Here, we show the Rb level is higher in early-passage MSCs, compared to late-passage MSCs. Rb knockdown induces premature senescence and reduced differentiation potentials in early-passage MSCs. Rb overexpression inhibits senescence and increases differentiation potentials in late-passage MSCs. The expression of DNMT1, rather than DNMT3A and DNMT3B, is also higher in early-passage MSCs than late-passage MSCs. Besides, DNMT1 knockdown in early-passage MSCs induces senescence and reduces differentiation potential, while DNMT1 overexpression in late-passage MSCs has opposite effect. These results demonstrate that Rb expressed in early-passage MSCs up-regulates DNMT1 expression and inhibits senescence in MSCs. Therefore, genetic modification of Rb could be a way to improve the efficiency of MSCs in clinical use.

W-3086
BONE MARROW DERIVED CLONAL MESENCHYMAL STEM CELLS INHIBIT OVALBUMIN INDUCED ATOPIC DERMATITIS

Jeon, Myung-Shin[1], Na, Kwangmin[1], Yoo, Hyun Seung[2], Zhang, Yong Xu[3], Lee, Hyun-Joo[4], **Kim, Junghee[4],** Yi, TacGhee[1], Song, Sun[1]
[1]Translational Research Center, Inha University School of Medicine, Incheon, Republic of Korea, [2]Drug Development, Inha University School of Medicine, Incheon, Republic of Korea, [3]Molecular Biomedicine, Inha University School of Medicine, Incheon, Republic of Korea, [4]Drug Development, Inha University School of Medicine, Incheon, Republic of Korea

Mesenchymal stem cells (MSCs) possess immunomodulatory activities, including suppression of T-and B-cell activation. However, their effects on atopic dermatitis (AD) have not yet been studied. Using an ovalbumin-induced AD mouse model, we investigated whether MSCs can be used as therapeutics in AD. We isolated both allogeneic and syngeneic clonal MSCs (cMSCs) from mouse bone marrow according to the sub-fractionation culturing method. Our cMSCs suppressed both T- and B-cell activation. T-cell proliferation and cytokine production, including interferon (IFN)-γ and interleukin (IL)-4, were suppressed by inhibition of transcription factors, such as T-bet, GATA-3, and c-Maf. Those transcription factors were nitric oxide dependent. Immunoglobulin E (IgE) suppression occurred through downregulation of AID and BLIMP-1, which are important regulators for isotype class switch and B-cell differentiation. AID expression was post-transcriptionally regulated, whereas BLIMP-1 was transcriptionally regulated. cMSCs were injected intravenously into ovalbumin-induced AD mouse model, and the therapeutic effects were analyzed. Injection of both allogeneic and syngeneic cMSCs in an AD mouse model inhibited cell infiltration in skin lesions and decreased the serum level of IgE. IL-4 expression was also suppressed by cMSCs both in the spleen and skin. cMSCs migrated to skin lesions and draining lymph nodes. Taken together, these data demonstrated that cMSCs, which suppressed T- and B-cell functions, can be used for the treatment of AD in mice.

W-3087
IMPACT OF MESENCHYMAL STEM/PROGENITOR CELL FATE IN ORTHOPAEDIC TRAUMATIC COMBAT BLAST INJURY INDUCED HETEROTOPIC OSSIFICATION

Ji, Youngmi[1], Christopherson, Gregory[1], Jones, Patrick[2], Shin, Emily[2], Patel, Nayha[1], Cirino, Carl[1], Patel, Vyomesh[1], Nesti, Leon[1]
[1]NIH, Bethesda, MD, USA, [2]Walter Reed National Military Medical Center, Bethesda, MD, USA

Modern war-trauma frequently results in severely damaged or loss of muscle tissue resulting in impaired or loss of limb function. Arising from these injuries is heterotopic ossification (HO) an aberrant mature bone formation in non-osseous tissues. Notably, the high (64%) prevalence of HO in the combat-wounded during recent military operations has raised key questions to its clinical and molecular basis. Symptomatic HO severely impacts morbidity, pain, prosthetic fitting, often requiring additional surgery and delaying recovery as leading to limit patient's functional level. Normal wound healing is largely initiated by elevated levels of TGF-β and the recruitment of various cells including immune cells, fibroblasts and mesenchymal progenitor cells. By contrast, abnormal healing results in tough and fibrous scar tissue accumulating at injury site and manifestes as fibrosis. The healing response following combat blast traumatic injury also results in tissue fibrosis, a salient feature in HO lesions. Repeated surgical observations have further linked areas of abundant fibrotic scarring within the wound to an abnormal healing process. As stated, the mechanism of HO formation is unclear; we thus hypothesized that cells within the war-trauma muscle tissue may become dysregulated by conflicting wound-healing signals resulting in osteogenesis. Work from our laboratory have isolated and identified a population of mesenchymal progenitor cells (MPCs) within war-trauma muscle tissues on the basis of in vitro adherence selection. Characterized MPCs were similar to Mesenchymal stem cells (MSCs) from bone marrow by morphology, cell surface epitopes, and tri-lineage differentiation capacity (into osteoblasts, adipocytes, and chondrocytes). Notably, MPCs could be identified by their expression of CD105 using immunohistochemstry; these cells were seem to be localized within damaged muscle fibers of war-trauma muscle. To understand the etiology of HO after combat trauma, we explored: 1) Histopathological and scanning electron microscope (SEM) analysis, to determine the pathophysiology and evaluating the physical microenvironment of HO and war-trauma muscle, respectively; 2) Analysis of the molecular signature of war-trauma muscle by gene and microRNA expression; 3) Functional validation of MPCs using in vitro fibrotic nodulogenesis assay; 4) Functional validation of MPCs using collagen nanofiber matrix, and 5) Evaluating blast amputated limbs of a rat model of HO, reliably shown to produce HO by prescribed-intensity blast injury and comparable to human HO etiology. Our results found that Histopathological and SEM analysis of fibrotic muscle tissue revealed an abundant fibrous network, indicating a sufficient microenvironment to promote cellular osteogenic differentiation in vitro. Furthermore, we found that several fibrotic markers are elevated in tissue samples that precede the development of HO. We also examined MPCs behavior after exposure to fibrotic signals and found this resulted in an enhanced ability to form bone. Analysis of rat blast amputated limbs showed very similar histopathological and molecular features to human HO. Emerging data from our studies using in vitro and in vivo model systems will likely help to further our understanding of HO pathogenesis and find suitable therapeutic strategies to reduce fibrosis and prevent HO, and with the potential to accelerate desirable muscle regeneration using MPCs to facilitate repair and healing.

MESENCHYMAL STEM CELL DIFFERENTIATION

W-3088
SILENCING VEGFR2 HAMPERS ODONTOBLAST DIFFERENTIATION OF DENTAL PULP STEM CELLS

Janebodin, Kajohnkiart[1], Parent, Sara[2], Ieronimakis, Nicholas[1], Monis, Grace[3], Hays, Aislinn[1], Reyes, Morayma[1]
[1]Pathology, University of Washington, Seattle, WA, USA, [2]Oral Health Sciences, University of Washington, Seattle, WA, USA, [3]Laboratory Medicine, University of Washington, Seattle, WA, USA

Dental pulp stem cells (DPSCs) give rise into odontoblast-like cells and form dentin/pulp-like structures, which is important in dentin and tooth regeneration. In addition, we have demonstrated that DPSC-induced angiogenesis is VEGFR2-dependent. However, the role of VEGFR2 signaling in odontoblast differentiation of DPSCs is still not well understood. Therefore, we aimed to study whether VEGFR2 plays an important role in odontoblast differentiation of DPSCs both in vitro and in vivo. DPSCs were induced by VEGFR2-shRNA viral vectors transfection (MOI = 20:1) to silence the expression of VEGFR2. The efficiency of viral transfection was demonstrated by positive GFP expression in DPSCs induced by the control CopGFP viral vectors under the same condition. VEGFR2 shRNA-DPSCs which were selected in puromycin-supplemented media showed no difference in

cell proliferation when compared to CopGFP-DPSCs. Interestingly, VEGFR2 shRNA-DPSCs expressed not only very low level of VEGFR2, but also that of its ligand, VEGF-A, compared to CopGFP-DPSCs in both transcriptional and translational levels. In vitro differentiation of DPSCs in osteo-odontogenic media supplemented with BMP-2 (100ng/ml) for 21 days demonstrated that CopGFP-DPSCs, but not VEGFR2 shRNA-DPSCs, were positive for ALP staining and formed mineralized nodules indicated by positive Alizarin Red S staining. The expression levels of dentin matrix proteins, dentin matrix protein-1 (DMP-1), dentin sialoprotein (DSP), and bone sialoprotein (BSP), were also up-regulated in CopGFP-DPSCs, compared to those in VEGFR2 shRNA-DPSCs, suggesting an impairment of odontoblast differentiation in VEGFR2 shRNA-DPSCs. In vivo subcutaneous transplantation of DPSCs with hydroxyapatite (HAp/TCP) for 5 weeks demonstrated that CopGFP-DPSCs were able to differentiate into elongated and polarized odontoblast-like cells and form well-organized dentin/pulp-like structures with abundant blood vessels, as demonstrated by H and E, Alizarin Red S, and dentin matrix staining. On the other hand, in VEGFR2 shRNA-DPSC transplants, odontoblast-like cells were not observed. Collagen fibers were seen in replacement of dentin/pulp-like structures. In conclusion, these results indicate that VEGFR2 may play an important role in dentin regeneration and tooth formation. To prove this concept, a transgenic mouse model using the inducible Cre-lox system to knock down the expression of VEGFR2 will be generated, and tooth development across different ages and dentin regeneration after an injury in those mice will be examined, compared to the wild type mice. These results would provide pivotal knowledge to enhance dentin regeneration and tissue engineering as a promising clinical application.

W-3089
FGF2: AN INDEPENDENT AND EFFICIENT INDUCER OF DOPAMINERGIC NEURONS DERIVED FROM HUMAN BONE MARROW MESENCHYMAL STEM CELLS

Mohanty, Sujata[1], Singh, Manisha[1], B Nandy, Sushmita[2]
[1]Stem Cell Facility, All India Institute of Medical Sciences, New Delhi, India, [2]Department of Biomedical Sciences, Texas Tech University, El Paso, TX, USA

Bone Marrow (BM) is a very rich source of Mesenchymal Stem Cells (MSC), which hold a great potential of differentiation into various cells types. BM-MSCs have been reported to be differentiated successfully into dopaminergic (DA) neurons, whose degeneration is the main cause of Parkinson's disease. Different inducers have been employed for in vitro differentiation of BM-MSCs into DA neurons, like ATRA, basic fibroblast growth factor (FGF2), fibroblast growth factor- 8 (FGF8), sonic hedgehog (SHH), Forskolin, brain derived growth factor (BDNF), Oxysterols, safrole oxide, insulin- transferring- selenium, Dimethyl sulfoxide (DMSO), etc. However, the efficiency of generation of DA neurons is low and there is great variation of it in literature; with either growth factors/chemicals alone or combination of them. In this study, we selected FGF2, BDNF and Oxysterol (22-HC) and explored their potential as inducers to generate DA neurons from BM-MSC, efficiently and economically. Institute Ethical Clearance was obtained before the study was initiated. Cryopreserved BM-MSCs were revived and expanded in vitro in DMEM-LG with 10% FBS and processed for induction from 3rd to 5th passage. The basic constituents of the induction media (IM) consisted of neurobasal media with B27 supplement, L- glutamine, penstrep and epidermal growth factor; however, IM-1 consisted of FGF2, IM-2 consisted of BDNF and IM-3 consisted of Oxysterol (22-HC) as the main DA neuronal inducers. Induction period of all the three study groups was 12 days. BDNF was added to the culture media on 9th day of induction. Uninduced MSCs in expansion medium were treated as control group. Post induction period, the induced cells were evaluated for neuron and dopaminergic neuron specific markers, Nestin, β- tubulin III (Tuj1) and Tyrosine Hydroxylase (TH) by immunoflorescence (IF) assay. The transcriptome level of TH gene was quantified and compared in cells induced by IM-1, IM-2, IM-3 with those of control sample. Observations under light microscope revealed that the cells treated by different inducers showed a remarkable change in their morphology from spindle shaped to elongated cells bearing cell body and dendritic outgrowths. Expression of cytoplasmic proteins- Nestin, Tuj1 and TH, as assayed by IF, was more in induced groups as compared to that in uninduced cells. On qRT-PCR analysis, increase in the relative expression of TH in induced groups as compared to control group was found to be 22 folds in cells induced with IM-1, followed by those with IM- 2 (8 folds) and IM- 3 (4 folds). Overall, the morphological changes, IF assay and qRT-PCR analysis suggest that IM-1 fairs better than IM-2 and IM-3. Based on the above evaluations, i.e., morphological analysis, expression of cytoplasmic markers (Nestin, Tuj1 and TH) by IF and qRT-PCR, it is indicated that FGF2 is a better inducer of BM-MSC into DA neurons, than BDNF and Oxysterol (22-HC). Western blotting experiments to characterise the neuronal specific proteins are underway to substantiate the above data. Further characterization of the induced cells with more number of DA neuronal specific markers and their evaluation by different techniques needs to be done. Combinations of these inducers may also exhibit synergistic effect, and can serve as an alternate approach, leading to maximum generation of DA neurons in vitro.

W-3090
THE ROLE OF DERMAL SHEATH CELLS IN ADULT HAIR FOLLICLE REGENERATION

Abbasi, Sepideh[1], Rahmani, Waleed[1], Hagner, Andrew[2], Stykel, Morgan[2], Biernaskie, Jeff[3]
[1]University of Calgary, CALGARY, AB, Canada, [2]University of Calgary, Calgary, AB, Canada, [3]University of Calgary, Calgary, AB, Canada

The dermal papillae (DP) and dermal sheath (DS) are the two main mesenchymal compartments of the hair follicle. In vivo lineage tracing studies in our lab have shown that a population of dermal stem cells envelop the quiescent telogen DP, are activated at the onset of anagen (growth phase), regenerate the DS and most importantly, are recruited into the DP. Furthermore, a recent study showed that the DP cell number specifies the three hair types (zigzag, auchene, awl) generated in the next cycle. Individual hair follicle can switch from small hair types (zigzag) to a larger hair type (auchene or awl) if the number of the DP cells increase beyond a certain threshold. However, the functional role of these dermal stem cells (DSC) during hair follicle regeneration remains poorly understood. Based on our previous work, we hypothesize that hair follicle DSCs play a critical role in the regeneration of a hair follicle and the production of normal hair fibres. Therefore, we asked whether ablating DSCs within the adult hair follicle would impair subsequent hair follicle regeneration. We crossed αSMA-CreERT2-ROSA26eYFP mice to iDTR mice, such that YFP and dyptheria toxin receptor is expressed upon administering tamoxifen in DS cells, blood vessels and arrector pili muscle. To minimize potentially confounding vascular effects, we grafted backskin of these transgenic mice to nude mice. After tamoxifen application, our results show that administering Diphtheria toxin during late telogen and early anagen promotes the reversion toward zigzag (smaller) hair fibres ($p<0.05$). At the same time, the newly produced hair fibers were shorter compared to control ($p = 0.0002$ in zigzag hairs and $p = 0.01$ in awl/auchene hair). Ours results show that dermal stem cells contribute cells into the adult DP to maintain the normal cycling of hair follicles and support the production of larger and longer hair fibres.

W-3091
SPATIAL CONTROL OF ADULT STEM CELL FATE USING NANOTOPOGRAPHIC CUES

Ahn, Eun Hyun[1], Kim, Younghoon[2], Kz, Kshitiz[1], An, Steven S.[2], Afzal, Junaid[2], Lee, Suengwon[2], Kwak, Moonkyu[3], Suh, Kahp-Yang[3], Kim, Deok-Ho[1], Levchenko, Andre[2]
[1]University of Washington, Seattle, WA, USA, [2]Johns Hopkins University, Baltimore, MD, USA, [3]Seoul National University, Seoul, Republic of Korea

Adult stem cells hold great promise as a source of diverse terminally differentiated cell types for tissue engineering applications. However, due to the complexity of chemical and mechanical cues specifying differentiation outcomes, development of arbitrarily complex geometric and structural arrangements of cells, adopting multiple fates from the same initial stem cell population, has been difficult. Here, we show that the topography of the cell adhesion substratum can be an instructive cue to adult stem cells and topographical variations can strongly bias the differentiation outcome of the cells towards adipocyte or osteocyte fates. Switches in cell fate decision from adipogenic to osteogenic lineages were accompanied by changes in cytoskeletal stiffness, spanning a considerable range in the cell softness/rigidity spectrum. Our findings suggest that human mesenchymal stem cells (hMSC) can respond to the varying density of nanotopographical cues by regulating their internal cytoskeletal network and use these mechanical changes to guide them toward making cell fate decisions. We used this finding to design a complex two-dimensional pattern of co-localized cells preferentially adopting two alternative fates, thus paving the road for designing and building more complex tissue constructs with diverse biomedical applications.

W-3092
INTEGRATED MICRORNA AND GENE EXPRESSION ANALYSES REVEALED MICRORNA-320/RUNX2 AXIS AS KEY REGULATOR OF ADIPOCYTIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS (MSCS)

Alajez, Nehad M.[1], Hamam, Dana[1], Ali, Dalia[1], Vishnubalaji, Radhakrishnan[1], Kassem, Moustapha[2], Aldahmash, Abdullah M.[1]
[1]King Saud University College of Medicine, Riyadh, Saudi Arabia, [2]Department of Endocrinology, University of Southern Denmark, Odense, Denmark

The molecular mechanisms promoting lineage-specific commitment of human mesenchymal (skeletal or stromal) stem cells (hMSCs) into adipocytes are not fully understood. Thus, we performed global microRNA (miRNA) expression profiling during adipocytic differentiation of hMSC, and utilized bioinformatics as well as functional and biochemical assays to identify the functionally-relevant miRNAs exclusively expressed during adipogenesis. We identified several novel miRNA candidates differentially expressed during adipogenesis. Among these, miR-320 family (miR-320a, 320b, 320c, 320d, and 320e) were ~2.2-3.0 fold upregulated. Over-expression of miR-320c in hMSCs enhanced adipocytic differentiation and early formation of mature adipocytes in ex vivo cultures accompanied with enhanced expression of adipocyte-specific genes (PPARg, AP2, and AdipoQ). Microarray gene expression analysis performed on MSCs over-expressing miR-320c combined with target prediction revealed several potential gene targets for miR-320 family (i.e. RUNX2, KITL, BMPR1A and others). Functional annotation and gene ontology analysis on the predicted targets indicated a role for miR-320c in regulating several biological processes such as gene expression, cell differentiation, and cell fate commitment. siRNA-mediated knockdown of selected miR-320c gene targets (such as RUNX2) phenocopied the effects of miR-320c over-expression on promoting adipogenesis, suggesting biological relevance of the identified genes in miR-320-mediated adipogenesis. Interestingly, RUNX2 had four predicted miR-320 binding sites on its 3'UTR located between nucleotides 1175 and 3142. To confirm that RUNX2 is indeed a direct target for miR-320 family, a reporter vector carrying the predicted binding site(s) of RUNX2 downstream of the firefly luciferase gene in the pMIR-REPORT™ miRNA expression reporter vector was constructed. Co-transfection experiments demonstrated significant regulation of the RUNX2 reporter by miR-320c miRNA (~ 50%). The regulation of RUNX2 UTR by miR-320c was specific, as mutating the seed region completely abrogated this effect, indicating RUNX2 as a bona fide target for miR-320 family during adipogenesis.Conclusion: Our data revealed for the first time a novel role for miR-320 family in promoting adipocytic differentiation of hMSCs via suppression of multiple genes, including RUNX2. Our data suggest the utilization of miR-320 mimics and/or inhibitors as potential therapeutic modality for regulating hMSC fate into fat. Acknowledgment: This work was supported by grant (No. 11-BIO1941-02) from the National Plan for Sciences and Technology Program, King Saud University, kingdom of Saudi Arabia.

W-3093
MAJOR ANTIPROTEASE ALPHA-1-ANTITRYPSIN ENHANCES MSC SURVIVAL ON DECELLULARIZED HUMAN LUNG MATRIX: A NOVEL ROLE IN COPD-RELATED REGENERATION

Aldonyte, Ruta
State Research Institute, Vilnius, Lithuania

Regeneration mechanisms of an adult lung tissue remain enigmatic. In healthy lungs tissue destruction and regeneration/reparation processes are maintained in a fine balance. This balance is susceptible to inflammation-related and other insults. In patients with COPD this balance is shifted towards proteolysis and resulting tissue destruction. Indeed, unopposed proteolysis is recognised as one of the major pathways to develop emphysematous changes in COPD lungs. The major human antiprotease involved is the multifunctional protein alpha-1-antitrypsin (AAT). We have assessed some potential therapeutic effects of this major human antiprotease on progenitor cells in a novel native in vitro model of COPD. We have employed dental pulp-derived mesenchymal stem cells (MSC). Cells were cultured on decellularized cadaver lung matrix in the presence of native AAT at physiologic concentrations. AAT internalization by the MSCs and their proliferation traits under AAT influence are presented. Native AAT significantly increased proliferation rate of the lung matrix-seeded cells in our native 3D model. We also present internalisation of AAT by MSC and its subcellular localization to endoplasmic reticulum. AAT is up taken by progenitor cells and positively modulates their proliferation on decellularized 3D lung tissue matrices in vitro. These results suggest the novel and important role of AAT in the maintenance and regulation of tissue regeneration processes in lungs in vivo.

W-3094

COMPARATIVE CHARACTERIZATION OF MESENCHYMAL STEM CELLS FROM TISSUES OF HUMAN UMBILICAL CORD: A SEARCH FOR RELIABLE CELL SOURCE FOR REGENERATIVE MEDICINE

Baghabaneslaminejad, Mohamadreza[1], Zare, Mohamadali[1], Hosseini, Ahmad[2]

[1]Department of Stem Cells and Developmental Biology at Cell Sciences Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Teheran, Iran, [2]Shahid Behesti University, Department of Anatomical Science, Teheran, Iran

The purpose of this study was to isolate and characterize MSCs from human umbilical cord blood (UCB), umbilical vein (UV) as well as Wharton's jelly (WJ) in the search for an appropriate source containing MSCs with high capacity of proliferation and good potential of osteogenic and chondrogenic differentiation for use in regenerative medicine. In this study, 35 samples of human umbilical cord from healthy full-term newborn were investigated. In the laboratory, the UCB was loaded onto Lymphodex solution, centrifuged and its mononuclear cells were collected. The UV was catheterized, its distal end was clamped and filled with a 1% collagenase solution. After incubation at 37°C for 20 min, released cells from the endothelial and subendothelial layers were collected. The WJ was minced into small pieces, subjected to the collagenase digestion for 20 min and the released cells were collected. The cells were propagated with three successive subcultures. Passaged-3 cells from each tissue were examined and confirmed according to the minimal criteria (including morphological and immunophenotypical characteristics as well as the differentiation potential into skeletal cell lineages) described for MSCs in literatures. Then the proliferation and differentiation cultures were established to compare the cells in terms of their colonogenic property, population doublings time (PDT), culture mineralization, alkaline phosphtase (ALP) activity and the expression level of bone- and cartilage-specific genes. From the 35 samples of each tissue source some, but not all, succeeded in establishing culture. The success rate of establishing culture for WJ samples was 80% while this rate tended to be 65.7% and 32% for UV and UCB samples respectively. Regarding the colonogenic activity, the number of the colonies, produced in low density culture of the cells, was roughly the same for all the cultures, but there was a significant difference in terms of dimensions of the colonies. The cells from WJ tended to produce relatively larger colonies (1600±70μm) compared with that of UV and UCB (1300±100μm and 950±120μm respectively, $p<0.05$) cells. Accordingly, PDT for the culture of WJ cells was significantly shorter (51±4 hours) than that for the culture of UV and UCB cells (60±7 and 65±5 hours, $p<0.05$). At the osteogenic cultures, the amount of mineral deposition for the WJ cells appeared to be 0.33±0.04 μM. This was in significant difference with that for UV (0.09±0.01) and UCB (0.14±0.03) cells. Furthermore, there was a significantly higher ALP activity at the culture of WJ than UV and UCB cells ($p<0.05$). These findings were in accordance with qPCR data for bone-specific gene expression. The relative level of Runx2 and osteocalcin expression was significantly higher at the culture of WJ compared with that of UV and UCB cells ($p<0.05$). Regarding the chondrogenic differentiation, the sections prepared from the differentiation culture of the WJ cells stained more intense than that of UV and UCB cells. Accordingly, there was a statistically higher expression level of coll-II, sox9, and aggrecan genes at WJ versus UV or UCB cell culture ($p<0.05$). Taken together, our findings demonstrated that MSCs from the tissue constituting human umbilical cord represent good alternative sources of adult MSCs. Among these tissues, Wharton's jelly seems to be much promising as it contains MSCs with high capacity of proliferation and good potential of bone and cartilage differentiation.

W-3095

DISTINCT TEMPORAL PATTERNS FOR 'STABLE' AND 'TRANSIENT' 5HMC ACQUISITION DURING CHONDROGENIC DIFFERENTIATION

Bhutani, Nidhi[1], Taylor, Sarah[1], Smeriglio, Piera[1], Li, Henry[2], Rath, Madhusikta[1], Wong, Wing H.[2]

[1]Orthopedic Surgery, Stanford University, Stanford, CA, USA, [2]Statistics, Stanford University, Stanford, CA, USA

Endochondral ossification during cartilage and bone development involves the stepwise differentiation of mesenchymal stem and progenitor cells towards a chondrogenic fate followed by hypertrophy and eventual formation of bone. Epigenetic regulation of chondrogenic differentiation especially the role of DNA methylation and demethylation is little understood. Such an understanding will aid in enhancing cartilage regeneration and tissue engineering, an unmet clinical need. Recent studies have highlighted the critical role of the ten-eleven-translocation (TET) proteins in regulating stem/progenitor cell differentiation. The TET proteins can oxidize methylated cytosines, 5mC, to their modified derivatives, 5hmC, 5fC and 5caC eventually leading to DNA demethylation. 5hmC is present stably in DNA and functions as an 'epigenetic' mark as well, however the delineation of its role as a 'stable' or 'transient' entity is not well understood. Here, we report that chondrogenic differentiation *in vivo* and *in vitro* is accompanied by global changes in 5hmC. Interestingly, we observe distinct temporal patterns of 5hmC acquisition leading to stable 5hmC patterns; and 5caC acquisition and loss leading to subsequent DNA demethylation during chondrogenic differentiation. While 5caC is acquired and lost early during chondrogenic differentiation, 5hmC is stably maintained showing a clear temporal decoupling of 'transient' 5hmC acting as a DNA demethylation intermediate and 'stable' 5hmC. Global analysis of the 5hmC- and 5mC-enriched sequences showed that the stable 5hmC acquisition rather than the early loss of methylation is associated with regulatory factors essential to cartilage development and chondrogenic differentiation. Therefore, the critical function of 5hmC during chondrogenic differentiation is as a stable mark rather than as an intermediate to DNA demethylation. In addition, our studies provide the first comprehensive analysis of DNA demethylation and hydroxymethylation dynamics during chondrogenic differentiation of mesenchymal progenitor cells.

W-3096

S-NITROSYLATION REGULATES THE BALANCE OF ADIPOGENESIS AND OSTEOGENESIS IN MESENCHYMAL STEM CELLS

Cao, Yenong, Gomes, Samirah A., Rangel, Erika B., Paulino, Ellena, Balkan, Wayne, Hare, Joshua M.

University of Miami, Miller School of Medicine, Miami, FL, USA

Osteoblasts and adipocytes arise from the same progenitor, multipotent bone marrow-derived mesenchymal stem cell (BMMSCs). This balance is maintained in large part by the activity of peroxisome proliferator- activated receptor γ (PPARγ), a member of the steroid-thyroid nuclear receptor family of transcription factors and nitric oxide (NO), an important regulator of skeletal homeostasis. Disruption of the differentiation balance between adipocyte and osteoblast lineages underlies numerous bone and metabolic diseases. We hypothesized that S-nitrosylation mediated-NO signaling controls the activity of PPARγ and therefore the balance between adipogenic and osteogenic differentiation of BMMSCs. Body weight and bone mineral density were measured in wild type mice (WT) and mice deficient in S-nitrosoglutathione reductase ($GSNOR^{-/-}$), a denitrosylase that regulates the level of protein S-nitrosylation. BMMSCs were isolated

from WT and GSNOR$^{-/-}$ mice and were grown in either adipogenic or osteogenic differentiation media followed by functional and gene expression assays. S-nitrosylation was tested by SNO-RAC assay and transcriptional activity was mearsured by luciferase reporter and chromatin immunoprecipitation (ChIP) assays. GSNOR$^{-/-}$ mice had decreased body weight (22.53±0.45g in WT vs. 18.62±0.51g in GSNOR$^{-/-}$ mice, P<0.001) and bone mineral density (46.18±0.66 mg/cm^2 in WT vs. 42.08± 0.61 mg/cm^2 in GSNOR$^{-/-}$ mice, P<0.05) compared to WT cells. BMMSCs derived from GSNOR$^{-/-}$ mice had reduced adipogenesis with decreased fat droplet formation and expression of the adipogenic markers *PPARγ* (1329±415.3-fold increase in WT vs. 158±65.61-fold in GSNOR$^{-/-}$, P<0.05) and *FABP4* (11.06±3.29-fold in WT vs. 4.06±0.62-fold in GSNOR$^{-/-}$, P<0.05). Conversely, GSNOR$^{-/-}$ BMMSCs exhibited enhanced osteogenic differentiation as indicated by greater calcium deposition and higher expression of the osteogenic marker *Osteopontin* (1.48±0.17-fold in WT vs. 16.18±5.26-fold in GSNOR$^{-/-}$, P<0.05). GSNOR$^{-/-}$ cells had higher baseline expression of the osteogenic markers *Osteopontin, Osteocalcin* and *Runx2*. Treatment of WT BMMSCs with a GSNOR inhibitor increased osteogenic differentiation and decreased gene expression of adipogenic markers without affecting fat droplet formation in WT cells. The abundance of S-nitrosylated PPARγ was 1.4-fold greater at baseline in GSNOR$^{-/-}$ cells (P<0.05) which led to a lower binding affinity for its downstream target FABP4. PPARγ binding to FABP4 was also reduced in WT BMMSCs treated with the NO donor GSNO (8.2-fold, P<0.05). Similarly, 293T cells when treated with GSNO exhibited decreased PPARγ transcriptional activity (1.3-fold, P<0.05). Denitrosylation via GSNOR alters the transcriptional and post-translational activity of PPARγ and the balance between adipogenic and osteogenic differentiation of BMMSCs. GSNOR-mediated S-nitrosylation is a pivotal, yet previously unrecognized, regulator of skeletal homeostasis, which may facilitate the development of new therapeutic approaches against bone and metabolic diseases.

W-3097
EFFECT OF DONOR AGE ON THE PROLIFERATION AND MULTIPOTENCY OF CANINE ADIPOSE DERIVED MESENCHYMAL STEM CELLS

Cha, Sang-Ho, Lee, Keum Sil, Lee, So Hyun, Song, Jae-young, Kim, Chan-Lan
Animal and Plant Quarantine Agency, Anyang, Republic of Korea

Adipose-derived mesenchymal stem cells (AD-MSCs) have demonstrated the feasibility of the usage in clinical application, due to ease of isolation and abundance in the tissue without age barrier. In spite of the broad interest, little is known about the variability of characteristics of canine MSCs in relation to the age of the donor. In this study we isolated MSCs from adipose tissues of young (7 months, n=3) and old (10-11years, n=3) dogs, which were subjected to sequential passages (P1 to P7). The MSCs were examined for proliferation kinetics, and expression of molecules associated with self-renewal, expression of MSCs-specific surface markers and differentiation capabilities at passage 3. In results, the proliferative activity was significantly higher in MSCs of young donors than in those of old donors. In addition, expression of molecules (Oct3/4, Nanog) associated with self-renewal in young donors was significantly higher (P<0.05) when compared with that of old donors. However, no difference in the expression of MSC-specific surface markers between them was observed. For differentiation into mesodermal cell lineages (adipocyte, osteocyte, and chondrocyte), the capacity of osteogenic and chondrogenic differentiation was considerably decreased (P<0.05) in old donors than in young donors, while adipogenic differentiation was not. In conclusion, this study suggests that donor age would affect multipotent characteristics of canine MSCs derived from adipose tissue, which should be considered in clinical application using the MSCs.

W-3098
DUAL FUNCTION OF MIRNA199A HIF1A TWIST1 CYCLIC PATHWAY IN STAGE SPECIFIC OSTEOGENESIS OF MESENCHYMAL STEM CELLS

Chen, Xiao[1], Gu, Shen[2], Ouyang, Hong Wei[3], Lee, Tin Lap[2], Chan, Wai Yee[2]
[1]School of Medicine, Zhejiang University, Hang Zhou, China, [2]The Chinese University of Hong Kong, Hong Kong, [3]Zhejiang University, Hang Zhou,Zhe Jiang, China

Introduction: The emergence of stem cell research started a new era in clinical medicine - the era of regenerative medicine. Elucidating the molecular mechanisms that regulate osteogenesis of human mesenchymal stem cell (hMSC) is important for the development of cell therapies for bone regeneration. MSC differentiation processes involve complex pathways that are regulated at both transcriptional and posttranscriptional levels. However, the key regulator(s) of MSC differentiation has not been identified. MicroRNAs (miRNAs) are small non-coding RNAs that bind to target mRNA leading to translational arrest or mRNA degradation. It is known that miRNAs are involved in the regulation of a number of biological processes, including stem cell differentiation. Here, we show that hsa-miR-199a-5p (miR-138) modulates osteogenic differentiation of hMSCs. Methods: Hsa-miR-199a expression were validated by quantitative RT-PCR during MSC osteogenesis. The function of hsa-miR-199a-5p in osteogenesis of MSC were evaluated by upregulating and inhibition of hsa-miR-199a-5p. We investigated the HIF1a-Twist1-miR-199a cyclic pathway activty during differentiation of human MSCs to findout the mechanism of hsa-miR-199a-5p on osteogenesis. Results: Hsa-miR-199a expression were validated by quantitative RT-PCR. revealed that miR-199a was up-regulated during osteogenesis of hMSCs. Overexpression of miR-199a-5p not 3p enhance osteoblast differentiation of hMSCs in vitro, whereas inhibition of miR-199a-5p function by antimiR-199a-5p reduce osteoblast-specific genes, alkaline phosphatase (ALP) activity, and matrix mineralization. Furthermore, overexpression of miR-199a ehnace ectopic bone formation in vivo. Target prediction analysis and experimental validation by WB confirmed HIF1a-Twist1 pathway play a key role in promoting osteoblast differentiation, as a target of miR-199a-5p. We show that HIF1a-Twist1 pathway have dual function on osteogenesis both in vitro and in vivo at least in part through miR-199a-5p. at early stage , HIF1a-Twist1 pathway activity enhance osteogenesis by upregulating miR-199a-5p while miR-199a-5p enhance osteogensis maturation by inhibiting HIF1a-Twist1 pathway. Conclusion: In conclusion, Our findings for the first time demonstrated that HIFα-Twist1-miR-199a cyclic pathway could regulate MSC osteogenesis at different differentiation stage, which could represent a therapeutic strategy for enhancing bone formation in vivo.

W-3099
TOWARDS AN EFFECTIVE STEM CELL-BASED THERAPY FOR GLIOBLASTOMA: APPLICATION OF DEDIFFERENTIATION-REPROGRAMMED MSCS

Chen, Rui, Zhang, Xiaohu, Zhang, Jieting, Tsang, Lailing, Chan, Hsiaochang, Jiang, Xiaohua
School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong

Glioblastoma is the most aggressive malignant primary brain tumor. Although lots of progress has been made in traditional therapies such

as surgery, radiotherapy and chemotherapy, the outcome of patients is still dismal. Mesenchymal stem cells (MSCs) have inherent tumor targeting ability, which makes them a promising gene delivery vehicle for improving the efficacy and minimizing the toxicity of gene therapy in glioma treatment. However, for clinical applications, sufficient mobilization of engineered MSCs to tumors is critical. Therefore, the development of methods to improve the migratory capacity of MSCs to tumors, which thereby increase the delivery of therapeutic genes is in burning need. Interestingly, our previous study has demonstrated that neuronal-differentiated-MSCs could be reverted back to MSCs morphologically under appropriate condition. These dedifferentiated MSCs (DeMSCs) present a variety of distinguishing genetic and phenotypic characteristics distinct from their original counterparts. After dedifferentiation, DeMSCs acquired enhanced anti-apoptosis ability, higher neuronal differentiation potential and improved therapeutic efficiency in ischemic brain damage model. Recently, we have found beside increased survival and neuronal differentiation ability, DeMSCs show enhanced migration ability. Through wound healing experiment, DeMSCs migrate faster than MSCs. In addition, transwell assay indicates more DeMSCs can migrate toward a set of tumor cells, including gliomablatoma cell line U87. Furthermore, our PCR array has demonstrated many chemokine and cytokine are up-regulated in DeMSCs. Among them, chemokine (C-C motif) ligand 5 (ccl5) is the most significantly increased chemokine. Besides, the very important inflammatory factor TNFα is also up-regulated. Subsequently, we have found that TNFα increases ccl5 expression in MSCs. Recombinant CCL5 increases migration ability in MSCs. In addition, CCL5 induces ERK phosphorylation through CC chemokine receptor 1 (CCR1) but not CCR5. Inhibition of ERK phosphorylation significantly reversed ccl5-stimulated migration in MSCs. Therefore, we conclude that TNF-α/CCL5/CCR1/MAPK pathway is involved in enhanced migratory ability in DeMSCs . Taken together, after dedifferentiation, MSCs acquired enhanced migration ability. Use DeMSCs to deliver therapeutic gene to brain tumors will represent a major step forward in the treatment of patients with the deadly disease.

W-3100
THE IDENTIFICATION AND HIERARCHY OF BM-DERIVED ARTERY-RESIDENT MESODERMAL PROGENITOR CELLS AND THEIR KINETICS IN ATHEROSCLEROSIS

Cho, Hyun-Ju[1], Cho, Hyun-Jai[2], Kim, Ju-Young[3], Hur, Jin[3], Kwon, Yoo-Wook[3], Yang, Han-Mo[4], Koo, Bon-Kwon[4], Oh, Byung-Hee[4], Park, Young-Bae[2], Kim, Hyo-Soo[5]

[1]Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, and College of Medicine or College of Pharmacy, Seoul National University(SNU), Seoul, Republic of Korea, [2]Cardiovascular Center, Department of Internal Medicine, Innovative Research Institute for Cell Therapy (IRICT), Seoul National University Hospital(SNUH), Seoul, Republic of Korea, [3]Innovative Research Institute for Cell Therapy (IRICT), Seoul National University Hospital(SNUH), Seoul, Republic of Korea, [4]Cardiovascular Center, Department of Internal Medicine, Seoul National University Hospital(SNUH), Seoul, Republic of Korea, [5]Department of Internal Medicine, Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, and College of Medicine or College of Pharmacy, SNU, Cardiovascular Center, IRICT, SNUH, Seoul, Republic of Korea

Background: We recently identified two types of bone marrow (BM)-derived vessel resident vascular calcifying progenitor cells. Sca-1+/PDGFRα- cells possess bi-directional (osteoblastic/osteoclastic) differential potentials whereas Sca-1+/PDGFRα+ cells possess uni-directional (only osteoblastic) potential. However, the nature of bi-directional progenitor cells is under debate. Therefore, we investigated cellular characteristics and developmental hierarchy of Sca-1+/PDGFRα- and Sca-1+/PDGFRα+ cells in BM and artery, and compared their mobilization and infiltration in atherosclerosis. Methods and Results: We harvested mononuclear cells from BM and artery of C57 mice. We first excluded mature hematopoietic lineage cells and included mesenchymal lineage cells using lineage antibody cocktail and CD29. We divided Lin-/CD29+ cells into two groups thereafter. In BM, Lin-/CD29+/Sca-1+/PDGFRα- cells showed hematopoietic colony forming potential and differentiated into osteoclasts (OC). They also possessed mesenchymal stem cell property including osteoblastic (OB) differentiation. Sca-1+/PDGFRα+ cells only differentiated into OB. These results suggest that Sca-1+/PDGFRα- cells may possess the characteristics of mesodermal progenitor cells. In the artery, to exclude heterogeneity of BM-derived artery-resident cells, we established single cell-derived and clonally-expanded cells. Sca-1+/PDGFRα+ cells only showed OB differentiation. Sca-1+/PDGFRα- cells maintained bi-directional potentials but lost hematopoietic characteristics. Intriguingly, during OB differentiation, Sca-1+/PDGFRα- cells shifted into Sca-1+/PDGFRα+ cells. Therefore, we examined the functional significance of PDGFRα expression during differentiation. When we overexpressed or knocked down PDGFRα, there was no alteration in OB or OC differentiation of Sca-1+/PDGFRα- cells and no effect on OB differentiation of Sca-1+/PDGFRα+ cells, indicating that PDGFRα is a surface marker but not a functional player. To examine *in vivo* kinetics of these cells, we used hyperlipidemic ApoE-KO mice. In the atherosclerotic group compared with control group, Sca-1+/PDGFRα- cells were less mobilized from BM to peripheral circulation (13±1.5% *versus* 21±2.6%, P=0.007) and less infiltrated into the vasculature (61.7±8.2% *versus* 76.5±4.4%, P<0.001), whereas Sca-1+/PDGFRα+ cells were not significantly affected. Multiplex bead assay of serum and artery revealed that IL-1β, IL-10 and IL-12 were significantly increased (P<0.05) and IL-5 was markedly decreased (P<0.01) in atherosclerotic group. IL-1β decreased the migration of Sca-1+/PDGFRα- cells by 5 folds compared with positive control (TNFα), and IL-5 increased the migration as much as TNFα. But the migration of Sca-1+/PDGFRα+ cells was not altered by these cytokines. These data suggested that atherosclerosis-related humoral factors mainly regulated vascular protective and bi-directional mesodermal progenitor cells' kinetics. Conclusions: We demonstrate that Sca-1+/PDGFRα- cell is a mesodermal progenitor cell that possesses both the hematopoietic potential including OC differentiation and the mesenchymal potential including OB differentiation. In atherosclerosis, the mobilization and infiltration of Sca-1+/PDGFRα- progenitor cells were regulated by IL-1β and IL-5. These data provide a pivotal mechanism regarding the role of vascular protective bi-directional progenitor cells in atherogenesis.

W-3101
EFFECTS OF RHOA/ROCK INHIBITORS ON CHONDROGENESIS OF HUMAN PERIODONTAL LIGAMENTDERIVED MESENCHYMAL STEM CELLS

Cho, JaeJin

School of Dentistry, Seoul National University, Seoul, Republic of Korea

We previously demonstrated that periodontal ligament-derived mesenchymal stem cells (PDLSCs) can be induced to undergo chondrogenesis by TGF-β3. Additionally, several researches reported that the control of cytoskeleton activity via RhoA-ROCK may promote the chondrogenic differentiation of stem cells. Therefore, we determined whether the inhibition of RhoA-ROCK pathway by ROCK inhibitor Y-27632 and actin polymerization inhibitor cytochalasin D can promote chondrogenesis in PDLSCs in the

presence or absence of TGF-β3. PDLSCs were isolated andpurified from the periodontal ligament of the human third molar teeth. After initiating chondrogenesis with 3D cell cluster formation, the clusters were maintained under 10 ng/mL TGF-β3, 3 μM cytochalasin D, 10 μM Y-27632, TGF-β3+cytochalasin D, TGF-β3+Y-27632 and without treatment as negative control. We analyzed the chondro-clusters by glycosaminoglycan assay (GAG), histology evaluation, safranin O and von Kossa staining and PCR. Immunohistochemistry were performed to measeure the expression for Collagen I, II as well as aggrecan. Clusters treated with TGF-β3 and Y-27632 had the highest level of GAG synthesis. Unlike cytochalasin D, Y-27632 was found to exert a synergistic effect as well. Combination treatment of TGF-β3 and Y-27632 increased the expression of the chodro-related genes, collagen type II and sox9 while decreasing expression of the osteogenic gene, Runx2. On the other hand, treatment with TGF-β3 and cytochalasin D let do the greatest expression of Runx2 and collagen X, a hypertrophic chondrocyte marker gene. Calcium deposits were visualized by von Kossa staining. The least amount of calcium deposition was found in clusters exposed to TGF-β3 with Y-27632. In conclusion, our data suggest that the treatment of TGF-β3 along with RhoA/ROCK inhibition using Y-27632 induced more selective chondrogenesis. Furthermore, PDLSCs and control of the RhoA/ROCK pathway have the potential to be for cartilage tissue repair including the temporomandibular joint disc regeneration.

W-3103
THERAPEUTIC EFFECTS OF ADULT HUMAN BRAIN-DERIVED PERIVASCULAR MESENCHYMAL STEM CELLS IN STROKE-DAMAGED RATS

Choi, Chunggab[1], Özen, Ilknur[2], Oh, Seung-Hun[3], Paul, Gesine[2], Chang, Da-Jeong[1], Lee, Nayeon[1], Jeon, Iksoo[1], Kim, Hyun Sook[3], Roh, Jeong-Eun[1], Lee, Hyunseung[4], Hong, Kwan Soo[5], Song, Jihwan[6]
[1]CHA Stem Cell Institute, CHA University, Seoul, Republic of Korea, [2]Translational Neurology Group, Department of Clinical Sciences, Lund University, Lund, Sweden, [3]Department of Neurology, CHA Bundang Medical Center, CHA University, Gyeonggi-do, Republic of Korea, [4]Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Republic of Korea, [5]Division of Magnetic Resonance, Korea Basic Science Institute, Gyeonggi-do, Republic of Korea, [6]CHA University, Seoul, Republic of Korea

We have recently isolated and characterized perivascular mesenchymal stem cells (PV-MSCs) derived from adult human brain. In this study, we investigated the long-term therapeutic effects of PV-MSCs following transplantation into a rodent model of middle cerebral artery occlusion (MCAo). PV-MSCs were injected into the contra-lateral side of striatum at 7 days after MCAo, and the transplanted animals were examined up to 8 weeks using 4.7T animal MRI and various behavioral tests, and immunohistochemical analysis was performed afterwards. First of all, transplanted animals exhibited significant behavioral improvements in rotarod, stepping and modified neurological severity score (mNSS) tests, and to a lesser extent, improvement was also observed in apomorphine-induced rotation and staircase tests. Interestingly, animal MRI results indicate that a high proportion of contra-laterally transplanted PV-MSCs were migrated to the infarct region, exhibiting a patho-tropism. We also observed that the transplanted human cells were co-localized with Nestin, GFAP, vWF, BDNF and CXCR4, implying that transplanted PV-MSCs may be involved in the formation of perivascular network or vessels, the production of neurotrophic factors, and the cell migration, although they are not directly involved in neural regeneration. We also found that transplanted PV-MSCs can contribute to the reduction of glial scar formation and apoptosis, while they can lead to the increase of microvessel density in the damaged area. They were also shown to play an important role in the modulation of inflammatory response. Although no overall changes of inflammatory response were observed, more detailed analyses revealed that while M1 markers (pro-inflammatory) were reduced, M2 markers (anti-inflammatory) were increased significantly by transplanted PV-MSCs. We also observed that the number of proliferating neuroblasts significantly increases in the subventricular zone (SVZ), implying that transplanted PV-MSCs also promotes some aspects of neurogenesis. Histologically, transplanted animals showed that the corpus callosum density was restored, indicating the improvement of inter-hemispheric neuronal network, although no significant change of infarct size was observed following transplantation. Taken together, these results provide strong evidence that PV-MSCs are functional in vivo and may serve as useful tools to treat various brain diseases, including stroke. This study was supported by a grant of the Korea Healthcare technology R and D project, Ministry for Health, Welfare and Family Affairs (A111016). We are grateful to the MRI facility in the Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Korea.

W-3104
ENRICHMENT OF MESENCHYMAL STROMAL CELLS WITH CLONAL MULTIPOTENT CAPACITY FROM HUMAN SKELETAL MUSCLE

Downey, Jennifer[1], Lauzier, Dominique[2], Richter, Martin[3], Hamdy, Reggie[2], Scime, Anthony[4], Balg, Frédéric[5], Grenier, Guillaume[1]
[1]Department of Surgery, Orthopedic Service, University of Sherbrooke, Sherbrooke, QC, Canada, [2]Shriners Hospital for Children, Montreal, QC, Canada, [3]Department of Pediatrics, University of Sherbrooke, Sherbrooke, QC, Canada, [4]York University, Toronto, ON, Canada, [5]Department of Orthopedic Surgery, University of Sherbrooke, Sherbrooke, QC, Canada

Mesenchymal stromal cells (MSCs) are classically defined by the ability to differentiate into multiple lineages and with cell surface markers that include CD73, CD105 and CD90. Although mouse studies suggest their presence in every adult tissue, currently classical human MSCs have been isolated mainly from bone marrow and adipose tissue. We show for the first time the enrichment of a human muscle resident MSC (hmrMSC) population. Freshly digested human muscle derived cells were grown as adherent cells in culture and further enriched using fluorescent activated cell sorting (FACS). hmrMSCs represented by the $CD73^{+}CD105^{+}CD90^{-}$ subpopulation, displayed robust trilineage potential under appropriate differentiation conditions. Clonal differentiation assays confirmed that all three lineages stem from a single multipotent progenitor within this population. Furthermore, they were able to differentiate into brown adipocyte-like cells, expressing UCP1 at the RNA and protein levels. Interestingly the full multipotent capacity of hmrMSCs could be denoted within heterotopic ossification within skeletal muscle. Hence, the identification of hmrMSCs within the adult human skeletal muscle will enable a better understanding of non-myogenic stem cell contribution to skeletal muscle regeneration as well as in different myopathies where aberrant tissue formation is present. Finally, considering the relatively important compartment that skeletal muscle represents, this newly identified MSC population may hold great promise for future clinical applications.

W-3105

HYPOXIA INCREASES PROLIFERATION AND OSTEOGENIC DIFFERENTIATION POTENTIAL OF MUSCLE RESIDENT STROMAL CELLS FOLLOWING MUSCLE TRAUMA

Drouin, Geneviève[1], Couture, Vanessa[1], Daviau, Alex[2], Faucheux, Nathalie[3], Grenier, Guillaume[1]

[1]Department of Orthopedic Surgery, Université de Sherbrooke, Sherbrooke, QC, Canada, [2]Department of Chemical Engineering, Université de Sherbrooke, Sherbrooke, QC, Canada, [3]Department of Chemical Engineering, Université de Sherbrooke, Sherbrooke, QC, Canada

Muscle resident stromal cells (mrSCs) are multipotent cells known to contribute to several muscle pathologies such as heterotopic ossification (HO). HO is a debilitating condition characterized by bone formation in soft tissue. It can be due to a genetic disease, or more often, as the result of a severe traumatic injury or surgery. Following an acute muscle damage, mrSCs can differentiate into chondrocytes and osteoblasts and form aberrant endochondral bone in the muscle. The mechanism(s) triggering the chondrogenic and osteogenic differentiations of mrSCs in vivo remains to be identified. We hypothesized that cells within severely damaged muscle are exposed to hypoxia due to the blood vessels breakdown and affects the activation, proliferation and differentiation potential of mrSCs. The hypoxic state of damaged muscle was shown using Tie2LacZ mice. In CTX-damaged muscle, blood vessels were disrupted, consistent with the upregulation of hypoxia marker HIF-1α 3.5 days after the injury. The effect of hypoxia on mrSCs activation and proliferation was evaluated by colony forming and 3H-thymidine incorporation assays. In hypoxic condition (0.7% O2) mrSCs formed more colonies (2.9-fold; $p<0.0001$) than in normoxic condition (21% O2). Moreover, the average colony area was 6.1 fold greater in hypoxia than normoxia ($p<0.0001$). The increase of mrSCs proliferation in hypoxia was confirmed by a significantly higher incorporation of 3H-thymidine after 24 and 72 hours (1.6-fold and 2.4-fold, respectively) in comparison to normoxia. The effect of hypoxia on the adipogenic, chondrogenic and osteogenic differentiation potential of mrSCs was also determined in vitro. Results indicated that hypoxia promoted chondrogenic and osteogenic differentiations to the detriment of adipogenic differentiation. As some BMPs are known to be strongly osteogenic and chondrogenic growth factors, the expression of BMP2, 7 and 9 by mrSCs under hypoxia was evaluated and compared to normoxic condition. Real time PCR analyses showed only a significantly increase in BMP9 gene expression after 24 hours exposure of mrSCs to hypoxia (3.6-fold; $p<0.0001$). The increase of BMP9 protein expression was also confirmed by Western blot. Finally, the effect of BMP9 on mrSCs differentiation was assessed. BMP9 had no effect on adipogenic differentiation, while it induced a strong chondrogenic and osteogenic differentiation of mrSCs. Together, our results suggest that oxygen levels influence mrSC behavior; cells that are cultured under hypoxic conditions showed increased capacity to proliferate and to develop cartilage and bone. This effect may be mediated by the expression of BMP9 by mrSCs under hypoxia but the underlying mechanisms still need to be studied. Recently, we have shown the presence of BMP9 in osteoblasts of a patient with early HO formation. This is the first study to demonstrate the implication of BMP9 in HO and support our results that hypoxia could be a key factor involved in the process of HO via the expression of BMP9 by mrSCs.

W-3106

OSTEOGENIC DIFFERENTIATION OF HIGHLY MINERALIZING HUMAN BONE MARROW-DERIVED MSC IS MODULATED BY ALP, IGFBP5, AND LRP3 THROUGH TGFB SIGNALING PATHWAY

Elsafadi, Mona A.[1], Muthurangan, Manikandan[2], Aldahmash, Abdullah M.[2], Mahmood, Amer[2]

[1]University of Southern Denmark, Odense, Denmark, [2]King Saud University, Riyadh, Saudi Arabia

Although mesenchymal stromal cells (MSCs) have demonstrated great therapeutic potential, the heterogeneity of MSC may cause the incongruent data of MSC-based preclinical and clinical trials. Previously we have shown that two human clonal hBMS-TERT lines were isolated and characterized. One of the clones exhibited higher proliferation and osteogenic differentiation potential with increased ALP activity. Gene expression microarray clarified the molecular basis of this heterogeneity by showing high up-regulation of several skeletal muscle, mineralization and bone-related genes in highly mineralizing cells proving the pronouncement of osteoblastic phenotype of these cells. Using RNA interference technique we studied the role of three highly up-regulated genes in high mineralizing cells including: alkaline phosphatase (ALP), insulin-like growth factor binding protein 5 (IGFBP5), and low-density lipoprotein receptor-related protein-3 (LRP3). The metalloenzyme ALP is known as an early marker of osteogenesis and its expression is regulated by BMP/RUNX2/Osterix system, and WNT signaling cascade. IGFBP5 is a member of IGFBP that modulate the multiple activities of IGF-I and II. It has a regulatory action in cell fate determination. IGFBP-5 in osteoblasts regulates bone formation in vivo and in vitro. LRP3 is a member of a highly conserved gene family, LDL receptor (LDLR) family. However LRP-5 regulates osteoblast proliferation and bone formation, the function of LRP3 especially in osteogenic regulation (if any) has not yet been explored. *ALP*, *IGFBP5* and *LRP3* siRNA transfected cells showed strong down-regulation of gene expressions of each corresponding gene and significant blockage of ALP activity. In addition, osteogenic differentiation was significantly inhibited proven by loss of mineralization and down-regulation of osteogenic markers after 7 days of osteogenic induction. By employing gene expression microarray technology on the three siRNAs transfected cells, Venn diagram of microarray analysis showed that many of the 110 common down-regulated elements of all transfected cells were involved in TGFB signaling pathway and were key osteogenic markers including *ALP, RUNX2, BMP4, NOTCH1, MAPK3, FOXO4.* By using functional protein association networks, string network identified TPM1, SERPINB2, ACTA2, ALP, and IGFBP5 as common elements involved in TGFB pathway. RT-PCR showed significant down-regulation of *TPM1*, *SERPINB2*, *ACTA2*, and *ALP* in both *IGFBP5* and *LRP3* knocked-down cells. Interestingly, knocking down *LRP3* down-regulated *IGFBP5* and vise versa. Treating cells with TGFB1 significantly induced *TPM1, ACTA2*, and *ALP* and reduced *SERPINB2* and *IGFBP5* gene expression levels, while blocking TGFB pathway by SB exhibited significant opposite effect. Thus, *TPM1, SERPINB2* and *ACTA2* are common down-stream gene involved in *IGFBP5*, and *LRP3* pathways which all hence are also modulated by TGFB pathway.In conclusion, in our high bone forming hBMSC, in vitro mineralization is proposed to be regulated by *IGFBP5, ALP* and *LRP3*, which are directly or indirectly involved in TGFB pathway. LRP3 is a suggested novel putative key player in osteogenesis, and its mechanism of action is related to *ALP* and *IGFBP5* that are involved in TGFB signaling pathway, however this has to be further investigated in details. This study provides an insight into the molecular basis of osteogenesis in vitro, for better understanding of pathological conditions related to

bone formation.

W-3107
EXTRACELLUAR MATRIX STIFFNESS BUT NOT TETHERING REGULATE DIFFERENTIATION

Engler, Adam
University of California San Diego, La Jolla, CA, USA

The extracellular matrix (ECM) is a 3D assembled protein scaffold which creates the niche in which stem cells engraft. ECM presents several important intrinsic signals to stem cells, e.g. stiffness and ligand type, which they integrating together along with soluble niche signals to regulate their fate. Recently, the degree of fibrous protein coupling to the surface of an underlying substrate, i.e., tethering and matrix porosity, has been proposed as a component of the niche that can mechanically regulate stem cell differentiation. To assess its contribution to the differentiation of a model adult stem cell, i.e. adipose-derived stromal cell (ASC), we modulated substrate stiffness, porosity, and ligand tethering to investigate how these factors collectively regulate cell fate. Varying substrate porosity did not significantly change protein tethering, substrate deformations, or osteogenic differentiation of sub-confluent human ASCs. Adjusting protein-substrate linker density did not affect osteogenesis or substrate deformations as cells deformed their tethers to a similar degree regardless of changes in how the protein was tethered to the substrate. To eliminate protein deformations, a short cell-adhesive peptide was incorporated into the substrate; ASC differentiation was unaffected by the absence of protein tethering or peptide density as only softer substrates prevented sub-confluent ASC osteogenesis. These data imply that cell-generated deformations of planar matrices of a specific stiffness regulate stem cell differentiation independently of protein tethering and porosity.

W-3108
EFFECT OF PURMORPHAMINE ON OSTEOGENIC DIFFERENTIATION OF HUMAN BONE MARROW- DERIVED MESENCHYMAL STEM CELLS IN A THREE-DIMENSIONAL DYNAMIC CULTURE SYSTEM

Faghihi, Faezeh[1], Papadimitripoulos, Adam[2], Martin, Ivan[2], Baghaban Eslaminejad, Mohamadreza[3], Mohammad Taghi, Joghataei[4], Jafar, Ai[5]
[1]Research Center for Cellular and Molecular Biology, Iran University of Medical Sciences, Tehran, Iran, [2]Departments of Surgery and of Research, University Hospital Basel, Basel, Switzerland, [3]Department of Stem Cells and Developmental Biology at Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, Tehran, Iran, [4]Research Center for Cellular and Molecular Biology, Iran University of Medical Sciences, Tehran, Iran, [5]Department of Tissue Engineering, Tehran University of Medical Sciences, Tehran, Iran

We have previously demonstrated that purmorphamine exerts an osteoinductive effect on human bone marrow mesenchymal stem cells (hBM-MSCs) in a two-dimensional (2D) static culture system. In the present study, we have evaluated the effect of purmorphamine on osteogenic differentiation of human mesenchymal stem cells (hMSCs) in a three-dimensional (3D) dynamic culture system versus a 2D static culture condition. In this study, hMSCs were seeded in either 3D collagen scaffolds using a perfusion bioreactor or on 2D culture plates. One day later, the medium was replaced with osteogenic medium supplemented with dexamethasone, L-ascorbic acid and β-glycerophosphate in the presence or absence of purmorphamine. Histological staining, flow cytometry, and real-time PCR were conducted for evaluation of osteogenesis in the study groups. The expressions of RUNX-2, alkaline phosphatase (ALP), osteocalcin (OC), collagen I (Col I), and bone sialoprotein (BSP) were compared between the 3D and 2D culture systems at 14 and 21 days post-induction of differentiation. Based on our results, alizarin red staining showed deposition of the mineralized matrix in the 2D static and 3D dynamic cultures. Our flow cytometric analysis indicated that the number of ALP+/OC- cells increased in the presence of purmorphamine in the 2D culture rather than the 3D system at day14. One week later, the numbers of OC+/ALP- and OC+/Stro-1- cells increased significantly in the 3D dynamic model compared with the 2D cultures. Expression of RUNX-2 was upregulated in the presence of purmorphamine in both cultures at day 14. The purmorphamine response gene, Gli-1, was upregulated during both the early and late culture periods in the 3D dynamic system, while similar up-regulation was observed only during the early culture period in 2D culture. No difference was observed in expressions of collagen type I and BSP between groups. In conclusion, the 3D dynamic culture model in the presence of purmorphamine might favor osteogenic differentiation of hMSCs by mimicking the physiological in vivo environment compared to a 2D static culture system.

W-3110
DOES MACROPHAGE DEPLETION AND CYTOKINE STIMULATION AFFECT THE PHENOTYPE OF SYNOVIAL MESENCHYMAL PROGENITOR CELLS IN VITRO?

Fichadiya, Akash, Frank, Cyril, Yates, Robin, Krawetz, Roman
University of Calgary, Calgary, AB, Canada

The aim of the present study was to elucidate the relationship between synovial mesenchymal progenitor cells (sMPCs) and macrophages of the knee joint in OA pathogenesis. Specifically, this study sought to assess how components of the macrophage secretome (pro- and anti-inflammatory activation and polarization factors) affect the chondrogenic capacity of sMPCs in vitro. In addition to this, the effects of macrophage depletion from OA and normal biopsied human synovium were also assessed. Patients with clinical and radiographic OA with no other co-morbidities consented and had synovial membrane biopsies obtained during knee arthroplasty or meniscal/ligamentous repair at the Peter Lougheed Centre, Canada. Synovial fluid and synovial membrane biopsies from macroscopically normal knees were obtained from cadavers less the 4hrs after death. Tissue donors were received by the Southern Alberta Organ and Tissue Donation Program. OA and normal biopsy samples were freshly plated and received 40ng/ml of IFN-γ, TNFα, Il-4, or IL-10, with or without Dichloromethylenediphosphonic acid disodium salt (Clodronate Disodium - Sigma) in solution every 4 days for 12 days post seeding (clodronate disodium is a first generation bisphosphonate utilized in research for the depletion of macrophages). Samples of the supernatant are also collected on days 4, 8, and 12 for proteomic assessment via Luminex. Following this treated 12 day outgrowth of sMPCs, cells are isolated, purified, expanded, and placed in 3 week chondrogenic differentiation (in pellet culture aggregates). qRT-PCR is conducted thereafter to assess gene expression levels of chondrogenic factors in addition to qualitative alcian blue staining. Our study revealed greater expression levels of Sox9, Col2a and Aggrecan on sMPCs which came from OA biopsy specimens that received the clodronate-only treatment in comparison to untreated biopsy specimens following chondrogenic differentiation. OA Biopsies which received anti-inflammatory cytokines (IL-10 and IL-4) during sMPC outgrowth also showed greater expression levels of Sox9, Col2a and Aggrecan following chondrogenic differentiation in comparison to those receiving pro-inflammatory cytokines (IFN-γ and TNFα). Additionally, OA biopsies receiving either pro-or anti-inflammatory cytokines alongside clodronate showed variable sMPC expression levels of chondrogenic factors following differentiation. The TNFα + Clodronate group showed the most reduced expression levels in

comparison to sMPCs from untreated biopsies. Normal biopsies which underwent the same treatment regimens demonstrated similar results in the way of reduced chondrogenic gene expression following pro-inflammatory exposure, and increased expression following clodronate treatment. Our findings shed light on macrophage effects on sMPC phenotype. Our results demonstrate that depletion of synovial macrophages increases the chondrogenic capacity of sMPCs and that pro-inflammatory cytokines which constitute an M1 macrophage secretome reduce chondrogenic capacity of sMPCs. Taken together, these findings raise interesting insight into the inflammatory influences on OA pathogenesis and cartilage regeneration.

W-3111
THE COMBINATION OF MESENCHYMAL STEM CELL PERIPHERAL BLOOD AND LOW GLYCEMIC INDEX DIET IN PATIENTS WITH TYPE II DIABETES

Gargiulo, Ciro
Regenerative Medicine, Humane International Medical Center, University of Fribourg, Switzerland, Ho Chi Minh, Vietnam

Autologous peripheral stem cell transplantation was first reported in 2007 to treat type 1 diabetes mellitus (DM) and achieved encouraging effect, but whether similar results can be achieved in type II DM is yet to be demonstrated. The main aim of this study is to determine the efficacy of autologous mesenchymal stem cells from peripheral blood (PB MSCs) in association with a low glycemic index (LGI) diet to treat patients diagnosed with DM type II. Peripheral blood mononucleated cells consist of haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). To date there is no well-defined isolation or characterisation protocol of stem cells from human adult peripheral blood mononucleated cells and their use in association with LGI diet for the treatment of diabetes type II. With no less impact, MSCs have been used as cell therapy to treat defects in bone and cartilage and to help in wound healing and they have been used in combination with biomaterials in tissue engineering procedures. Until now, the limitation in the number of totally characterized autologous MSCs available represents a major obstacle to their use for adult stem cell therapy. GI diet is a general term for weight-loss diets that are based on blood sugar level. The GI was originally developed to help improve blood sugar control in diabetes classifies carbohydrate-containing foods according to their potential to raise your blood sugar level. Present this study already includes 5 patients with type 2 DM (aged 48 to 96 years) during a period of 120 days in the Central Hospital of Ho Chi Ming city, Vietnam. Clinical variables (body mass index, duration of DM, oral hypoglycemic drugs, time free from oral drugs) and laboratory variables (hemoglobin A1c (HbA1c)), mononuclear cells infused are assessed. Purified PB MSCs are infused into major systemic vein (upper limbs or lower limbs). Follow-up is performed weekly after infusion. Preliminary results Mean HbA1c values showed a significant reduction during follow-up in all patients during the treatment. First patient, 96 years old, HbA1c decreased from 8.3 to 7.1; second patient male 54 years, HbA1c 8.9 to 7.1; third patient male 48 years HbA1c 8.6 to 6.8; fourth patient 54 years, HbA1c 9.6 to 5.2; fifth patient 53 years, HbA1c 10.2 to 5.4. Conclusions Combined therapy of intravenous PB MSCs and LGI diet treatment can improve glucose control and remove the dose of oral hypoglycemic drugs in type 2 DM patients. Further randomized controlled clinical trials, more follow up and involved more patients will be required to confirm these findings and the mechanism needs to be illustrated deeply.

W-3112
INHIBITION OF AKT/MTOR SUPPRESSES AGE-RELATED LOSS OF MESENCHYMAL STEM CELL SELF-RENEWAL AND FUNCTION.

Gharibi, Borzo, Ghuman, Mandeep, Hughes, Francis J.
Periodontology, Kings College London Dental Institute, London, United Kingdom

Decreased self renewal and differentiation potentials of mesenchymal stem cells (MSC) that occurs during aging, results in loss of their ability to maintain and repair tissues and may limit their potential for use in clinical applications . Therefore interventions to preserve the immature state of MSC and consequently maintain self-renewal and differentiation capacity during in vitro and in vivo aging may be of considerable significance. We have previously shown the involvement of AKT / mTOR signalling in fate decisions of MSC in vitro during long term culture. Therefore the aim of the study was to investigate the effect of inhibition of PI3K/Akt/mTOR signalling pathway on the maintenance of self-renewal and differentiation potentials of MSC during aging in culture. MSC aged in vitro in the presence of inhibitors of Akt (LY294002) and mTOR (rapamycin) maintained the morphology of cells in their early passages with low size and granularity. They demonstrated increased clonogenic frequency and enhanced proliferative capacity compared to untreated controls (4.1 and 6.6 fold increases respectively). Inhibiting Akt and mTOR also significantly reversed the age-dependent loss of osteogenic differentiation observed with long term culture, showing high expression of Runx-2, ALP and osteopontin and matrix mineralisation following osetogenic induction. Inhibition of mTOR was associated with increased expression of the stem-ness genes Nanog and Oct-4, and the reduction of accumulation of cytoplasmic reactive oxygen species (ROS) and ROS-associated DNA damage. Finally, inhibition of superoxide dismutase-1 (SOD-1), resulted in similar changes in MSCs to those seen following aging and these effects were reversed by mTOR inhibition by rapamycin. Taken together the results suggest that loss of Akt/mTOR may prevent MSC aging by regulating the expression of pluripotency genes Nanog and Oct-4, production of cytoplasmic ROS, and by reducing accumulation of DNA damage. The data enhance our understanding of the mechanisms involved in MSC aging and suggest possible interventions to maintain the functions of MSC prior to in vivo transplantation and for diseases associated with reduced stem cell capacity.

W-3113
ORGANIC AND INORGANIC ZINC HAVE DIFFERENT EFFECTS ON THE EXPRESSION OF GERM CELL SPECIFIC GENES IN RAM BONE MARROW DERIVED MESENCHYMAL STEM CELLS

Ghasemzadeh-Hassankolaei, Maryam[1], Baghaban Eslaminejad, Mohamadreza[2], Ghorbanian, Mohammad Taghi[3], Ghasemzadeh-Hasankolaei, Mohammad[4]
[1]School Of Biology, Damghan University, Damghan, Iran, [2]Stem Cells and Developmental Biology, Royan Institute, Tehran, Iran, [3]School of Biology, Damghan University, Damghan, Iran, [4]Fatemeh Zahra Infertility and Reproductive Health Research Center, Babol University of Medical Sciences, Babol, Iran

Zn is a well-known trace element that is essential for the activity of more than 300 metalloenzymes. It is necessary for normal spermatogenesis and has a critical role in testicular development. Zn mainly accumulates in germ cells (GC) but is not located in interstitial tissue or Sertoli cells and our previous study revealed that zinc sulfate can alter the expression of male GC-specific genes in bone marrow-derived mesenchymal stem cells (BM-MSCs). Thus, this study was fabricated

to determine the effects of different levels of zinc acetate (organic zinc) and zinc sulfate (inorganic zinc) on the expression of GC-specific genes in passaged-3 ram BM-MSCs. Studies like this can lead to better understanding of the role of zn in spermatogenesis procedure. For this goal, ram BM-MSCs were isolated from the rams bone marrow samples and then treated with six different concentrations ($0.1,1,10,10^2,10^3,10^4$ μM) of either organic or inorganic zinc supplements for a period of 7 and 14 days. MTT assay were employed to investigate the effects of different concentrations of zinc on cells viability. Moreover, changes in the expression of the GC genes were evaluated with real time reverse transcription polymerase chain reaction (real time RT-PCR). MTT results showed that 10^3 and 10^4 μM of both organic and inorganic zinc were cytotoxic, so these concentrations were eliminated from the study. Changes in the expression of molecular markers of primordial germ cells such as Oct4 and Vasa; as well as molecular markers of spermatogonia including Dazl, beta Integrin (ITG b1), Piwil2 and also meiotic marker, Acrosin were evaluated after one and two weeks treatment. The results demonstrated that two-week treatment with organic and inorganic zinc supplements caused downregulation in almost all genes, while, one week treatment, induced upregulation in some genes including ITGb1, Piwil2 and Vasa and downregulation of the others. Totally, from the results it could be concluded that inorganic zinc has more considerable effects than the organic zinc on the expression of GC-specific genes in ram BM-MSCs.

W-3114
AMELIORATION OF PANCREATIC ENDOCRINE FUNCTION: ENHANCING HOMING AND REPAIR OF MOUSE BONE MARROW MESENCHYMAL STEM CELLS BY USING BIOACTIVE COMPOUND IN STREPTOZOTOCIN INDUCED DIABETIC BALB/C MICE.

Gupta, Sarita Sharadchandra
Biochemistry, M.S University, Vadodara, India

For over a decade now researchers have been exploring the potential of Mesenchymal stem cells (MSCs) in preclinical and clinical studies to explore improved therapies that can resolve injuries by enhancing endogenous repair opening a new paradigm in stem cell therapy. MSCs having both regenerative and immunomodulatory properties have been of keen interest among the researchers to explore possibilities of a stem cell cure for type I diabetes mellitus. Bone marrow mesenchymal stem cells (BMSCs), being having wider clinical acceptability as their clinical harvesting protocols are already in place, serves as a wonderful source of pluripotent/multipotent stem cells, have the potential to undergo multiple lineages and can generate insulin producing cells. From our in-vitro study, bone marrow mesenchymal stem cells derived islet like cell clusters were found to be very efficient in diabetes reversal upon transplantation in streptozotocin treated diabetic balb/c mice. Hence, we created GFP labelled mouse BMSCs and performed in-vivo lineage tracing experiment, where we successfully demonstrated endogenous differentiation of GFP+ve mBMSC into newly generated islets in STZ induced mice pancreas. The transplantation in combination with bioactive compound enhanced the homing of mBMSCs to the damaged pancreas in turn boosting pancreatic regeneration. Thus, the potential of mBMSCs along with our bioactive compound can be effectively translated as therapeutic tool for the treatment of type 1 diabetes.

W-3115
FUNCTIONAL LINEAGE POTENTIAL WITHIN THE HAIR FOLLICLE MESENCHYMAL STEM CELL NICHE

Hagner, Andrew, Raharjo, Eko, Biernaskie, Jeff
Comparative Biology and Experimental Medicine, University of Calgary, Calgary, AB, Canada

Dermal stem cells within the hair follicle (HF) function to regulate morphogenesis and cyclic regeneration (Biernaskie et al. 2009). The HF mesenchyme is comprised of the dermal papilla (DP), the inductive signaling centre that orchestrates follicular regeneration, and the dermal sheath (DS) that is thought to play a structural role. The functional relationship between these compartments during HF regeneration remains unclear. Here we show that proliferation in the hair follicle dermis is largely restricted to the sheath, during a specific phase of the hair cycle. We have hypothesized DS cells may represent a renewable source of cells that replenish the inductive cells of the dermal papilla, as well as reconstituting the dermal sheath. DP and DS cells were isolated by FACS from adult mouse hair follicles and compared for: 1) capacity for self renewal in vitro, 2) expression of markers consistent with a signature previously described for dermal stem cells, and 3) their capacity to reconstitute one or both mesenchymal hair follicle compartments in vivo. Results show that prospectively isolated DS cells can be expanded in vitro over multiple passages, and proliferate to produce cells exclusively expressing markers of either the DS or DP. Both isolated DP and DS cells are capable of generating de novo hair upon transplantation, but contribution is biased toward their prior residence. Together, these results suggest that maintenance and regeneration of the mesenchymal compartments of the hair follicle are dynamic, niche-dependent and may be driven by a common dermal progenitor residing in the dermal sheath.

W-3116
ENHANCED STEMNESS AND HEPATOGENIC DIFFERENTIATION OF HUMAN ADIPOSE TISSUE MESENCHYMAL STEM CELLS BY GENE ENGINEERING WITH OCT4 AND SOX2

Han, Sei-Myoung[1], Coh, Ye-Rin[1], Ahn, Jin-ok[1], Kim, So-Rae[1], Jeon, Ki-Ok[1], Park, Sang-Cheol[1], Kim, Hyeon-Tae[1], Song, Woo-Jin[1], You, Min-Ok[1], Jang, Goo[2], Lee, Hee-Woo[3], Youn, Hwa-Young[1]
[1]Seoul National University, Seoul, Republic of Korea, [2]Department of Theriogenology and Biotechnology, Seoul National University, Seoul, Republic of Korea, [3]Research Institute for Veterinary Science, Seoul, Republic of Korea

Adipose tissue mesenchymal stem cells (ATMSCs) represent an attractive tool for the establishment of a successful stem cell-based therapy in the field of liver regeneration medicine. Oct4 and Sox2, which are essential transcription factors for pluripotency and self-renewal, are naturally expressed in MSCs at low levels in early passages, and their levels gradually decrease as the passage number increases. Therefore, to improve MSC proliferation and stemness, we introduced human Oct4 and Sox2 for conferring higher expansion and differentiation capabilities. The Oct4-IRES-Sox2 vector was transfected into human adipose tissue MSCs (ATMSCs) by liposomal transfection and used directly. Oct4 and Sox2 were successfully transfected into ATMSCs, and we confirmed maintenance of MSC surface markers without alterations in both RFP (control) and Oct4/Sox2-ATMSCs. Enhanced proliferative activity of Oct4/Sox2-ATMSCs was shown by WST-1 assay, and this result was further confirmed by cell counting using trypan blue exclusion for a long period. In addition, FACs cell cycle analysis showed that there was a reduction in the fraction of Oct4/Sox2-ATMSCs in G1 with a concomitant increase in the fraction

of cells in S, compared to RFP-ATMSCs. Increased levels of cyclin D1 were also seen in Oct4/Sox2-ATMSCs, indicating acceleration in the transition of cells from G1 to S phase. Furthermore, Oct4/Sox2-overexpressing ATMSCs showed higher differentiation abilities for adipocytes or osteoblasts than controls. The markers of adipogenic or osteogenic differentiation were also upregulated by Oct4/Sox2 overexpression. Based on those results, we hypothesized that *Oct4* and *Sox2* can increase the "trans-differentiation" of ATMSCs into hepatic lineage. After the induction of differentiation into hepatocyte-like cells, the cell morphology changes into round or polygonal epithelioid cells. In RT-PCR results to evaluate hepatic markers, albumin was expressed strongly in hepatogenic differentiated Oct4/Sox2-ATMSCs while the expression level of a-fetoprotein was lower than that of RFP-ATMSCs. From the results of periodic acid-Schiff (PAS) staining and urea assay to evaluate the capabilities of functional hepatocytes, PAS-positive cells were significantly higher and the urea production was significantly increased in Oct4/Sox2-ATMSCs than that of RFP-ATMSCs. Taken together, the hepatocyte-like cells from Oct4/Sox2-ATMSCs are mature hepatocytes which might have capabilities of functional hepatocytes, and have enhanced capacity to store glycogen and to produce urea. In this study, we demonstrated promoted transdiffrentiation into hepatocyte-like cells which have enhanced hepatocyte-specific functions from ATMSCs overexpressing Oct4 and Sox2. Therefore, we expect that the improvement in cell proliferation and differentiation using Oct4/Sox2 expression in ATMSCs may be a useful method for expanding the population and increasing the stemness of ATMSCs and Oct4/Sox2-ATMSCs may become very useful source to hepatocyte regeneration or liver cell transplantation.

W-3117
ESTABLISHING QUIESCENCE IN HUMAN BONE MARROW STROMAL STEM CELLS LEADS TO ENHANCED OSTEOBLAST DIFFERENTIATION

Harkness, Linda[1], Rumman, Mohammad[2], Dahwan, Jyotsna[2], Kassem, Moustapha[3]
[1]KMEB, Odense University Hospital, Odense C, Denmark, [2]Instem, Bangalore, India, [3]KMEB, Odense University Hospital, DK-5000 Odense C, Denmark

Human bone marrow stromal (skeletal) stem cells (hBMSC) are multipotent stem cells that comprise approximately 0.001% of bone marrow mononuclear cells and are thought to maintain their 'stemness' in the niche through quiescence (G0). However, current in vitro culture conditions lead to cellular proliferation and differentiation. Thus, we examined the effects of quiescence on the biological characteristics of BM-hMSC. Induction of quiescence was carried out in a telomerised model of in an immortalised BM-hMSC, hMSC-TERT, and in primary BM-hMSC using cellular suspension in 2% methyl cellulose. The quiescence G0 state was confirmed by proliferation markers, such as: Ki67 staining and BRDU incorporation, that were down regulated within 24hrs of cell suspension, absence of staining for the senescence marker b-Galactosidase and down-regulation of cyclin (CCNA2, CCND1, CCNE1, CCNB1) gene expression. These changes were reversed during exit, re-plating of cells in normal media, of G0. During quiescence, up-regulation of the osteogenic genes and protein expression of RUNX2, SPARC and OPN was observed. Additionally, osteoblastic differentiation of BM-hMSC post quiescence revealed an increase in ALP activity and earlier mineralized matrix deposition. Adipocytic differentiation was also maintained. In conclusion, establishing quiescence of BM-hMSC lead to cellular synchronization with respect to proliferation and differentiation and is a better approach for OB induction.

W-3118
REPRODUCIBILITY IN SCIENCE: HOW VIDEO JOURNALS INCREASE RESEARCH VALIDITY AND PRODUCTIVITY

Henderson, Kira M.
Journal Development, JoVE, Journal of Visualized Experiments, Cambridge, MA, USA

Several high-impact studies indicate that an astoundingly low 11-30% of published scientific research is reproducible. The media has implied that scientists are actively practicing poor conduct and falsifying data under the pressure of career considerations. We reject this speculation and instead question the traditional, text-based format of scientific communication. As research methods incorporate new technologies and become increasingly complex, the platform for sharing new techniques remains relatively unchanged. Researchers currently present their dynamic methods as static snapshots manipulated to fit within the limitations of text-based journals. A new generation of science journals is changing that - it employs video technology to capture and share complex research techniques in a dynamic format. Here, we present an overview of the growing field of video publication and discuss its technical challenges, implications for scholarly communication and its adoption by the scientific community. Results from recently conducted case studies will be shared, such as the experiences of research groups at Purdue University and University of Alaska which indicate that video publications can save a lab up to $15,000 per experiment.

W-3119
ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELLS DIMINISH ACETAMINOPHEN-INDUCED LIVER INJURY IN MICE

Huang, Yu-Jen[1], Wu, Yao-Ming[2], Lee, Hsuan-Shu[1]
[1]Institute of Biotechnology, National Taiwan University, College of Bio-Resources and Agriculture, Taipei, Taiwan, [2]National Taiwan University Hospital, Taipei, Taiwan

Background: Acetaminophen (APAP) overdose is the most frequent cause of acute liver failure (ALF). Liver transplantation is the most effective strategy for ALF but limited by the availability of donor organs. Omentum adipose tissue-derived mesenchymal stem cells (MSCs) are good alternative source for cell therapy. In this study, we aimed at investigating omentum adipose tissue-derived MSCs may exert therapeutic effects on APAP-induced liver injury. Methods: MSCs are isolated from mice omentum adipose tissue and transplant into mice with ALF induced by APAP. We analyzed the therapeutic effect by the survival, histology and liver function test at different time points after transplantation. We also analyzed the GSH levels and antioxidant ability at 8 and 24 hours after omentum adipose tissue-derived MSCs transplantation. The effect of cell transplantation on the activation of MAPK signal and inflammatory cytokines are also analyzed. Results: Omentum adipose tissue-derived MSCs have fibroblast-like morphology and express multipotent stem cell properties, which positive for CD29, CD44, CD90, CD105 and negative for CD31 and CD34, and differentiated into adipocytes, osteoblasts, and hepatocytes in respective induction medium. Transplantation of omentum-derived adipose MSCs can improve significantly the survival and the liver function in ALF mouse model induced by APAP. Omentum adipose tissue-derived MSCs diminish APAP hepatotoxicity by increasing hepatic GSH content and antioxidant enzyme activity (SOD, GPx, catalase). Further, immunohistology and western blot results show omentum adipose tissue-derived MSCs alter metabolism of APAP and decrease the expression of cytochrome P450 2El, nitrotyrosine , 4-hydroxynonenal in APAP induced liver injury. In addition, omentum-derived MSCs therapy can decrease the expression of inflammation

cytokine (IL1α, IL1β, IL6, IL10) and suppress MAPK family protein levels (JNK/ERK/p38). Conclusion: These studies demonstrated the omentum adipose tissue-derived MSCs are novel cell source for cell therapy. And the omentum adipose tissue-derived MSCs diminish the APAP hepatotoxicity by increasing the antioxidant enzymes ability, changing metabolism of APPA and attenuating the MAPK mediated signal expression.

PRE-CLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL CELLS

W-3121
SUBCUTANEOUS AND VISCERAL ADIPOSE-DERIVED STEM CELLS HAVE SIMILAR BIOLOGICAL PROPERTIES AND BOTH IMPROVE CARDIAC FUNCTION OF INFARCTED RAT HEARTS

Chi, Chao[1,2,3], Xiang, Bo[2,7], Deng, Jixian[1,2], Wang, Fei[1,2,3], Natarajan, Kanmani[4], Hung-Yu, Lin[1], Hongyu, Liu[3], Lin, Francis[4], Freed, Darren H.[6], Arora, Rakesh C.[1,2,5], Tian, Ganghong[1,2]

[1]National Research Council Canada, Winnipeg, MB, Canada, [2]Department of Physiology and Pathphysiology, Faculty of Medicine, University of Manitoba, Winnipeg, MB, Canada , [3]The Departmetn of Cardiac Surgery, The First Affiliated Hospital of Harbin Medical University, Harbin, China, [4]Department of Physics and Astronomy, University of Manitoba, Winnipeg, MB, Canada, [5]St. Boniface Research Centre, University of Manitoba, Winnipeg, MB, Canada, [6]University of Alberta, Edmonton, AB, Canada, [7]Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg, MB, Canada,

Background: Adipose-derived stem cells (ASC) from both subcutaneous and visceral adipose tissues have been studied individually and separately. No studies have been performed to directly compare their biological properties and therapeutic function in treatment of congestive heart failure (CHF). It is still unclear whether ASC from the two different sources have significantly different biological properties and, more importantly, whether one sub-type of ASC is more effective in treatment of CHF. This study was therefore designed to comparatively analyze their biological properties and cardiac therapeutic function. Methods: Rat subcutaneous and visceral adipose were excised for isolation of ASC. Morphology, yield, proliferation, surface markers, differentiation potential, and cytokine secretion of the subcutaneous ASC (S-ASC) and visceral ASC (V-ASC) were analyzed. To assess their therapeutic capacity, a rat model of myocardial infarction (MI) was established by occlusion of the left anterior descending coronary artery. Seven days after MI, S-ASC (n = 11), V-ASC(n = 11), and cell culture medium (Control, n = 7)were injected into the infarct border zone, respectively. Cardiac function of the infarcted hearts was then monitored with MRI for six months. Results: Both S-ASC and V-ASC exhibited a fibroblast-like morphology and expressed stromal cell markers (CD29, CD90 and CD105). No significant expression of hematopoietic markers (CD11b, CD34 and CD45) was found. Under appropriate conditions, both cells could differentiate to adipocyte- and osteocyte-like cells. Both of them expressed a significant level of Hepatocyte growth factor (HGF), Insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF). As to their differences, V-ASC had approximately 3-times greater cell yield ($65.4\pm5.8\times10^4$/gram vs. $18.7\pm2.9\times10^4$/gram) and a lower colony-formation rate (9.8±1.0% vs.13.5±2.6%) relative to S-ASC. In contrast, S-ASC showed a significantly greater growth rate (Doubling Time:17.9±0.9hours/first 14 days culture vs. 26.0±2.6hours/first 14 days culture) relative to V-ASC. Both S-ASC and V-ASC-treated hearts showed a significantly greater left ventricular ejection fraction (LVEF, 58.3±14.5% and 56.7±3.1%) than the control group (LVEF, 47.2±15.9%) at end of six months of recovery period. LVEF between the two ASC-treated groups was not significantly different. Finally, the implanted stem cells were readily detected *in vivo* with MRI for at least 6 months. Myocardial tissue sections showed existence of ASC and their locations matched with MRI signals. Conclusions: S-ASC and V-ASC share several major biological characteristics. Both provide comparable significant improvement on cardiac function. Moreover, these implanted cells can be reliably tracked for at least 6 months using MRI. We conclude that the subcutaneous and visceral adipose tissues are equally effective cell sources for cell therapy of congestive heart failure. MRI is a reliable modality for longitudinal monitoring of implanted stem cells.

W-3122
GILZ-DEPENDENT ACTIVIN A PRODUCTION BY MESENCHYMAL STEM CELLS INHIBITS TH17 DIFFERENTIATION

Luz-Crawford, Patricia Alejandra[1], Gautier, Tejedor[1], Ipseiz, Natacha[2], Pène, Jerome[1], Morand, Eric[3], Beaulieu, Elaine[3], Christian, Jorgensen[1], Noel, Daniele[1], Djouad, Farida[1]

[1]INSERM U844, Montpellier, France, [2]Institute for Clinical Immunology, University of Erlangen-Nürnberg, Erlangen, Germany, [3]Monash University, Melbourne, Australia

Together with B cells, Th1 and Th17 cells are the immune cell subsets mainly associated with the development of autoimmune diseases. Mesenchymal stem cells (MSC) display immunosuppressive properties via the inhibition of T cell activation and proliferation. Recent evidences have shown that MSC negatively regulate both Th1 and Th17 responses and restore the balance between T-helper and T regulatory cells. However, the molecular mechanisms by which mouse MSC exert their immunosuppressive effect on Th1 and Th17 cells and the nature of T cells generated have not been fully elucidated. We used MSC isolated from the bone marrow of wild-type (WT MSC) or Gilz-deficient (Gilz-/- MSC) mice. T cells obtained from the spleen of WT mice were differentiated into Th1 or Th17 cells. After 6 days of T cell/MSC coculture, proliferation was quantified and the number of IFN-γ- and IL-17-producing Th1 and Th17 cells, respectively, was measured by flow cytometry. Using ELISA, we quantified the production of Activin A, PGE2 and NO2 in the supernatants of differentiated Th1 or Th17 cells in the presence or absence of WT or Gilz-/- MSC.. Phosphorylation of Smad-2/-3 in differentiated Th17 cells was determined using an ELISA-based assay. Here we show that the inhibition of differentiation of CD4+ naive T cell towards Th1 and Th17 lineages by MSC is accompanied by increased levels of NO2 and Activin A which were diminished in Gilz-/-, compared to WT, MSC. Also, under Th17 skewing conditions, the release of Activin A by MSC repressed the Th17 differentiation program through Smad3/2 activation and promoted the generation of IL-10 producing CD4+ T cells. Our data indicate that Gilz-dependent production of Activin A participates in the inhibition of Th17 cell development by MSC through the generation of IL-10 producing cells. Thereby, via Gilz expression, MSC offer a potential means of impacting on autoimmune diseases mediated by Th17 cells.

W-3123

EFFECTS OF VITAMIN C ON TRANSPLANTATION OF ADIPOSE-DERIVED STEM CELLS IN BONE FRACTURED SMP30 MICE

Min, Chang-Woo, Kim, Ah-Young, Lee, Eun-Mi, Lee, Eun-Joo, Kang, Kyung-Ku, Lee, Myeong-Mi, Kim, Sang-Hyeob, Sung, Soo-Eun, Ghim, Soong-Koo, Hwang, Meeyul, Jeong, Kyu-Shik

Department of Pathology, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea

To improve bone fracture healing prognosis and healing rate, various therapies were developed. Stem cell therapy (bone marrow derived stem cell, etc.) is also known as effective method. Vitamin C is also plays important role in fracture healing process. So, in present study, we aim to reveal the complex effects of vitamin C and adipose-derived stem cells (ASCs) in fracture healing process. Vitamin C deficient mouse model (SMP30 mice) that induced femur fracture bilaterally were divided into four groups and treated with combination of vitamin C and ASCs; None treated group (N), ASCs treated group (C), vitamin C treated group (V), ASCs and vitamin C treated group (VC). Twenty four-gauge needle was implanted intramedullary and the diaphysis was fractured. 3 x 10^5 ASCs mixed with matrigel were injected locally and the wound was closed. Mice were sacrificed at 10 days. The samples were analyzed in histology and molecular level. In vitamin C and ASCs treated group, callus were bigger than non-treated group which means its strong bridging structures characterized by soft tissue, cartilage and bone. On histological analysis, however, C group showed more necrosis of callus. VC group showed most severe necrosis and infiltration of inflammatory cells. Expression of osteopontin and IL-1β were up-regulated in vitamin C and ASCs group than other groups. We demonstrated that each of vitamin C and ASCs play important role in fracture healing and vitamin C enhances efficiency of stem cell therapy using ASCs but also induces necrosis by stimulating immunological reaction.

W-3124

PLX-RAD IN MITIGATION OF ACUTE RADIATION SYNDROME

Ofir, Racheli[1], Pinzur, Lena[1], Zahavi, Efrat[1], Aberman, Zami[1], Volk, Hans-Dieter[2], Reinke, Petra[2], Akyüz, Levent[2], Gaberman, Elena[3], Gorodetsky, Raphael[3]

[1]Pluristem Therapeutics Inc., Haifa, Israel, [2]Charité - Universitätsmedizin Berlin Institute of Medical Immunology and the Brandenburg Center of Regenerative Therapy (BCRT), Berlin, Germany, [3]Sharett Institute of Oncology, Hadassah - Hebrew University Medical Center, Jerusalem, Israel

Acute Radiation Syndrome (ARS), also referred to as an acute radiation sickness or radiation poisoning, is a multiple organ damage caused by a large dose of ionizing radiation received over a short period of time (acute), resulting in a DNA damage which first affects the proliferating cells of the organism. Symptoms start to appear within 24 hours from exposure and involve multiple systems such as hematological, gastrointestinal, and neurovascular. Hematological failure, being induced by lower radiation doses, would likely affect the largest proportion of an exposed population. Additionally, gastrointestinal damage is always accompanied by the hematopoietic, which eventually could be the cause of death. Hence, this study has focused on bone marrow (BM) protection and regeneration for ARS mitigation. PLacenta eXpanded (PLX)-RAD are placental derived mesenchymal-like adherent stromal cells designated for the treatment of hematological indications. The cells are expanded in Pluristem's proprietary bioreactor system using a three-dimensional culture method. PLX-RAD cells are suitable for allogeneic administration without HLA-matching due to their low immunogenicity thus defined as "of the shelf", ready to use, product and compatible for use in a nuclear disaster scenario. PLX-RAD cells have demonstrated in vitro immuno-modulatory properties, as they were found to inhibit activated T cell proliferation as well as to immuno-modulate the secretion profile of innate and acquired immune cell subsets. These immuno-modulatory capabilities of PLX-RAD cells make them a potential treatment option for radiation injuries resulting from immune stimulation. The angiogenic effect of PLX-RAD mediated by the secretion of VEGF, FGF and HGF may also influence the altered vascular barrier response seen in radiation damage. Finally, PLX-RAD cells secretion profile includes cytokines known to be stimulators of hematopoietic progenitor cells or be involved in the reconstitution of the immune system. To assess the potential effect of PLX-RAD in ARS, PLX-RAD cells were administered intramuscularly to C3H/HeN male mice one and five days following lethal dose (7.7 Gy) total body irradiation. Mice body weight and survival were monitored for 3 weeks. On the 23rd day of the study all the animals were euthanized and their BM and blood were sampled for cellularity evaluation. In this study PLX-RAD treatment resulted in a ~70% increase in survival rates as well as in a higher and faster weight gain, compared to the vehicle control group. Similarly preferable trend was seen when looking at complete blood and BM counts: PLX-RAD treated mice manifested significantly increased, close to normal, BM and blood counts of all the three lineages. Moreover, the presence of human BM regenerative proteins was detected in the mouse plasma following PLX-RAD administration. Thus, suggesting PLX-RAD mediated endocrine therapeutic effect as a possible cause of the higher hematopoietic progenitor portion in the BM consequently resulting in increased BM and blood cellularity and therefore reduced mortality. Based on this data, PLX-RAD may be a novel, highly effective therapy for radiation induced BM damage in general and particularly in a nuclear disaster scenario.

W-3125

INTRA ARTICULAR PLATELET-RICH PLASMA AND TRANSPLANTATION OF AUTOLOGOUS ADIPOSE DERIVED STEM CELLS IN ADVANCED OSTEOARTHRITIS OF THE KNEE

Dorea, Roberto[1], De Menezes, Maria Cristina Gil[2], Da Silva, Kátia Nunes[2], Sodré, Fabio[3], **Payão, Spencer Luiz Marques**[4], Dos Santos, Ricardo Ribeiro[2]

[1]Clínica de Terapia Celular, Salvador, Brazil, [2]Centro de Biotecnologia e Terapia Celular do Hospital São Rafael, Salvador, Brazil, [3]Instituto Cardio-Pulmonar, Salvador, Brazil, [4]Faculdade de Medicina de Marília, Marília, Brazil

Regenerative medicine aims to find new therapeutic options for chronic-degenerative diseases, like knee osteoarthritis in order to reduce pain and improve function. Mesenchymal stem cells (MSCs) and platelet-rich plasm (PRP) are candidates for regenerative therapies for knee osteoarthritis. We have investigated three Brazilian woman presenting with pain, edema and functional joint limitation on the knee. MRI analysis revealed findings of grade III osteoarthritis of both knee, with the presence of osteophytes, signals of joint degeneration, osteonecrosis of the medial condyle and plateau. Adipose tissue was aspirated and processed under GMP conditions in a cell culture facility for 30 days. MSCs were purified and expanded until the fourth passage, when the cells were characterized by confocal microscopy, FACs analysis and differentiation assays into chondrocytes, osteocytes and adipocytes. Absence of chromosomal alterations was verified by G-banding karyotype. The cells were dissociated, ressuspended in saline solution containing 20% human serum albumin and placed

in syringes. Under local anesthesia, MSCs were intra-articularly injected into the affected knee. The patients were also administrated with autologous activated platelet-rich plasma, intra-articularly, on the day of the lipoaspiration and on the day of MCS transplantation. The clinical parameters of evaluated showed amelioration of local pain, edema regression and increased joint mobility. The MRI of all patients were performed 6 months after PRP and MSCs injection and demonstrated attenuation of the signs of osteoarthritis and prominent reduction of the image of osteonecrosis.

W-3126
INTRAMUSCULAR TRANSPLANTATION OF PIG AMNIOTIC FLUID STEM CELLS HAS THERAPEUTIC POTENTIAL IN A MOUSE MODEL OF MYOCARDIAL INFARCTION

Shaw, S.W. Steven[1], Peng, Shao-Yu[2], Cheng, Po-Jen[1], Cheng, Winston TK[3], Wu, Shinn-Chih[2]

[1]Obstetrics and Gynecology, Chang Gung Memorial Hospital, Taoyuan, Taiwan, [2]Institute of Biotechnology, National Taiwan University, Taipei, Taiwan, [3]Department of Animal Science and Technology, National Taiwan University, Taipei, Taiwan

Acute myocardial infarction (MI) is a fatal event causing a large number of deaths world-wide. MI results in pathological remodeling and decreased cardiac function which could lead to heart failure and fatal arrhythmia. Cell therapy is a potential strategy to repair the damage through enhanced angiogenesis or by modulation of the inflammatory process via paracrine signaling. Amniotic fluid-derived stem cells (AFSCs) have been reported to differentiate to several lineages and can be used without ethical concerns or risk of teratoma formation. Since pigs are anatomically, physiologically and genetically similar to humans and pregnant pigs can be an abundant source of AFSCs, we used porcine AFSCs (pAFSCs) as our target cells. Intra-myocardial injection of AFSCs has been shown to cure MI in animal models. However, intramuscular transplantation of cells has not been extensively investigated. In this study, we investigated the therapeutic potential of intramuscular injection of pAFSCs on acute MI. MI mice were divided into 1) PBS control, 2) medium cell dose (1 x 106 cells per leg; cell-M), and 3) high cell dose (4 x 106 cells per leg; cell-H) groups. Cells or PBS were directly injected into the hamstring muscle 20 minutes after MI surgery. Four weeks after MI surgery, the cell-M and cell-H groups exhibited significantly better ejection fraction, significantly greater wall thickness, smaller infarct scar sizes and LV expansion index compared to the PBS group. Using in vivo imaging, we showed that the hamstring muscles from animals in the cell-M and cell-H groups had RFP positive signals. In summary, intramuscular injection of pAFSCs reduced scar size, reduced pathological remodeling, and preserved heart function after MI.

W-3127
BONE MARROW-DERIVED MESENCHYMAL STEM CELLS (BMSCs) REVITALIZE AUTOGRAFT BONE STERILIZED BY LIQUID NITROGEN WITH AUGMENTED OSTEOGENIC PROPERTY IN RAT MODEL

Iwata, Eiichiro[1], Tohma, Yasuaki[2], Honoki, Kanya[1], Tanikake, Yohei[3], Kura, Tomohiko[1], Tsukamoto, Shinji[1], Akahane, Manabu[4], Tanaka, Yasuhito[1]

[1]Orthopaedic Surgery, Nara Medical University, Kashihara, Japan, [2]Orthopaedic Surgery, National Hospital Organization Nara Medical Center, Nara, Japan, [3]Orthopaedic Surgery, Otemae Hospital, Osaka, Japan, [4]Public Health, Health Management and Policy, Nara Medical University, Kashihara, Japan

Background: Autograft bone treated by liquid nitrogen is an oncological sterilization method and used for reconstruction of limb with large bone defects following tumor exicision. This oncological sterilization method has a number of advantages such as simplicity, no need of bone bank and no elicitation of immune reaction. However, the reduced osteogenic activity is considered as the disadvantage of the liquid nitrogen - sterilized frozen bone. Objective: To develop the augmented osteogenic activity in autograft bones treated by liquid nitrogen using bone marrow-derived mesenchymal stem cells (BMSCs). We evaluated the capacity of BMSCs whether augment osteogenesis of graft bone. Materials and methods: We used F344 syngeneic rats in this study. Bone marrow cells were cultured as follows. Briefly, fresh bone marrow plugs were obtained from femoral shafts of 6 week-old male rats and BMSCs harvested from the plugs were cultured in minimal essential medium(MEM) with 15% fetal bovine serum and mixture of antibiotics, then cells were adjusted at the concentration of 1×10^7 cells/ml in MEM. Autogenic bone fragments, a cylindrical shape, 4mm in diameter × 5mm in length, were prepared from femora of 10 week-old male rats for liquid nitrogen treatment. The bone fragments were frozen in liquid nitrogen for 20 minutes, then thawed at room temperature for 20 minutes. We compared two groups; autogenic bone treated by liquid nitrogen without BMSCs (non-BMSCs group) and autogenic bone treated by liquid nitrogen with BMSCs (BMSCs group), n=4 for each experiment. For preparation of BMSCs group, we poured 20μl BMSCs in MEM (1×10^7 cells/ml) into the bone fragments, then soaked these bone fragments in MEM containing the BMSCs and incubated at 37°C for 30min. in a CO_2 incubator. The bone grafts for two experimental groups were transplanted subcutaneously into the back of the 8 week-old male rats. ALP activity in the grafted bone was measured at 2 and 4 weeks after transplantation. Histological bone formation was evaluated with haematoxylin and eosin (H and E)-stained sections. Results: The ALP activity in BMSCs group was significantly higher than in non BMSCs group, both at 2 weeks and 4weeks after transplantation. At 2 weeks after transplantation, in both groups, almost all histological sections showed empty lacunae, indicating necrotic bone. At 4 weeks after transplantation, non BMSCs group still showed only empty lacunae. However, BMSCs group showed fresh bone areas, evidenced by the appearance of many osteocytes in osteocytic lacunae, indicating the appearance of active newly formed bone. Discussion: Graft bone sterilized by liquid nitrogen has a number of advantages utilizing the resected bone for a biological reconstruction of large bone defects after bone tumor resection, for instance. However, there is a disadvantage of reducied osteogenic activity. In contrast, BMSCs have the multipotent capacity including osteogenic property. The current study showed cultured BMSCs could augment the osteogenic activity to autograft bone treated by liquid nitrogen, evidenced by elevated ALP activity and histological bone formation. These results indicate that BMSCs can revitalize the graft bone treated by liquid nitrogen, and possibly other mode of stored bank bone. Further study will be needed on this matter. In conclusion, our proposed technique, a revitalization of autologous bone graft treated by liquid nitrogen with BMSCs, will be useful for reconstruction of large bone defects.

W-3128
COMPARISON OF PREPARATION TECHNIQUES FOR LIPOMODELLING: EFFECT OF DECANTATION, CENTRIFUGATION, AND MEMBRANE-BASED TISSUE FILTRATION ON CONTENT, VIABILITY, AND MORPHOLOGY OF HUMAN ADIPOSE TISSUE STEM CELLS

Jaros, Josef[1], Streit, Libor[2], Jurtikova, Veronika[1], Pospisil, Jakub[1], Sedlackova, Miroslava[1], Drazan, Lubos[2], Hyza, Petr[2], Dvorak, Zdenek[2], Stupka, Igor[2], Jiri, Vesely[2], Hampl, Ales[1]

[1]Department of Histology and Embryology, Faculty of Medicine, Masaryk

University, Brno, Czech Republic, [2]Clinic of Plastic and Aesthetic Surgery, St. Anne University Hospital, Brno, Czech Republic

Lipomodelling (fat-grafting) is a promising surgical technique using patient's own fat for tissue regeneration and augmentation. Adipose-derived stem cells (ASCs) are reported to be the most potent fat tissue cells responsible for the regeneration. The percentage of the fat resorption is mainly related to the viability of processed fat tissue. The aim of our study was to compare selected properties of recovered fat tissue and isolated ASCs when processed by commonly used techniques of fat preparation. Adipose tissue was harvested from hypo-gastric region in 8 healthy female donor patients by manually operated liposuction. Aspirated tissue was processed by decantation, centrifugation, and membrane-based tissue filtration, respectively. In centrifugation protocol, the sample was divided to 3 fractions (low density LD - upper 2/3 of adipose fraction; high density HD - bottom 1/3 of adipose fraction; pellet). The morphology of individual samples was evaluated by scanning electron microscopy and its overall viability was assessed by live-dead assay. Then each tissue sample was collagenased and the isolated cells were counted and seeded on tissue culture plates. The number of adherent cells and their proliferation was assessed using nuclear staining followed by fluorescent microscopy. The stem cell character was confirmed by specific surface markers (CD105+, CD90+, CD45-) and by their ability to differentiate into adipo- and osteogenic lineage. The highest number of ASCs was isolated from LD fraction of centrifuged fat and then from the membrane-based processed adipose tissue. Overall viability as well as adherence and proliferation were about the same in all samples. Similarly, the presence of stem cell-specific markers and differentiation potential were indistinguishable among the samples. Still, we have observed significant changes to the submiscroscopic structure of the tissue samples associated with the preparation procedure. Specifically, we have seen that the extracellular matrix was best preserved by decantation technique, however the sample encompassed oil drops and residues of disrupted adipocyte membranes after operated liposuction. The cell debris was significantly eliminated with both other techniques and samples retained considerable part of fibrillar extracellular matrix. During centrifugation, the LD and HD fractions also contained oil drops, which were separated by membrane-based filtration procedure. Taken together, we may conclude that the decantation is very gentle technique, however the samples contain lowest number of ASCs and the technique is time-consuming, which is unsuitable for clinical practice. The centrifugation provides to isolate the highest numbers of ASCs, and with membrane-based tissue filtration are effective and comparable techniques of fat tissue preparation.

W-3129
MESENCHYMAL STROMAL CELLS FROM DIFFERENT SOURCES MAY LEAD TO DIFFERENT THERAPEUTIC RESULTS AGAINST 4T1 MURINE BREAST TUMOR

Jazedje, Tatiana[1], Ribeiro, Aline Lopes[1], Pelatti, Mayra Vitor[1], de Siqueira Bueno, Heloísa[2], Nagata, Gabriela Sanchez[3], Trierveiler, Marília[3], Rodrigues, Elaine Guadelupe[1], Zatz, Mayana[2]

[1]Experimental Oncology Unit (UNONEX), Federal University of Sao Paulo (UNIFESP), Sao Paulo, Brazil, [2]Human Genome and Stem Cell Research Center, University of Sao Paulo, Sao Paulo, Brazil, [3]Stomatology Department, University of Sao Paulo, Sao Paulo, Brazil

The use of Mesenchymal Stromal Cells (MSCs) aiming to treat cancer has shown contradictory results which could be due to different protocols, types of tumors, animal models or human and animal MSCs sources that were used. Some studies reported worsening of the disease after co-injection of MSCs and tumor cells in animal models, while others described clinical improvement when MSCs were injected intravenously or intraperitoneally in tumor-bearing animals. In an attempt to understand the possible role of MSCs in cancer, we are studying the therapeutic effect of human and murine MSCs in murine mammary adenocarcinoma. In a first study we observed that MSCs from the same cell line when injected in the same murine model with mammary adenocarcinoma can lead to opposite results. Mice co-injected with tumor cells and human tube Mesenchymal Stromal Cells (htMSCs) presented premature death. On the other hand, tumor bearing animals injected intraperitoneally with 2 doses of 10e6 htMSCs had a survival 50% higher than the untreated group, reduced number of metastases and lower levels of lung inflammation. The aim of the present study was to evaluate the effect of MSCs obtained from both human and murine bone marrow and adipose tissue as a potential treatment of the murine mammary adenocarcinoma 4T1. A total of 72 immunocompetent mice Balb-c females (12 weeks-old) were included in the present study. For analysis of a possible clinical effect of human and murine MSCs, 36 mice were injected with 10e4 tumor cell into the mammary adipose tissue. Then, mice were divided into 2 experimental groups: Bone Marrow (BM) and Adipose Tissue (AT). Animals of each group were further divided in 3 sub-groups (6 per group): (1) injected intraperitoneally with 10e6 human MSCs, 7 and 14 days after tumor cells inoculation (2) injected intraperitoneally with 10e6 murine MSCs, 7 and 14 days after tumor cells inoculation and (3) untreated control group. All groups were analyzed 30 days after tumor cells inoculation, in order to evaluate the presence of pulmonary metastasis. With the aim of analyze the effect of this experiment on survival, the same experiment was repeated in another group of 36 mice. Pulmonary tissue HE analysis showed a reduced number of metastasis and less inflammation in the injected mice, as compared to untreated group. However no relevant differences were observed among the sub-groups or between human versus murine MSCs regarding tumor growth and survival time. The present results, indicating no clinical effect of human and murine MSCs obtained from bone marrow and adipose tissue, as opposed to our first study with human htMSCs, give further evidence to the hypothesis that MSCs from different origins may lead different results for the treatment of the same disease. Other experiments on the possible beneficial immunomodulatory effect of htMSCs in tumor-bearing animals are currently underway.

W-3130
MSCS ISOLATED FROM MENSTRUAL BLOOD SHOW AN UNMATCHED ANTI-TUMORAL EFFECT ON A HUMAN PANCREATIC CARCINOMA CELL LINE

Alcayaga-Miranda, Francisca[1], Ainoa, Fernandez[2], Rosati, Antonella[3], Martin, Aldo[4], **Khoury, Maroun**[5]

[1]Faculty of Medicine, Universidad de Los Andes, Santiago, Chile, [2]Universidad Andres Bello, Santiago, Chile, [3]Universidad de Viña del Mar, Viña del Mar, Chile, [4]Cells for Cells-REGENERO, Santiago, Chile, [5]Universidad De Los Andes, Santiago, Chile

Introduction Mesenchymal stem cells (MSC) therapeutic effect on cancer cells remains controversial due to discrepancies in results. This controversy is thought to be due to the fact that tumor growth can both be stimulated and inhibited by various MSC secreted factors. Menstrual derived stem cells (MenSC) are a recently identified stem cell population, isolated from the menstrual fluids. We have showed that MenSC possess weaker immunomodulatory properties when compared to bone marrow MSCs (BM-MSC). While the lack of important immunosuppressive properties put the MensCs in a disadvantage for their therapeutical uses for inflammatory diseases, we investigate the direct effect of the lower immunosuppressive effect of MenSC on tumor growth. Methods: The tumor suppressive activity

of MenSC in comparison to BM-MSC was tested in co-culture at different ratio with various cancer cell lines (PC3, A549, MiaPaca-2, SkMel28, MBMD231, and PANC-1) through cell proliferation , tumor sphere and colony formation (CFU) assays. The effect of MSCs on cancer cell migration was evaluated in a transwell assay. The expression levels of TRAIL and DKK3 were determined by Q-PCR and Elisa. Immunodeficient NSG mice were then injected with pancreatic human cancer cell line alone or co-injected with MSCs at different time points. Tumor growth was then monitored and samples were collected, weighed and analyzed for histology and IHC. Results: The anti-tumoral effect of MSCs was variable and depended largely on the cell ratio and type of cell line tested. The most significant effect was observed on MiaPaca-2, a human pancreatic carcinoma, at a ratio of 2:1 (MiaPaca-2:MSCs). A 60% inhibition of the cancer cell CFU was observed in the presence of MenSCs , in comparison to a 40% inhibition when BM-MSCs were used. A significant reduction of the proliferation and the size of the tumor spheres was also noted. While the BM-MSCs conditioned media (CM) enhanced the migration of the cancer cells relative to a control media, MenSC-CM showed the opposite effect. Furthermore, the basal expression levels of TRAIL and DKK3 were 3 and 4-folds higher in MensCs. When MiaPaca-2 and MSCs were co-injected in NSG mice, a statistically significant delay of the incidence of the tumor apparition and of the tumor growth was observed only in MenSCs injected groups. In addition, when MenSCs were injected intratumorally at 10 days post-tumor engraftment a 6-fold decrease of the tumor growth was noted. We have demonstrated an anti-tumoral effect of MenSCs on a pancreatic tumor cell lines that was unmatched with the effect of BM-MSCs. Understanding the differential anti-tumoral effect of distinct sources of MSCs will greatly contribute in clearing the confusion looming around the use of Mscs in cancer, and unraveling the mechanism behind it, will advance to new approaches for anti-cancer treatments.

W-3131
THERAPEUTIC EFFICACY OF TRAIL EXPRESSING MESENCHYMAL STEM CELLS BY INTRA-ARTERIAL DELIVERY IN EXPERIMENTAL GLIOMA MODEL

Kim, Seong Muk, Jeong, Chang Hyun, Ryu, Chung Heon, Jeun, Sin Soo
The Catholic University of Korea, Seoul, Republic of Korea

Stem cell-based therapy using human mesenchymal stem cells (MSC) as delivery vehicles of therapeutic agents is a promising strategy to target brain tumor and currently under clinical investigation. However, optimized effective routes of cell delivery must be determined for the clinical setting since direct intracerebral (IC) injection is an invasive method and treatment options of gliomas are limited due to the blood-brain barrier. In this study, we evaluated the effects of engineered MSCs via various routes of administration in the intracranial xenograft glioma models. We showed the tumor targeting properties and therapeutic effects of the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-secreting MSC (MSC-TRAIL) delivered by different routes including IC, intraarterial (IA), intravenous (IV), or lumbar intrathecal (IT) injection. Tumor-targeting and migratory capacity of MSCs was investigated by In Vivo Imaging System and in histological analyses. IA delivery of MSCs displayed targeted tumor tropism with significant numbers of MSCs accumulating specifically at the tumor site. Real-time polymerase chain reaction experiments also revealed that IA application of MSCs leads to a rapid and targeted migration of cells toward intracerebral gliomas without cell loss into other systemic organs compared to the IV or IT delivery. Furthermore, *in vivo* survival experiments and bioluminescence imaging analyses showed that IA treatment of MSC-TRAIL had greater therapeutic efficacy than other treatment methods, although the direct treatment via IC delivery showed the most effective. These results suggest that IA delivery of stem cell-based therapeutics is a feasible and highly effective treatment modality, allowing for non-invasive and repeated application of engineered stem cells to target malignant glioma.

W-3132
STORAGE PROTEIN 2 FOR CRYOPRESERVATION OF MESENCHYMAL STEM CELLS

Kim, Sun-Mi, Kim, Mi Jin, Yun, Chang-Koo, Lee, Joon Ho, Choi, Yong-Soo
CHA University, Gyeonggi-do, Republic of Korea

Cryopreservation methods of human mesenchymal stem cells (hMSCs) typically depends on the presence of fetal bovine serum (FBS) with dimethyl sulfoxide (DMSO), which is not appropriate for its therapeutic application. Storage Protein 2 (SP2), natural products derived from silkworm, can inhibit reactive oxygen species (ROS) generation. We used the SP2 effective DMSO-low and FBS-free cryopreservation system for cryostorage and banking of hMSCs. We investigated the freeze/thaw viability and stem cell characterization of umbilical cord derived mesenchymal stem cells (UC-MSCs) in different freezing media, and then we observed its efficacy at 1 week and 1 year. Thawed samples were analyzed by RT-PCR, western blot, FACS for stem cell characterization. The percentage of viable cells obtained with 5 mg/ml SP2 protein with 4% DMSO was comparable with that obtained in freezing media with 90% FBS with 10% DMSO, that is, 96% ± 2% and 97% ± 1%, respectively. Adipogenic and osteogenic differentiation behavior of the frozen thawed cells was also assessed using histochemical staining. Post-thaw UC-MSCs viability, adipogenic and osteogenic differentiation ability can be maintained even when they are frozen in the absence of FBS. The results indicate that the SP2 protein could be used as an alternative to FBS for freezing medium.

W-3133
CONCERTED ACTION OF PGE_2 AND TGF-BETA1 FROM MESENCHYMAL STEM CELLS ALLEVIATES MOUSE ATOPIC DERMATITIS BY REGULATING MAST CELL DEGRANULATION

Kim, Hyung-Sik[1], Shin, Tae-Hoon[1], Lee, Sung-Hoon[2], Lee, Byung-Chul[1], Yu, Kyung-Rok[1], Seo, Yoojin[1], Lee, Seunghee[2], Choi, Soon Won[1], Shin, Ji-Hee[1], Kang, Tae-Wook[1], Lee, Jin Young[1], Kang, Insung[1], Kim, Jae-Jun[1], Koog, Myung-Guen[1], Sung, Eun-Ah[1], Kang, Kyung-Sun[1]
[1]Seoul National University, Seoul, Republic of Korea, [2]Institute for Stem Cell and Regenerative Medicine in Kang Stem Biotech, Seoul, Republic of Korea

Mesenchymal stem cell is a promising tool for the therapy of immune function disorders. However, their efficacy in treating allergic skin disorders is less verified, and their interactions with pathogenesis-related immune cells remain unknown. We sought to investigate the therapeutic efficacy of human umbilical cord blood-derived mesenchymal cells (hUCB-MSCs) or activated hUCB-MSCs for the treatment of a murine atopic dermatitis model and to explore the distinct mechanisms that enhance their efficacy. Atopic dermatitis (AD) was induced in mice by the topical application of *Dermatophagoides farinae* (Df). Naïve or activated-hUCB-MSCs were administered to mice, and clinical severity, histological damage and immunologic responses, such as immunoglobulin production and mast cell (MC) infiltration or degranulation, were determined. A β-hexosaminidase assay was used to evaluate the ability of hUCB-MSCs to suppress MC degranulation. The subcutaneous administration of nucleotide-

binding oligomerization domain 2 (NOD2)-activated hUCB-MSCs exhibited efficient protective effects against atopic dermatitis in mice, and suppressed the infiltration and degranulation of MCs to a greater extent than naïve hUCB-MSCs. NOD2-activated MSCs reduced the degranulation of mast cells by activating NOD2 signaling to COX-2. In contrast to hBM-MSCs, hUCB-MSCs exerted a cell-to-cell contact-independent suppressive effect on MC degranulation via the higher production of PGE2. TGF-β1 production from hUCB-MSCs in response to IL-4 also contributed to the attenuation of MC degranulation by down-regulating FCεRI expression in MCs. The subcutaneous application of NOD2-activated hUCB-MSCs can efficiently ameliorate AD, and concerted action of PGE_2 and TGF-β1 are required for the inhibition of MC degranulation.

W-3134

THE BASIC METABOLIC CHARACTERISTICS OF HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELL

Kim, Yu Mi, Yang, Yoon Sun, Oh, Wonil, Choi, Soo Jin, Jeon, Hong Bae, Kwon, Soon-Jae
Biomedical Research Institute, Medipost Co., Ltd, Seoul, Republic of Korea

Massive production of Mesenchymal stem cells (MSCs) is essential for their therapeutic applications and the necessity of optimization for the culture process are highly increasing. In this study, we investigated the level of basic metabolites during culture of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) and tried to optimize the culture process based on the metabolic characteristics. To investigate the critical factor on the growth of hUCB-MSCs, the basic metabolic characteristics were monitored and analyzed during cultivation based on the previous study (Deborah Schop, M.Sc. *et al.*). The yield of lactate from glucose ($Y_{lac/glc}$) and the glucose consumption rate (qGlc) were calculated from the glucose and lactate concentrations during exponential growth phase. hUCB-MSCs showed low yield of lactate form glucose and low cell-specific glucose consumption rate. UCB-MSCs seem to be different from human and rat bone marrow MSCs in the way of generation of cellular energy via indirect comparison. It was suggested that they utilize glucose for energy production mainly using the efficient oxidative phosphorylation pathway. Also, the half-exchange of culture media per 3 days enhanced cell proliferation compared to ones without exchange, which can be related with several factors such as increased glucose, reduced ammonia after exchange or other secreted growth-inhibitory molecules in media. First, we examined the effects of additional glucose without media exchange. But, it did not have any critical influence to the cell growth. Also, we evaluated the growth inhibitory effect of NH_3. To reduce the level of NH3 in the media during the cultivation, GlutaMAX™ media with a stabilized form of L-glutamine was used. Production used of ammonia was reduced as expected, but, there was no significant difference in growth rate between GlutaMAX and standard culture media. Also, the inhibitory concentration of NH_3 on hUCB-MSCs growth was examined by adding different concentrations of NH_4Cl (0~5mM). Growth inhibition was observed from 1.5mM NH3 concentration, which is higher level than NH_3 concentration of normal culture process. Therefore, ammonia seems not a major inhibitory factor during cultivation of hUCB-MSCs. We found that hUCB-MSCs use glucose efficiently as energy source from basic metabolic characteristics, but it was not enough to find clues for the optimization of culture process. More metabolic information are needed and have to be analyzed from LC-mass spectrometry with high content analysis for efficient optimization.

W-3135

MICRORNA-302 INDUCES PROLIFERATION AND INHIBITS OXIDANT-INDUCED CELL DEATH IN HUMAN ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS

Kim, Jee Young[1], Shin, Keun Koo[1], Kim, Young suk[1], Bae, Yong Chan[2], Jung, Jin Sup[1]
[1]Department of Physiology, School of Medicine, Pusan National University, Yangsan, Republic of Korea, [2]Department of Plastic Surgery, School of Medicine, Pusan National University, Pusan, Republic of Korea

Mesenchymal stem cells (MSCs) are a heterogeneous population of cells that proliferate in vitro as plastic-adherent cells, have a fibroblast-like morphology, form colonies in vitro and can differentiate into bone, cartilage and fat cells. The abundance, ease and repeatable access to subcutaneous adipose tissue and the simple isolation procedures provide clear advantages for the use of human adipose tissue-derived mesenchymal stem cells (hASDCs) in clinical applications. We screened microRNAs that affected the proliferation and survival of hADSCs. Transfection of miR-302d mimic increased cell proliferation and protected cells from oxidant-induced cell death in hADSCs, which was supported by. Flow cytometric analysis. miR-302d did not affect the expression of Bcl2 family members or anti-oxidant molecules. The Nrf2-Keap1 system, which is one of the major mechanisms for the cellular defense against oxidative stress, was not altered by transfection of miR-302d mimic. To identify the target of the miR-302d actions on proliferation and survival of hADSCs, a microarray analysis was performed using miR-302d-overexpressing hADSCs. Real-time PCR analysis showed that transfection of miR-302d mimic inhibited the CDKN1A and CCL5 expression. The downregulation of CDKN1A with a specific siRNA blocked the effect of miR-302d on hADSCs proliferation, but did not affect miR-302d-induced cell survival. Downregulation of CCL5 protected oxidant-induced cell death as like miR-302d and inhibited oxidant-induced ROS generation. This study indicates that miR-302 controls hADSCs proliferation and cell survival through different targets and that this microRNA can be used to enhance the therapeutic efficacy of hADSCs transplantation in vivo.

W-3136

CHARACTERISATION OF HUMAN ADIPOSE TISSUE DERIVED STEM CELLS WITH ENHANCED ANGIOGENIC AND ADIPOGENIC PROPERTIES

Kingham, Paul, Lauvrud, Anne Therese, Wiberg, Mikael
Umeå University, Umeå, Sweden

Autologous fat grafting is a popular method for soft tissue reconstructions. However, the success of such procedures relies on the graft surviving until a sufficient quantity of new blood vessels have revascularised the tissue. Survival can be improved by supplementing the fat graft with a stromal vascular fraction cell mix or isolated adipose tissue derived stem cells (ASC). With a view to identifying an optimal cell type for transplantation we have evaluated the angiogenic and adipogenic properties of CD146+ cells isolated from cultured ASC. Human abdominal fat (n = 6 female patients, mean age = 50 ± 1.2 years) was treated with collagenase type I followed by centrifugation to pellet the ASC which were then plated onto tissue culture plastic. The adherent cells at passages 1-2 were immunoselected for the CD146 surface antigen using a Miltenyi Biotech microbead kit. The mean yield of CD146+ cells was 18.16 ± 3.67%. Both CD146- and CD146+ cells expressed CD90 and alpha smooth muscle actin protein and were negative for CD31 and CD34. The CD146+ cells expressed more NG2 protein, consistent with an overall phenotype characteristic of pericytes. CD146- and CD146+ cells proliferated at similar rates over the first 10 passages (mean population doubling times of 40.87

± 1.31 and 40.49 ± 0.94 hours respectively) but thereafter the CD146- cells expanded significantly slower than the CD146+ cells. qRT-PCR and ELISA showed that CD146+ cells expressed higher levels of a number of angiogenic molecules including angiopoietin-1, FGF-1 and VEGF-A. Treatment of both CD146- and CD146+ cells with differentiating medium (DMEM with 10% serum plus dexamethasone, 3-isobutyl-1-methylxanthine, insulin and indomethacin) resulted in formation of Oil Red O positive adipocytes. After 3 weeks of treatment, CD146+ cells showed higher expression of adiponectin, a marker of mature adipocytes. Taken together, these results suggest that CD146+ cells selected from a heterogeneous mix of ASC have more favourable angiogenic and adipogenic properties, which might provide significant benefits when used for reconstructive and tissue engineering applications.

W-3137
AGE-RELATED REDUCTION OF MESENCHYMAL STEM CELL (MSC) IMMUNOMODULATORY FUNCTION: THERAPEUTIC IMPLICATIONS FOR THE USE OF MSC-BASED PRODUCTS

Kizilay Mancini, Ozge[1], Shum-Tim, Dominique[2], Lora, Maximillien[1], Colmegna, Ines[1]
[1]*Division of Rheumatology, McGill University, Montreal, QC, Canada,* [2]*Division of Cardiothoracic Surgery, McGill University, Montreal, QC, Canada*

Ischemic heart disease is the leading cause of death worldwide and atherosclerosis (AS) is the most common pathogenic mechanism underlying myocardial ischemia and infarct (MI). Although inflammation is seminal to the MI healing process, strong systemic and locoregional inflammatory responses promote myocardial necrosis, impairing MI-related outcomes. Mice depleted of T cells have been shown to have smaller infarct size and increased cardiac function, suggesting that T cells play a central role in this process. Therefore, modulation of T cell function has been proposed as a therapeutic strategy following acute MI. Mesenchymal stem cells (MSCs), multipotent cells that reside in almost all tissues, have the ability to migrate to inflammatory sites and exert potent immunomodulatory effects. MSCs modulate T cell proliferation, cytokine secretion and cytotoxicity. These properties provide the rationale for current therapeutic trials using MSCs for MI. In this context, the use of autologous MSCs is preferred to avoid immunogenicity. However, it is unknown whether advancing age affects the immunomodulatory functions of MSCs, thus reducing their therapeutic benefits. The aim of this study was to evaluate the impact of aging and age-associated diseases on MSC-dependent T cell modulation. MSCs were derived from human adipose tissue (aMSCs) obtained from patients undergoing cardiac surgery. The aMSCs phenotype was characterized according to the International Society for Cellular Therapy criteria, and their capacity to suppress activated human peripheral blood T cells was assessed in allogeneic aMSC:T-cell co-cultures. The mechanisms underlying the aMSC:T-cell effect were evaluated in trans-well experiments. The aMSCs secretome was profiled using cytokine arrays, and cytokine levels were determined by ELISA. aMSCs were obtained from 46 individuals (age range: 43 to 89 years). aMSCs showed an age-dependent decline in their capacity to suppress activated T cells (r=0.24, p=0.02). aMSCs from older donors (> 65 years) had a significantly lower T cell suppressive effect than aMSCs from younger donors (< 65 years) (p=0.02). Furthermore, aMSCs from patients with AS (n=18) were worse suppressors than those from age-matched non-AS patients (valve replacement surgery n=9) (p= 0.04). Similarly aMSCs from patients with type II diabetes (n=10) had impaired suppressive function compared to those from age-matched non-diabetic donors (n=10) (p=0.04). Soluble factors and, to a lesser extent, cell-cell contact mediated the MSC-dependent T cell effect. There was an age-dependent increase in aMSC secretion of pro-inflammatory senescence-associated cytokines IL-6 and MCP-1 (IL-6: n=15, p=0.01; MCP-1: n=11, p=0.004). IL-6 levels inversely correlated with the MSC immunomodulatory capacity (n=13, p=0.006, r=0.47) and IL-6 blockage reverted defective T cell effects of aMSCs from older donors (n=7, p=0.001). Aging reduces aMSC's capacity to suppress activated T cells. Similarly, aMSCs from individuals with accelerated aging conditions (e.g., atherosclerosis, diabetes) are functionally impaired. We propose that increased pro-inflammatory cytokine production (i.e., IL-6, MCP-1) by aged aMSCs accounts for their reduced capacity to suppress T cells. These findings suggest that modulation of the IL-6/MCP-1 MSC axis may provide a strategy for improving the immunomodulatory function of aged aMSCs for therapeutic purposes (i.e., MI).

W-3138
COMPARATIVE ASSESSMENT OF THE SAFETY OF STEM CELLS AND STANDARD ANTI-INFLAMMATORY THERAPY OF ULCERATIVE COLITIS

Knyazev, Oleg[1], Konoplyannikov, Anatoliy[2], Churikova, Alevtina[1]
[1]*Moscow Clinical Research Center, Moscow, Russian Federation,* [2]*Medical Radiology Center, Obninsk, Russian Federation*

Mesenchymal stromal cells (MSCs) are now widely used in clinical studies with various diseases, providing a positive effect due to the immunomodulatory and paracrine mechanisms. However, the profile of safety of these cells remains unproved. To compare the safety of treatment of the patients with ulcerative colitis (UC), receiving comprehensive anti-inflammatory therapy with the application of MSCs standard therapy with 5-aminosalicylic acid (5-ASA) and glucocorticosteroids (GSCs). Within the period from 2008 to 2013 the system transplantation of allogenic MSCs was carried out in 74 patients with UC. 56 patients were included in the first group, the average monitoring time averaged 62±4 months. 29 of them (51.78%) were men and 27 (48,22%) women. The average age was 35,4±1,42 years. 84 patients with UC, who received standard anti-inflammatory therapy with 5-ASA and GCSs, were included in the second, control group. Out of them 46 (54.8%) were men and 38 (45,2%) - women. The average age was 34,98±1,23 years. The patients, who received anti-cytokine therapy, were not included in this group. The safety of the used therapy was assessed by the presence of complications, arising during the observation, such as infectious complications, exacerbation of chronic inflammatory diseases, serious infectious complications, a malignant transformation, a lethal outcome. In the first group of patients with UC the development of non-severe infectious complications or exacerbation of chronic inflammatory diseases were registered in 7 patients out of 56, that totaled 12.5%, in the second - in 14 (16,7%) patients out of 84. When comparing the two groups, no differences were found in the risk of the development of infectious complications and exacerbation of chronic inflammatory diseases on the background of the standard anti-inflammatory UC therapy or with the introduction of the MSCs (OR-0.75, 95% Cl 1.5-23.58; x^2-0,16; p=0.66). Severe infectious complications (pneumonia, pleurisy, activation of latent TB) in the first group were detected in 1 patient (1,8%) out of 56, and in the second group in 5 (5,9%) out of 84. When comparing the two groups no differences in the risk of this type of complications were also found (RR-0,3; 95% Cl 0.04-2.5; x^2-0.59; p=0.44). Colorectal cancer was registered only in one she-patient from the first group (1,8%). The time between the introduction of the MSCs and diagnosed colon cancer was 10 days. In the second group of patients over the 5 years of follow-up, malignant transformation was observed in 4 (4,8%) patients out of 84 (RR-0.5, 95% Cl 0.05-4.96; x^2-0.01; p=0.97). Within

5 years of follow-up in the first and second groups of patients, fatal outcomes were registered on one occasion in each group, 1.8% and 1.2% respectively (RR-1.5, 95% Cl 0.1-23.49; x^2-0.19, p=0.66). The analysis did not reveal any differences in the development of infectious complications, exacerbation of chronic inflammatory diseases, serious infectious complications of malignant transformations and deaths in patients with UC, who received the MSCs and the standard anti-inflammatory therapy.

W-3139

CD200 POSITIVE HUMAN PLACENTA STEM CELLS EXHIBIT ANTI INFLAMMATORY AND IMMUNEMODULATORY EFFECT IN THE EARLY PHASE OF STROKE

Kong, TaeHo, Kim, Kyung-Sul, Park, Ji-Min, Kim, Han Wool, Bae, Sang-Hun, Lee, Hee Jeon, Kim, Hyun Sook, Chung, Sangsup, Moon, Jisook
CHA University, Seoul, Republic of Korea

Stroke is an important clinical neurologic disorder, and there is considerable interest in the protection of brain inflammation response to cerebral ischemia. Cell therapy is a promising treatment for serious neurologic disorders. Transplantation of mesenchymal stem cells (MSCs) has been shown to enhance the recovery of brain functions following ischemic injury. Although immune modulation has been suggested to be one of the mechanisms, the molecular mechanisms underlying improved recovery has not been clearly identified. Here, we report that MSCs express CD200 to suppress immune propagation in the ischemic rat brain. Interestingly, AMSCs showed greater inhibition of pro-inflammatory cytokines expression in co-cultures with LPS primed BV2 microglia when compared to LPS activated cells. Then, transplantation of MSCs increased immune-modulatory factors such as CD200, IDO and TGF-β and inhibited the subsequent upregulation of pro-inflammatory cytokines in the ischemic area. At day 1 and 7 post-ischemia, continuous increasing of pro-inflammatory cytokines was detected in brain of control group; however, this trend of cytokines expression was significantly attenuated by AMSCs transplantation. Moreover, reduced microglia activation in boundary region and improvements of motor function were confirmed in AMSC treated group. The results of this study suggest that CD200 positive AMSCs have the ability to modulate inflammatory cytokines and microglia activation in ischemia-induced brain inflammatory responses. This study thus offers a new insight into the mechanisms responsible for the immunomodulatory effect of AMSC transplantation, with implications for functional neurological recovery after stroke.

W-3140

THE USE OF SYSTEMIC TRANSPLANTATIONS OF MESENCHYMAL STEM CELLS ALONE OR COMBINED WITH PHYSICAL FACTORS (IR LASER RADIATION, LOW FREQUENCY ELECTRIC FIELD) IN THE TREATMENT OF DISEASES CAUSED BY THE DAMAGE OF THE HEART MUSCLE OF VARIOUS GENESIS

Konoplyannikov, Mikhail[1], Konoplyannikov, Anatoly[2], Kaplan, Mikhail[2], Popovkina, Olga[2]
[1]Federal Research Clinical Center, Moscow, Russian Federation, [2]Medical Radiological Research Center, Obninsk, Russian Federation

The application of systemic transplantations of mesenchymal stem cells (MSC) in the treatment of heart disease has already been widely practiced in different world clinical centers, and such a treatment brings a significant therapeutic effect in the majority of cases. Earlier we have shown in the animal experiments that a combination of the MSC transplantation and action of certain physical agents (such as low-intensity IR laser radiation (IRR) and low-frequency pulsed electric field with certain parameters (PEF)) enhances the therapeutic effects of MSC transplantation. In particular, we observed this effect in the model of the heart damage caused by the action of the doxorubicin antibiotic. Currently, we have obtained first results of the clinical trial on the application of IRR and PEF in the complex therapy of heart disease with an additional application of systemic MSC transplantation. In this case, the therapeutic effect of the applied MSC transplantation was markedly enhanced, with the increased myocardium contractility and perfusion in the first 3-6 months, and with the subsequent prolonged stable compensation of the heart function observed at the patients' follow-up during first 2-3 years. It may be assumed that the action of the physical factors improves the efficiency of the regenerative processes in the heart tissues, induced by the MSC-excreted paracrine factors.

W-3141

THE THERAPEUTIC EFFECT OF AUTOLOGOUS OR ALLOGENIC MESENCHYMAL STEM CELLS ON MDR-TUBERCULOSIS

Konoplyannikov, Anatoly[1], Vasilieva, Irina[2]
[1]Medical Radiological Research Center, Obninsk, Russian Federation, [2]Central Research Institute of Tuberculosis, Moscow, Russian Federation

Multi-drug-resistant (MDR) tuberculosis therapy presents significant difficulties, since the majority of anti-tuberculosis drugs are either entirely inefficient, or have only minor therapeutic effect in this case. In our pre-clinical and clinical studies, we used single or multiple transplantations of autologous or allogenic mesenchymal stem cells (MSC) as an additional modality in the treatment of resistant forms of tuberculosis. For such a combined therapy, we have demonstrated the enhancement of the therapeutic efficiency both in the experiments on laboratory mice and in the treatment of 57 patients with resistant forms of tuberculosis. The patients received chemotherapy before the systemic MSC transplantation. In the first 5 months after the transplantation, we observed essential improvement in the general health status of the patients. In 12-15 months, we observed a cessation of bacterioexcretion in 45 patients, marked decrease in the volume of the damaged lung tissue in 43 patients, complete healing of the lung cavities in 22 patients. 31 patients were followed up for more than 3-7 years from the beginning of the treatment, among them the clinical cure was achieved in 26 patients, 7 patients from this group being operated 6-12 months following the MSC transplantation, while in 3 patients the process was stabilized and their quality of life improved. Thus, systemic MSC transplantations in patients with resistant forms of tuberculosis is a novel and promising approach in the cell therapy. It can be assumed that the therapeutic effect of the systemically transplanted MSC is based upon their ability to migrate into the damaged lung regions and facilitate their healing by means of the paracrine factors excretion.

W-3142

BONE MARROW STROMAL CELL DERIVED HEPCIDIN SHOWS BOTH ANTI-INFLAMMATORY AND ANTI-MICROBIAL PROPERTIES

Krepuska, Miklos[1], Marko, Karoly[1], Mayer, Balazs[1], Nemeth, Krisztian[1], Rada, Balazs[2], Westerman, Mark[3], Ganz, Tomas[4], Mezey, Eva[1]
[1]Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD, USA, [2]University of Georgia, College of Veterinary Medicine, Athens, GA, USA, [3]Intrinsic LifeSciences LLC, La Jolla, CA, USA, [4]Division of Pulmonary and Critical Care Medicine, David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, CA, USA

Bone marrow stromal cells (BMSCs), also known as mesenchymal stem cells (MSCs) have been shown to improve GVHD (in humans) and sepsis and asthma (in mice). The production and release of anti-microbial agents surfaced as one of the numerous mechanisms how BMSCs fight infections. A possible candidate in this regulation is hepcidin (Hamp1), an anti-microbial peptide, which was widely studied in iron-regulation. Here we demonstrate the presence of hepcidin in BMSCs and describe some of its anti-inflammatory and anti-microbial functions. We used RT-PCR, immunocytochemistry and ELISA to show the presence of hepcidin in mouse and human BMSCs (NCT01071577). The ability of BMSCs to elevate the IL-10 production and to reduce TNF alpha production in co-cultured macrophages was used as a marker of their anti-inflammatory effects. IL-10 and TNF alpha were measured from supernatants of these co-cultures. WT and Hamp1-KO BMSCs, hepcidin blocking antibodies and a variety of macrophages (both primary and cell line) were used. The anti-microbial properties were studied by an in vitro E. coli killing assay using human and mouse BMSC supernatants. We demonstrated the presence of hepcidin and its mRNA in both human and murine BMSCs with a variety of techniques. Our results show that wild type BMSCs induce significantly more IL-10 production from co-cultured macrophages than Hamp1 KO BMSCs do (279.2 pg/ml ± 25.80 vs. 139.2 pg/ml ± 16.22, $p<0.01$). When anti-hepcidin antibody was added to the co-cultures the WT BMSCs induced elevation of IL-10 levels was decreased, further confirming the role of hepcidin in the effect (269.9 pg/ml ± 12.12 vs. 216.7 pg/ml ± 5.092 $p< 0.01$). Hepcidin KO BMSCs were also less efficient in suppressing TNF alpha compared to WT BMSCs (270.4 pg/ml ± 13.05 vs. 33.74 pg/ml ± 10.22, $p< 0.0001$). Supernatants of huBMSCs suppressed the number of bacteria in an in vitro E. coli killing assay, while the addition of anti-hepcidin Ab eliminated the effect (OD 650 nm 0.01057 ± 0.0005 vs. 0.1333 ± 0.0231, $p< 0.01$). Similarly, Hamp1 KO BMSC supernatants were less effective than the WT controls. We conclude that hepcidin plays an important role in the anti-inflammatory and anti-microbial functions of BMSCs. Further in vivo studies are needed to confirm the relevance of our in vitro findings.

W-3143

SECRETOME ANALYSES OF HUMAN BONE MARROW DERIVED MULTIPOTENT STROMAL CELLS WITH ISLET REGENERATIVE CAPASITY

Kuljanin, Miljan[1], Lajoie, Gilles[1], Hess, David A.[2]
[1]Biochemistry, Western University, London, ON, Canada, [2]Krembil Centre for Stem Cell Biology, London, ON, Canada

Diabetes is a metabolic disease that affects millions of people worldwide, and is projected to drastically increase in the up-coming years. Recently, multipotent Stromal cells (MSC) have surfaced as an attractive strategy to combat diabetes. MSC have the ability to differentiate into various cell types and more importantly have been shown to recruit to the injured pancreas and induce endogenous beta cell regeneration to improve islet function. It is believed that these cells secrete previously undetermined cytokines and at early passage, which help activate endogenous repair mechanisms. Further study of these secreted factors is needed to develop future therapies to treat diabetes. Our research aims to detect these signaling proteins that are secreted, by regenerative and non-regenerative cells, into the microenvironment using amino acid labeling combined with mass spectrometry techniques. Human bone marrow derived cells were isolated using florescence activated cell sorting into distinct population based on their aldehyde dehydrogenase activity (ALHD). Cells that retained high ALDH activity were grown in culture flasks in defined SILAC media that either contained heavy [13C,15N], or intermediate [13C] arginine and lysine. The media was also supplemented with excess proline to prevent the conversion of heavy arginine into heavy proline. Cells were grown to early/late passage and the condition media plus the cell pellets were collected. Protein samples were fractionated using a combination of techniques which included: FASP, GELFrEE and 1D-SDS-PAGE. Peptides were analyzed using by LCMS/MS and identified using PEAKS 7.0, as well as MaxQuant. The above approach was designed to answer two fundamental questions: 1) what factors are secreted by regenerative cell lines vs. non-regenerative cell lines, 2) what factors are being lost as the cells move through early to late passage. Cells were labeled using SILAC, and the labeling efficiency was deemed to be >95% after culturing the cells for ~ 6 days. Flow cytometry was used to determine the presence of multipotent markers while being exposed to the defined SILAC media. Cells were positive for CD73, 146,105 and 90, while being negative for CD 14 and 45, and differentiated efficiently into bone, cartilage and adipose tissue. By combining the data obtained from cell lysates and condition media a better understanding of the factors at play can be achieved. Fractionation of condition media using in-gel digestions had yielded over 1500 validated proteins, of which 770 are unique to each condition being evaluated. Regenerative cell lines have been shown to secret more proteins into the condition media with a total of 664 validated hits. Using gene ontology to filter the data for known secreted proteins or associated with extracellular matrix revealed some interesting results. These included: proteins associated with the β-catenin pathway, EGF associated proteins, cytokine receptor binding proteins, retinoic acid binding, SCD factors as well as proteins downstream of the Wnt pathway. This research a global approach to analyze the factors secreted from human bone marrow derived MSC that impact islet regeneration using quantitative proteomic techniques.

W-3144

THE NEUROPROTECTION EFFECTS OF TOPICALLY APPLIED ADIPOSE-DERIVED MESECHYMAL STEM ON EXPERIMENTAL TRAUMATIC BRAIN INJURY

Lam, Pk Kuen[1], Wang, Kevin KW[2], Lo, Anthony[3], Ching, Don[1], Tongy, Cindy[1], Lau, Henry[1], Kong, Themis[1], Lai, Paul[1], Wong, George Kc[1], Poon, Ws[1]
[1]Surgery, The Chinese University of Hong Kong, Hong Kong, [2]Center of Neuroproteomic and Biomarkers, University of Floria, Florida, FL, USA, [3]Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Hong Kong

Background: The neuroprotection effects of mesenchymal stem cells (MSCs) have been reported in animal and preliminary clinical studies. MSCs are usually administrated to patients by means of systemic infusion. However, the therapeutic potential is compromised by the pulmonary trap and blood-brain barrier as only a few of the infused MSCs are delivered to the brain. In this study, MSCs were directly applied to the injured site of experimental traumatic brain injury (TBI)

and the neuroprotection effects were evaluated. Materials and Methods: TBI was induced in SD rats with an electromagnetically controlled cortical impact device after craniotomy was performed between the bregma and lambda, 1 mm lateral to the midline. 1.5 million MSCs, derived from the adipose tissue of transgenic green fluorescent protein (GFP)-SD rats, were applied to the exposed cerebral cortex at the injured site (N=10). The MSCs were held in position by a thin layer of fibrin. Motor and cognitive behavior testings were performed to evaluate functional recovery at days 3, 7, 14 post cell therapy. Results: Within 5 days following topical application, immunohistochemistry staining showed GPP-positive cells in the brain parenchyma. These cells co-expressed with markers of GFAP, Nestin and NeuN. Compared with the control without MSC treatment (N=10), there were less neuronal death in CA1 and CA3 of hippocampus. Neurological functional recovery was significantly improved in Motor Rod test, Water Maze and Gait Analysis. Conclusion: Topically applied MSCs can migrate to the injured brain parenchyma and offer neuroprotection effects.

W-3145
THE EFFECT OF ADIPOSE STEM CELL THERAPY ON PULMONARY FIBROSIS INDUCED BY REPETITIVE INTRATRACHEAL BLEOMYCIN IN MICE

Lee, Eun Joo[1], Lee, Sang Hoon[2], Lee, Sang Yeub[1], Je Hyeong, Kim[1], Shin, Chol[1], In, Kwang Ho[1], Uhm, Chang Sub[2], Kim, Han-Kyeom[2], Yang, Kyung-Sook[2], Park, Sanghoon[3], Kim, Hyun Soo[4], Kim, Yong Man[4], Yoo, Tai June[5]

[1]Korea University Anam Hospital, Korea University College of Medicine, Seoul, Republic of Korea, [2]Korea University College of Medicine, Seoul, Republic of Korea, [3]KEPCO Medical Center, Seoul, Republic of Korea, [4]Pharmicell Co.,Ltd., Seongnam-si, Republic of Korea, [5]University of Tennessee Health Science Center, Memphis, TN, USA

Adipose stem cells (ASCs) are detectable in the parenchyma and large airways of lungs after systemic administration, and ameliorate inflammatory infiltration and cell death in animal models of emphysema. We evaluated whether ASC treatment could attenuate lung fibrosis induced by repetitive intratracheal bleomycin administration. Male 8-week-old C57BL/6J mice (control group, bleomycin-only group, and bleomycin-plus-ASC group) were used. Eight biweekly doses of bleomycin were injected intratracheally via an intubation procedure at a dose of 0.04 units in a total volume of 100 μl of sterile saline. During the latter 2 months of the 4-month bleomycin exposure, human ASCs (3×10^5 cells) were administered repeatedly via intraperitoneal injection at the same time as bleomycin. Lung tissues were evaluated for histology, collagen content, TUNEL staining, and TGF- β levels. Bronchoalveolar lavage (BAL) was performed for cell counting. Administrations of ASCs ameliorated the deleterious effects of repetitive intratracheal instillation of bleomycin, namely hyperplasia of Club cells (Clara cells) and cuboidal alveolar epithelial cells, infiltration of the perialveolar ducts by inflammatory cells, septal thickening, enlarged alveoli, and extensive fibrosis. Addition of ASC lead to suppression of bleomycin-induced epithelial cell apoptosis and expression of TGF- β. These results suggest a useful therapeutic effect of ASCs on pulmonary fibrosis induced by repetitive bleomycin administration. Further studies will be required to evaluate the efficacy of ASC therapy for the treatment of idiopathic pulmonary fibrosis.

W-3146
BONE MARROW MESENCHYMAL STEM CELLS EXPRESSED SECRETED ANTIVIRAL ENTRY INHIBITORY(SAVE) PEPTIDES INHIBIT IN VITRO HIV INFECTION.

Lee, Narae[1], Walker, Edith[1], Egerer, Lisa[2], Laer, Dorothee von[2], Bunnell, Bruce A.[1], Braun, Stephen E.[1]

[1]Regenerative Medicine, Tulane National Primate Reserach Center, Covington, LA, USA, [2]Department of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University, Innsbruck, Austria

Mesenchymal stem cells (MSCs) have increased in use with their unique properties in cellular therapies and regenerative medicine. In addition, MSCs can be a vehicle for delivering genes to specific tissues in the body as many studies have reported in anti-cancer research. Infection with human immunodeficiency virus (HIV) results in CD4+ T cells depletion and the subsequent loss of immune function has led to the death of over 25 million people from AIDS. In combination, antiretroviral therapies control viremia; however, drug regimens are complex and expensive, require life-long intervention with potential side effects. Engineered MSCs to deliver desired vector and to enable them to express, it is possible to significantly improve the variety of antiretroviral drug therapy for which MSCs could provide benefit. Herein, we have shown the potential therapeutic role of the Secreted Anti-Viral Entry inhibitory (SAVE) peptide in transduced MSCs was efficiently block infection of new cells by interfering with the function of HIV-1 gp41. We measured the inhibition of HIV infection in vitro with C46-transduced MSCs. First, we transduced human and rhesus MSC (bone marrow-derived) with retroviral and lentiviral vectors (LV) expressing GFP (LZRS-GFP: murine leukemia virus, [MLV] and HRST-GFP [LV]), membrane-bound C46 (M218: MLV), or the secreted C46 (T-60: MLV and T-42: LV). Fluorescent microscopy and flow cytometry demonstrated that up to 69% of LV-transduced MSCs and 293T control cells expressed GFP. Molecular analysis of MLV ψ packaging element revealed that up to 25.5% of the human MSCs, rhesus MSCs, and 293T cells were transduced with the T-60 and M218 vectors. C46 was detectable in SAVE-transduced MSCs by western blot using 2F5 antibody. We sequentially conducted the single round infection assay to measure the inhibition of viral infection in vitro with conditioned medium from the SAVE-transduced MSCs. The data showed that conditioned medium from C46 and SAVE transduced rhesus bone marrow derived-MSC blocked the infection of the HIV vectors by from 60-75%. In order to test whether transduction and the insertion of the trans-gene affect the differentiation of MSCs potency, we conducted osteogenic, adipogenic, and chondrogenic differentiation assay on SAVE-transduced rhesus bone marrow derived MSCs. As a result, all the transduced rhesus bone marrow derived MSCs maintained differentiation potential. Thus, SAVE peptides expressed by MSCs may provide an alternative in vivo drug delivery system to AIDS patients and, in combination with other anti-retroviral therapies, thus promising to provide a permanent clinical efficacy for a diverse range of diseases.

W-3147
COMPLEMENT C3 SECRETED FROM MYCOPLASMA-INFECTED MESENCHYMAL STEM CELLS REGULATES ANTIBODY PRODUCTION IN B CELLS

Lee, Hyun-Joo[1], Kim, Junghee[1], Lee, Dong-Seok[1], Yi, TacGhee[2], Kim, Si-Na[1], Jeon, Myung-Shin[3], Song, Sun[4]
[1]Inha University School, Incheon, Republic of Korea, [2]INHA University School of Medicine, Incheon, Republic of Korea, [3]Inha University Hosp, Incheon, Republic of Korea, [4]Inha University School of Medicine, Incheon, Republic of Korea

Mesenchymal stem cells (MSCs) have immunomodulatory functions such as the suppression of T and B cells. MSCs suppress immunoglobulin (Ig) production by B cells via cell-cell contact as well as via secretion of soluble factors. Our study showed that the conditioned medium (CM) of MSCs infected with a mycoplasma strain, Mycoplasma arginini, has marked inhibitory effects on Ig production by lipopolysaccharide/interleukin 4-induced B cells compared with mycoplasma-free MSC-CM. We analyzed mycoplasma-infected MSC-CM by fast protein liquid chromatography and liquid chromatography to screen the molecules responsible for Ig inhibition. Complement C3 (C3) was the most critical molecule among the candidates identified. C3 was shown to be involved in the suppression of the Ig production of B cells. C3 was secreted by mycoplasma-infected MSCs, but not by mycoplasma-free MSCs or B cells. It was able todirectly inhibit Ig production by B cells. In the presence of a C3 inhibitor, Ig inhibition by MSC-CM was abrogated. This inhibitory effect was concomitant with the downregulation of B cell-induced maturation protein-1, which is a regulator of the differentiation of antibody-secreting plasma cells. These results suggest that C3 secreted from mycoplasma-infected MSCs plays an important role in the immunomodulatory functions of MSCs. However, its role in vivo needs to be explored

W-3148
MESENCHYMAL STEM CELLS DELIVER EXOGENOUS MIRNAS TO NEURAL CELLS AND IMPACT THEIR DIFFERENTIATION AND FUNCTIONS

Lee, Hae Kyung[1], Finniss, Susan[1], Xiang, Cunli[1], Cazacu, Simona[1], Brodie, Chaya[2]
[1]Henry Ford Hospital, Detroit, MI, USA, [2]Henry Ford Hospital, Southfield, MI, USA

MicroRNAs (miRNAs) emerge as potential therapeutic targets in a variety of pathological conditions in the central nervous system; however, their clinical application is hampered by lack of efficient delivery modes. Mesenchymal stem cells (MSCs) have been shown to migrate to sites of injury and inflammation in the brain and to exert therapeutic effects in a variety of pathological conditions in the CNS. We recently demonstrated the ability of MSCs to functionally deliver miRNA mimics to glioma cells in vitro and in vivo. In this study we examined the ability of MSCs to deliver exogenous miRNA mimics and pre-miRNAs to human neural progenitor cells (NPCs) and astrocytes and characterized the functional impact of this delivery. Using fluorescent labeled miR-124 mimic, we found that MSCs derived from different sources efficiently delivered this miRNA mimic to co-cultured NPCs and astrocytes after 24 hr in co-culture and this effect was sustained up to five days thereafter. We further demonstrated the delivery of miR-124 using novel reporter plasmids which contain a sequence fully complementary to miR-124 downstream of luciferase or mCherry, so that binding of miR-124 to this sequence results in decreased luciferase activity or mCherry fluorescence. These plasmids enable analysis of miRNA delivery in living cells both in vitro and in vivo. The delivered miR-124 mimics significantly decreased the expression of the target gene Sox-9 by targeting its 3'-UTR, and increased the neuronal differentiation of the NPCs as determined by the increased expression of β3-tubulin and the decreased expression of nestin. In addition, the delivered miR-124 increased the expression of the glutamate transporters, EAAT1 on NPCs and EAAT2 in both NPCs and astrocytes. Similar results were obtained with MSCs transduced with pre-miR-124. The increased expression of the glutamate transporters increased the glutamate uptake by the cells and was induced by a yet unknown indirect effect of miR-124. We found that the miRNA delivery was mostly mediated by MSC-derived exosomes and was not gap junction dependent. In vivo studies demonstrated beneficial therapeutic impact of MSCs transduced with pre-miR-124 in mouse models of ALS and Parkinson's disease. These results suggest that MSCs can functionally deliver exogenous miRNAs to neural cells and provide an efficient route of therapeutic miRNA delivery to the brain in pathological conditions with clinical implications for regenerating medicine.

W-3150
THE MECHANISM OF IMMUNOMODULATION OF MESENCHYMAL STEM CELLS ISOLATED FROM HUMAN AMNION, PLACENTA AND UMBILICAL CORD

Manochantr, Sirikul[1], Meesuk, Ladda[1], Kheolamai, Pakpoom[1], Tantrawatpan, Chairat[1], U-pratya, Yaowaluk[2], Issaragrisil, Surapol[2]
[1]Division of Cell Biology, Department of Preclinical Sciences, Faculty of Medicine, Thammasat University, Pathumthani, Thailand, [2]Division of Hematology, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Mesenchymal stem cells (MSCs) basically isolated from bone marrow (BM) are considered as adult stem cells that can be differentiated to become bone, cartilage and other mesenchymal tissues under appropriate culture conditions. In addition to multilineage differentiation capacity, BM-MSCs also have an ability to promote hematopoietic stem cell engraftment and prevent graft versus host disease (GvHD) in patients who receive allogeneic stem cell transplantation. However, the collection of bone marrow is an invasive and painful procedure. Therefore, seeking for an alternative source of MSCs for transplantation is of a great value. MSCs derived from umbilical cord (UC-MSCs), placenta (PL-MSCs) and amnion (AM-MCSs) are suitable substitutes for BM-MSCs because of their non-invasive procedure for collection. However, the biological and immunoregulatory properties of UC-MSCs, PL-MSCs and AM-MSCs are still poorly characterized. Particularly; the underlying mechanisms of this immunomodulation remain undefined. Recent research demonstrated that BM-MSCs express the tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO), known to suppress T-cell responses. This study was designed to address whether IDO contributes to the immunoregulatory functions of UC-MSCs, PL-MSCs and AM-MSCs *in vitro*. To isolate UC-MSCs, PL-MSCs and AM-MSCs, umbilical cord, placenta and amnion were digested with collagenase and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum. The expression profiles of several MSC markers were examined by flow cytometry. UC-MSCs, PL-MSCs and AM-MSCs from passage 3-5 were used for *in vitro* adipogenic and osteogenic differentiation assays by culturing in appropriate induction media. The results demonstrated that UC-MSCs, PL-MSCs and AM-MSCs were easily expanded to 18-20 passages while maintaining the undifferentiated state and exhibiting MSC markers CD73, CD90, and CD105 but do not express the hematopoietic markers CD34 and CD45. Similar to BM-MSCs, UC-MSCs, PL-MSCs and AM-MSCs were able to differentiate to several mesodermal-lineages including adipocytes and osteoblasts. Fascinatingly, these cells also exhibited comparable immunomodulatory effects when they

were co-cultured with activated T-cells in dose-dependent manner. In addition, IDO secreted by UC-MSCs, PL-MSCs and AM-MSCs was responsible for induction of immunosuppressive activities in the same manner as BM-MSCs. Taken together; the results of the present study demonstrate that while UC-MSCs, PL-MSCs, AM-MSCs and BM-MSCs show similar immunomodulatory effects, UC-MSCs, PL-MSCs, AM-MSCs may have additional advantage over the BM-MSCs in terms of availability. Therefore, UC-MSCs, PL-MSCs, AM-MSCs might be considered a potential source for therapeutic applications.

W-3151
ENVIRONMENTAL CONDITIONS AFFECT THE ANTI-INFLAMMATORY ACTION OF BONE MARROW STROMAL CELLS

Marko, Karoly, Krepuska, Miklos, Mezey, Eva
Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research, Bethesda, MD, USA

Bone marrow stromal cells (BMSCs) are considered to be promising candidates in cell therapy. In addition to their potential in tissue regeneration, their immune-modulatory (anti-inflammatory) effects are thought to help in the treatment of diseases such as sepsis, asthma or GVHD. Since inflammation is usually accompanied by elevated temperature (fever) and local hypoxia, we tested the possible role of these conditions on the immune-modulatory effect of human BMSCs. We used co-culture assays to study how human BMSCs (from healthy volunteers based on protocol NCT01071577) effect cytokine production by macrophages following LPS stimulation. We cultured two different human macrophage lines (THP-1 and U-937) with and without the BMSCs. We first confirmed the already described anti-inflammatory effect in control conditions, i.e. upon co-culture with BMSCs and LPS stimulation, the TNF-alpha and IL-10 production of macrophages were greatly decreased and increased, respectively. Both hypoxia (1% pO2) and elevated temperature (38.5 °C) alone and additively decreased the TNF-alpha production of both human macrophage cell lines. This effect was further decreased following co-culture with BMSCs. In addition, pretreatment of the BMSCs in a hypoxic environment before the co-culture was set up further decreased the TNF-a production of macrophages. On the other hand, IL-10 production of macrophages was not detectable in the absence of BMSCs in either condition using ELISA. However, upon co-culture with BMSCs and stimulated with LPS, the macrophages produced a significant amount of IL-10, which was further increased by elevated temperature. Our in vitro data suggest multiple effects of environmental conditions on the inflammatory responses of macrophages and suggest an important interaction between the BMSCs and the macrophages. Further in vitro experiments are necessary to clarify the underlying mechanisms and also in vivo studies to confirm the physiological relevance of the in vitro findings.

W-3152
AUTOLOGOUS ADIPOSE STEM CELLS IN TREATMENT OF FEMALE STRESS URINARY INCONTINENCE - RESULTS OF A PILOT STUDY

Miettinen, Susanna
BioMediTech, University of Tampere, Tampere, Finland

Urinary incontinence is a common health problem affecting a large number of women. Adipose stem cells (ASCs) provide a novel alternative to invasive surgical treatment of stress urinary incontinence (SUI). The objective of our study was to find out if transurethral injections of autologous ASCs in combination with injectable bulking agent are an effective and a safe treatment for female SUI. Five SUI patients were treated with ASCs combined with bovine collagen gel and saline solution between. The subcutaneous fat from lower abdomen was collected under local anaesthesia. The ASCs were isolated and expanded for three weeks. Multipotency (adipogenic, osteogenic, chondrogenic and myogenic differentiation) and mesenchymal stem cell surface marker profile (CD14, CD19, CD34, CD45, CD73, CD90, CD105, HLA-ABC-PE, and HLA-DR) of ASCs was analysed. Moreover, chromosomal integrity, sterility, endotoxins and mycoplasma were determined before cell transplantation. Mixture of ASCs and collagen gel was injected transurethrally via cystoscope. The patients were followed at 3, 6 and 12 months after the injections. The primary end point was cough test to objectively measure the effect of the treatment. Validated questionnaires were used for determining the subjective cure rate. At six months, one out of five patients displayed a negative cough test with full bladder filled with 500ml saline. At one year, the cough test was negative with three patients; two of them were satisfied with the treatment and didn t wish for further treatment for SUI. There was some subjective improvement with all five patients. This is the first study describing use of autologous ASCs in combination with collagen gel in treating SUI in women. So far the treatment with autologous ASCs has proven safe and well tolerated. However, feasibility and efficacy of the treatment were not optimal and more studies are needed to find a better carrier material for ASCs and the ideal technique for the transurethral injections.

W-3153
AGE-RELATED CHANGES IN BONE MARROW STEM CELL MOBILIZATION AND PERIPHERAL RECRUITMENT ARE IMPROVED BY MESENCHYMAL STEM CELLS OR HYPERBARIC OXYGEN

MilovanovaI, Tatyana N.[1], Sorokina, Elena M.[1], Moore, Jonni S.[2], Thom, Stephen R.[1]
[1]*Emergency Medicine, University of Pennsylvania, Philadelphia, PA, USA,* [2]*Abramson Cancer Center and Flow Cytometry Core Facility, University of Pennsylvania, Philadelphia, PA, USA*

Introduction: Hyperbaric oxygen (HBO_2) stimulates mobilization and peripheral recruitment of bone marrow stem/progenitor cells (SPCs) in animals, but the effect of age is unknown. As 1 mouse year is ~40 human years, most studies are conducted on animals equal to ~ 7 year old humans. We hypothesized that HBO_2 has similar effects on SPCs and adipose-derived mesenchymal stem cells (MSCs) in old animals. Materials and methods: Studies involved 'young' (8 weeks) and 'old' (17 months) mice. SPCs ($Sca\text{-}1^+/CD34^+$) and MSCs ($Sca\text{-}1^+/CD34^-/CD31^-/DAPI^-$) were analyzed by flow cytometry and MSCs stained with carboxyfluorescein-succinimidyl-ester (CFSE). Two Matrigel pillows (1 ml) injected subcutaneously in lateral sides of mice, one containing 9,000 MSCs, were harvested 18 hours later. Mice were kept in room air (control) or received HBO_2 (2.8 ATA O_2 for 90 minutes) immediately and again 12 hours after implantation. Results: Matrigel implants from young control mice had 1.4 +/- 0.10 (n=4) x 10^6 SPCs; old mice had 0.54 +/- 0.06 (n=4, $p<0.05$) x 10^6 SPCs. Implants in young mice receiving HBO_2 had 3.3 +/- 0.17 x 10^6 SPCs (n=4, $p<0.05$ vs control); implants from old mice receiving HBO_2 had 1.3 +/- 0.09 x 10^6 SPCs (n=4, $p<0.05$ vs young HBO_2, NS vs young control). Cell counts in harvested Matrigel with MSCs were insignificantly different between young and old mice. Young control mice had 2.2 +/- 0.21 x 10^6 CFSE-CD34+ cells and 2.2 +/- 0.09 x 10^6 CFSE+ CD34+ cells; old mice had 1.6 +/- 0.07 x 10^6 CFSE- and 1.9 +/- 0.05 x 10^6 CFSE+ cells. HBO_2 more than doubled cell counts in MSCs-containing implants from young and old mice: 8.7 +/- 0.33 x 10^6 CD34+ cells (young), 9.6 +/- 0.25 x 10^6 (old). Conclusions: HBO_2 or MSCs normalize SPCs recruitment to Matrigel implants in old mice, in combination they are synergistic.

Financial Disclosure: This work was supported by funds provided by NIH grant R01-DK094260 and the Office of Naval Research to SRT.

JUNE 19, 2014

THURSDAY POSTER PRESENTATIONS

6:00 - 7:00 ODD numbered posters presented
7:00 - 8:00 EVEN numbered posters presented

PANCREATIC CELLS

T-1007
MACROPHAGE AND EPITHELIUM CROSS TALK REGULATES CELL CYCLE PROGRESSION AND MIGRATION IN PANCREATIC PROGENITORS

Mussar, Kristin E.[1], Tucker, Andrew[2], McLennan, Linsey[2], Gearhart, Addie[2], Jimenez-Caliani, Antonio[2], Cirulli, Vincenzo[2], Crisa, Laura[2]
[1]Pharmacology, University of Washington, Seattle, WA, USA, [2]Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA, USA

Macrophages populate the mesenchymal compartment of all organs during embryogenesis and have been shown to support tissue organogenesis and regeneration by regulating remodeling of the extracellular microenvironment. Whether this mesenchymal component can also dictate select developmental decisions in epithelia is unknown. Using the embryonic pancreatic epithelium as model system, we found that macrophages drive the epithelium to execute two developmentally important choices, i.e. the exit from cell cycle and the acquisition of a migratory phenotype. We demonstrate that these developmental decisions are effectively imparted by macrophages activated toward an M2 fetal-like functional state, and involve modulation of the adhesion receptor NCAM and an uncommon "paired-less" isoform of the transcription factor PAX6 in the pancreatic epithelium. Over-expression of this PAX6 variant controls both cell motility and cell cycle progression in a gene-dosage dependent fashion. Importantly, induction of these phenotypes in embryonic pancreatic transplants by M2 macrophages in vivo is associated with an increased frequency of endocrine-committed cells emerging from ductal progenitor pools. In addition, analysis on the expression of paired-less PAX6 in embryonic stem cells undergoing directed differentiation toward pancreatic lineages reveals induction of this isoform at select stages of differentiation. Further experiments are in progress to determine developmental effects of its modulation in these cultures. These results identify M2 macrophages as key effectors capable of coordinating epithelial cell cycle withdrawal and cell migration, two events critical to pancreatic progenitors' delamination and progression toward their differentiated fates. The functional properties of macrophages identified here may be exploited for the development of improved protocols of in vitro directed differentiation of pancreatic islet cells from stem cells.

T-1008
CHROMATIN REMODELING ESTABLISHES ENDOCRINE LINEAGE COMMITMENT IN MULTI-POTENT PANCREATIC PROGENITORS

Patel, Nisha A.[1], Nguyen, Tung T.[2], Ai, Rizi[2], Xie, Ruiyu[1], Wang, Allen[1], Benthuysen, Jacqueline R.[1], Rosenfeld, Michael G.[3], Wang, Wei[2], Sander, Maike[4]
[1]Pediatrics, University of California, San Diego, La Jolla, CA, USA, [2]Chemistry and Biochemistry, Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, USA, [3]Medicine, Howard Hughes Medical Insitute, University of California, San Diego, La Jolla, CA, USA, [4]Pediatrics, Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, USA

Much effort is being expended on attempts to derive transplantable insulin-producing beta-cells from human embryonic stem cells for treating diabetes mellitus. However, current protocols fail to support the generation of functional beta-cells, necessitating a better understanding of the molecular mechanisms underlying beta-cell development. The transcription factor network regulating genesis of beta-cells and other endocrine cell types in the embryonic pancreas has been comprehensively dissected. In contrast, the chromatin remodeling events regulating pancreatic gene expression and orchestrating proper endocrine cell development remain largely unexplored. Here, we used genome-wide analysis of chromatin modifications to map and compare active regulatory elements in multi-potent pancreatic progenitors and terminally-differentiated endocrine cells. While chromatin modifications at promoters were remarkably similar between progenitors and differentiated endocrine cells, we found that enhancer usage between the two cell types was distinct. Further cistrome analysis demonstrated that endocrine lineage-determining transcription factors were recruited to progenitor-specific enhancers in pancreatic progenitors and these enhancers were subsequently inactivated upon endocrine differentiation. Using in vivo and in vitro models of pancreas development, we were able to identify an uncharacterized chromatin remodeling factor that is recruited to these progenitor-specific enhancers. Pancreatic progenitors lacking this factor's activity do not form endocrine cells and fail to undergo chromatin remodeling events associated with endocrine cell differentiation. Spatially-controlled inactivation of this factor during pancreas development further revealed that the requirement for this factor precedes the onset of endocrine cell differentiation by several days. This is the first demonstration that early chromatin remodeling events are necessary in order for endocrine lineage induction to occur later in development. Together, our findings suggest that chromatin remodeling events prime development of the endocrine lineage early during pancreatic development.

T-1009
INTRAPANCREATIC TRANSPLANTATION OFFERS AN ALTERNATIVE SITE FOR CHEMICALLY REPROGRAMMED INSULIN PRODUCING CELLS

Rueda-Argumedo, Nelson A.[1], Pereyra-Bonnet, Federico[2], Hyon, Sung H.[1], Gimenez, Carla[1], Argibay, Pablo F.[1]
[1]Hospital Italiano de Buenos Aires, Buenos Aires, Argentina, [2]ICBME, Hospital Italiano, Buenos Aires, Argentina

Studies on stem cells (SC) show that SC functions are determined, among other factors, by the extracellular microenvironment known as the "niche". The pancreas represents a strategic location for providing all the extracellular cues to induce beta cell maturation. Insulin producing cells reprogrammed from different cell sources have been transplanted successfully in several ectopic niches; however,

the intrapancreatic transplantation efficiency remains yet to be proven. We transplanted PDX1+ NGN3+ chemically reprogrammed fibroblasts from patients with diabetes type 1 intrapancreatically (n= 8) and in the kidney capsule (n= 4) of streptozotocin(STZ)-treated nude mice. Before transplantation, we demonstrated by RT-PCR that chemically reprogrammed cells expressed, in vitro, most of the beta cell transcription factors (PDX1, NGN3, GLUT2, PAX4, PAX6, ISL-1), but not insulin, compared with the human pancreas. After transplantation, the mean mortality rate in mice was measured to assess operative feasibility between the two transplant locations. The mean mortality rate was 16.6% and 20% for pancreas and kidney capsule, respectively. There was no significant difference ($P<0.05$) in blood glucose kinetics or weight loss between the pancreas and kidney capsule, although an amelioration of a hyperglycemia state was evidenced in comparison to sham controls. Moreover, monitoring of the blood glucose levels revealed that the mouse endogenous beta cells were destroyed by the STZ treatment. After 1-month transplantation, we detected the presence of human insulin transcripts by qPCR with specie-specific primers in 1/8 (12.5%) of the mice in the intrapancreatic location and 1/4 (25%) of mice in the kidney location. Upon glucose stimulation of the implanted mice, human insulin hormone was only detected by Inmunolite® assay in mice transplanted intrapancreatically (1/8). Moreover, we tested in a non-streptozotocin nude mouse (n=7), possible damages caused by long exposure of cells to the pancreatic niche (during 3 months). As a result, we did not find any signal of pancreas damage nor teratoma formation, and these mice also tested positive for human insulin in blood by ELISA assay (2/7; 28.5%). These findings suggest that intrapancreatic transplantation may be an approach that could somewhat induce rapid insulin hormone release in blood in vivo and offer an equally safe surgical alternative in comparison to the kidney capsule transplantation.

T-1010
VMAT2 IDENTIFIED AS A REGULATOR OF LATE-STAGE PANCREATIC BETA-CELL DIFFERENTIATION

Sakano, Daisuke[1], Shiraki, Nobuaki[1], Kikawa, Kazuhide[1], Yamazoe, Taiji[1], Kataoka, Masateru[1], Umeda, Kahoko[1], Araki, Kimi[2], Mao, Di[3], Matsumoto, Shirou[4], Nakagata, Naomi[5], Andersson, Olov[6], Stainier, Didier Y.[7], Endo, Fumio[4], Kume, Kazuhiko[1], Uesugi, Motonari[3], Kume, Shoen[1]

[1]Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan, [2]Laboratory of Developmental Genetics, Institute of Resource Development and Analysis, Kumamoto University, Kumamoto, Japan, [3]Kyoto University, Uji, Kyoto, Japan, [4]Department of Pediatrics, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan, [5]Division of Reproductive Engineering, Center for Animal Resources and Development, Kumamoto University, Kumamoto, Japan, [6]Department of Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden, [7]University of California San Francisco, San Francisco, CA, USA

The pancreatic transplantation and the pancreatic islet transplant are known as effective treatments for type I diabetes. Donor shortage is an obstacle to the treatment of type I diabetes. Despite numerous efforts, it is still difficult to efficiently produce mature insulin-secreting beta cell from ES cells. Recently, we established an efficient β-cell induction system for the screening of low molecular compounds. Previously several reports focused the differentiation process from ES cells into definitive endoderm or pancreatic progenitor cells. We focused on the late stages of the differentiation from pancreatic progenitor into insulin producing beta cells and performed a cell-based screening of chemical library. We identified reserpine as a small molecule that inhibits vesicular monoamine transporter 2 (VMAT2)-mediated secretion of monoamine that promoted differentiation of Pdx1-positive progenitors into insulin-expressing cells. And we also observed the VMAT2 inhibition increased insulin producing cells as well as in vitro cell differentiation assay. Our analyses suggest that one of the VMAT2-controlled monoamines, dopamine, serotonin and histamine negatively regulates beta cell differentiation. Exogenous addition of a cell-permeable cAMP analogue, dBu-cAMP, canceled these inhibitory effects on beta cell differentiation and synergized with VMAT2 inhibition. The ES cell derived beta cells showed glucose-sensitive insulin secretion ability, and reversed hyperglycemia upon engraftment into AKITA diabetic model mice.

T-1011
CHARACTERIZATION OF MURINE PANCREAS-RESIDENT MESENCHYMAL STROMAL CELLS USING A NEW MARKER, EXPRESSED IN MESENCHYMAL CELLS (EMC)

Schreiner, Petra[1], Scott, Wilder[2], Ng, Betina[2], Wong, Derek[2], Choi, HeeJung[2], Underhill, Michael[2]

[1]Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada, [2]University of British Columbia, Vancouver, BC, Canada

Mesenchymal stromal cells (MSC) play fundamental roles in tissue development, homeostasis and regeneration. In homeostasis, MSCs are in a quiescent state and become "activated" in response to various signals. Activation is associated with MSC entry into the cell cycle where they contribute to the microenvironment and support tissue regeneration. These cells are associated with pathological conditions including accelerated aging syndromes, cancer and fibrosis. Previous studies analyzing MSCs in skeletal development identified a gene (Expressed in mesenchymal cells, Emc) that appears to be solely expressed within embryonic mesenchyme. To characterize the expression and function of Emc, a conditional knockout mouse line incorporating a nuclear LacZ reporter into the Emc locus was generated. Analysis of reporter gene activity in adult tissues, along with immunodetection, revealed that Emc expression is localized to interstitial stromal and perivascular cells in many tissues. In pancreas, EMC+ cells overlap extensively with platelet derived growth factor receptor alpha (Pdgfrα), Sca1 and CD34, which together identify putative MSCs in various tissues. To track the fate of Emc+ cells, a CreERT2 knock-in mouse has been generated; preliminary studies indicate this mouse is useful for identifying, manipulating and following the fate of MSCs. Without damage, these cells remain in a quiescent state within the pancreas, exhibiting little perceived turnover or activity over six months. Pancreatic EMC+ cells may be pancreatic stellate cells (PSCs), as they both express vimentin and nestin, and contain peri-nuclear lipid droplets consistent with retinol stores once cultured. PSCs are suggested to be effector cells, becoming activated in conditions such as pancreatic cancer, fibrosis or pancreatitis. Upon cerulein administration to induce pancreatitis, EMC+ cells become activated, enter the cell cycle and express α-smooth muscle actin (αSMA) within the damaged areas, presumably creating an environment conducive to regeneration. As the damage is resolved, the labeled cells return to their normal frequency and distribution. This pattern of damage followed by activation is repeated in various damage models, including muscle damage and lung fibrosis. After conditional deletion of Emc in adult mice these pancreatic MSCs, as marked by Sca1 expression, display increased EdU incorporation. Within two weeks after Emc deletion the pancreas contains significantly increased numbers of perilipin+ adipocytes in comparison to controls. Studies are now ongoing to determine the impact of Emc deletion on pancreas regeneration following cerulein-induced damage. These experiments are complemented by analysis of the impact of EMC+-cell ablation on pancreas homeostasis and regeneration, along with RNA-seq-based

analyses of the transcriptome. These results will be presented, and together will provide critical insights into the nature of the trophic factor support provided by MSCs in the regenerating microenvironment.

LIVER CELLS

T-1012
ENHANCEMENT OF HEPATOCYTE DIFFERENTIATION FROM HUMAN EMBRYONIC STEM CELLS BY CHINESE MEDICINE FUZHENGHUAYU

Chen, Jiamei[1], Gao, Wei[1], Ma, Xiaocui[1], Tschudy-Seney, Benjamin[1], Hasan, Ahmad[1], Liu, Chenghai[2], Duan, Yuyou[1], Liu, Ping[2], Zern, Mark[1]
[1]University of California Davis Medical Center, Sacramento, CA, USA, [2]Institute of Liver Diseases, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China

Previous reports indicate that Chinese medicine, Fuzhenghuayu (FZHY), appears to prevent fibrosis progression and improve liver function in humans. In this study, we employed FZHY to evaluate whether hepatocyte differentiation from human embryonic stem cells (hESC) is enhanced. hESC line, H9 was used in our standard differentiate protocol, and FZHY was added to the culture medium at day 10 of differentiation at a final concentration of 50 μg/ml until day 27. Liver genes and epithelial mesenchymal transition (EMT) markers were evaluated in control and treated hESC-derived hepatocytes (hEH). Quantitative PCR (qPCR) results showed that albumin expressions increased at 2.0 fold and tyrosine aminotransferase expression 7 fold. qPCR revealed that expression of important phase I and II metabolizing enzymes, and a transporter were also increased: 2.0 fold for CYP3A4, 2.2 fold for CYP7A1, 2.0 fold for UGT1A1, 7.1 fold UGT1A3, 3.7 fold for UGT1A6, 14 fold for UGT1A8, 1.6 fold for UGT2B7, and 2.1 fold for glucose transporter 2. This indicated that biotransformation function of hEH would likely be increased. Asialoglycoprotein receptor, ASGPR, is a mature hepatocyte marker. qPCR results showed that the expression of ASGPR was increased by 2.2 fold, indicating that FZHY might promote the differentiation and maturation process. ELISA showed that albumin secretion in the medium was also increased over 35%, further indicating that FZHY enhanced the differentiation process. Importantly, we found that expression of EMT markers, α-SMA, vimentin, and N-cadherin (N-cad), were significantly reduced by 60%, 58%, and 71% at day 27. This suggests that one possible mechanism by which FZHY may promote hepatocyte differentiation and maturation is through inhibition of EMT. Our hEH have showed proliferation for two weeks in primary culture during differentiation, and they have maintained this proliferation in secondary culture after splitting, as determined by cell counting. This proliferative capacity also could be demonstrated that our hEH co-expressed albumin and Ki67, a proliferation marker at day 25 after differentiation. Moreover, we found that FZHY could enhance the proliferation of our hEH. qPCR results indicated that Ki67 expression was increased by 1.8 fold at day 20, and 2.2 fold at day 27 of differentiation. These results showed a decrease of EMT markers and an increase of hepatocyte markers after treatment with FZHY; these results further indicate that the enhanced proliferation involved hepatocytes rather than another cell type. Expression of wnt1, wnt3a, wnt7a, and wnt7b was increased by 2.5, 2.4, 2.2 and 3.5 fold, as determined by qPCR, this suggests that the enhancement of hepatocyte differentiation is likely mediated through activation of wnt signaling pathway by FZHY. In conclusion, our results indicate that FZHY not only promoted hepatocyte differentiation and maturation, but also enhanced hEH proliferation. They suggest that FZHY treatment may enhance our effort to generate mature hepatocytes with proliferative capacity for cell-based therapeutics and for pharmacological and toxicological studies.

T-1013
DIRECT CONVERSION OF MOUSE FIBROBLASTS INTO FUNCTIONAL INTEGRATION FREE HEPATOCYTE-LIKE CELLS

Kim, Jonghun
Konkuk University, Seoul, Republic of Korea

Recent works have been shown that fibroblasts can be directly converted into various types of cells with defined set of transcription factors, bypassing a pluripotent state. In addition, it was demonstrated that the combinations of hepatic transcriptional factors (Hnf4a plus Foxa1 or Foxa2 or Foxa3 and Gata4/Hnf1a/Foxa3) could directly convert fibroblasts into functional hepatocytes-like cells, namely induced hepatocytes (iHeps) using viral systems. However, insertional mutagenesis caused by viral integration into host genome increases the risk of tumorigenecity. Here, we describe that mouse fibroblasts can be converted into hepatocytes-like cells by using a set of hepatic transcriptional factors (Gata4, Hnf1a and Foxa3) based on integration-free vector systems. Integration-free iHeps are stably maintained in vitro and have hepatic features similar to hepatocytes in terms of morphology, marker expression, global gene expression pattern and functionality. Therefore, these results suggest that integration-free iHeps could be an alternative source for cell therapy and drug screening for hepatic disease.

T-1014
A NEW GENERATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED HEPATOCYTES DISPLAYS ADULT CHARACTERISTICS AND A SUBSTANTIALLY IMPROVED FUNCTIONALITY

Kuppers-Munther, Barbara[1], Asplund, Annika[2], van Giezen, Mariska[1], Bjorquist, Petter[3]
[1]Cellectis AB, Gothenburg, Sweden, [2]School of Biosciences, University of Skövde, Skövde, Sweden, [3]Novahep AB, Gothenburg, Sweden

Hepatocytes derived from human pluripotent stem cells have the potential to provide relevant human in vitro model systems for toxicity testing and drug discovery studies. However, until recently, the functionality of stem cell-derived hepatocytes has remained on a level that renders the cells sub-optimal for drug metabolism studies. In this study, we present a new generation of human induced pluripotent stem cell-derived hepatocytes, Enhanced hiPS-HEP2™, which display several adult hepatic features (e.g, expression of the adult enzymes CYP2C9, 2C19, 2E1 and 3A4 as well as LDL uptake). Importantly, the cells display substantial expression levels and functional activity of drug metabolizing enzymes relevant for toxicity testing and drug metabolism (e.g., CYP1A, 2C9, 2C19, 2D6, 2E1, and 3A4). To the best of our knowledge, this is the first time such improved functionality is described for stem cell-derived hepatocytes in a 2D culture system. Due to the substantially improved functionality, the Enhanced hiPS-HEP2™ are highly suitable for toxicity testing and drug metabolism studies, as well as other applications relying on an inexhaustible source of functional human hepatocytes.

T-1015

HEPATITIS C VIRUS-HOST INTERACTION WITH STEM CELL DERIVED HEPATOCYTES

Lee, Emily M.[1], Wu, Xianfang[1], Hookway, Tracy[2], Hammack, Christy[1], McDevitt, Todd[2], Tang, Hengli[1]

[1]Department of Biological Science, The Florida State University, Tallahassee, FL, USA, [2]The Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, USA

Primary human hepatocytes represent the most physiologically relevant cell culture model for hepatitis C virus (HCV) infection but are highly variable, not readily accessible and refractory to genetic manipulation. We have recently reported a novel, alternative cell culture model for HCV infection utilizing differentiated hepatocyte-like (DHH) cells derived from human embryonic or induced pluripotent stem cells (hESCs or iPSCs). DHHs are permissive to infection by both cell culture-adapted HCV (HCVcc) and serum-derived HCV (HCVser). In patients, treatment responsiveness and disease progression is correlated with HCV genotype. Of the seven reported genotypes of HCV, only a single isolate of genotype 2a has been made widely available for use in studying the full viral lifecycle in cell culture. We report the direct infection of DHHs by additional genotypes derived from patient sera, which are not infectious in the predominant cancer cell line system (HuH-7.5). In addition, we have identified a transition phase during differentiation of stem cells to hepatocyte-like cells that confers permissiveness to HCV infection. Transcription microarray analysis during this transition phase revealed novel potential proviral and antiviral factors in HCV infection, which are the subject of further study. Furthermore, we have applied novel three dimensional rotary culture techniques for the HCV-DHH model to further mimic physiological liver conditions and expand the robustness of the patient serum-derived infections. This rotary system has supported the formation of cell aggregates which may bridge the experimental systems gap between monolayer cell culture and small-animal model systems.

T-1017

MICRORNA 199A INHIBITION LEADS TO IMPROVED IN VITRO HEPATIC DIFFERENTIATION AND ENHANCED IN VIVO LIVER REPOPULATION FROM EMBRYONIC STEM CELL DERIVED HEPATOCYTE LIKE CELLS

Möbus, Selina[1], Yang, Dakai[1], Yuan, Qinggong[2], H.-W. Lüdtke, Timo[2], Balakrishnan, Asha[2], Sgodda, Malte[2], Rani, Bhavna[2], Kispert, Andreas[2], J. Araúzo-Bravo, Marcos[3], Vogel, Arndt[2], P. Manns, Michael[2], Ott, Michael[2], Cantz, Tobias[2], Deep Sharma, Amar[1]

[1]Hans-Borst-Zentrum, Hannover Medical School, Hannover, Germany, [2]Hannover Medical School, Hannover, Germany, [3]Max Planck Institute for Molecular Biomedicine, Münster, Germany

Currently, liver transplantation is the only life-saving procedure for patients suffering from end-stage liver disease. However, its potential benefits are mainly hampered by severe shortage of liver donors. Alternative therapeutic concepts such as cell therapies or tissue engineering also require access to large numbers of hepatocytes. Thus, the development of novel strategies, which can provide ample number of therapeutically relevant hepatocytes for transplantation, is of the utmost importance. One very attractive approach is the directed differentiation of embryonic stem cells (ESCs) into hepatocyte-like cells (HLCs) by mimicking the *in vivo* liver development *in vitro*. Nevertheless, current hepatic differentiation protocols for human ESCs require substantial improvements, as the resulting HLCs do not match the therapeutic potential of primary hepatocytes for cell-based therapies. It has been demonstrated that microRNAs (miRNAs), post-transcriptional regulators of gene expression, regulate hepatocyte cell fate during liver development. However, their precise role during hepatic *in vitro* differentiation and their utility for the generation of functional hepatocytes remain to be explored further. The aim of the present study was to identify and to analyze hepatogenic miRNAs for their potential to enhance the hepatic *in vitro* differentiation of ESCS. Based on miRNA profiling from ESCs, HLCs differentiated from ESCs, fetal liver and adult primary hepatocytes, we chose 20 conserved candidate miRNAs to test their hepatogenic potential. According to the following miRNA screening, we found that inhibition of miR-199a-5p in HLCs facilitates efficient hepatocyte differentiation from mouse as well as human ESCs. MiR-199a-5p is one of the most abundantly expressed miRNAs in HLCs, whereas in primary human or mouse hepatocytes it is barely detectable. Therefore, it can be hypothesized that by miR-199a-5p inhibition during hepatic differentiation the HLCs come closer to the natural hepatocyte state. Upon transplantation, miR-199a-5p inhibition in human ESCs-derived HLCs leads to their engraftment and repopulation in the liver of immunodeficient fumarylacetoacetate hydrolase knockout (*Fah*$^{-/-}$/*Rag2*$^{-/-}$/*Il2rg*$^{-/-}$) mice. Furthermore we show for the first time, that human ESCs-derived HLCs are able to engraft and to repopulate the liver of this certain mouse model. For the elucidation of the underlying molecular mechanism, we identified SMARCA4 and MST1 as two novel targets of miR-199a-5p that contribute to the improved *in vitro* HLCs generation as well as *in vivo* liver repopulation. Additionally, we provide first insights into the role of miR-199a-5p during fetal liver hepatoblast development. Taken as a whole, our findings suggest that miRNA modulation may offer a promising approach to generate more mature HLCs from stem cell sources for the treatment of liver diseases.

T-1018

HUMAN IPSC-DERIVED HEPATOCYTE SHEET TRANSPLANTATION ENHANCES THE SURVIVAL RATE OF ACUTE LIVER FAILURE MICE

Nagamoto, Yasuhito[1], Takayama, Kazuo[1], Ohashi, Kazuo[1], Sakurai, Fuminori[1], Tachibana, Masashi[1], Kawabata, Kenji[2], Mizuguchi, Hiroyuki[1]

[1]Osaka University, Suita, Japan, [2]National Institute of Biomedical Innovation, Osaka, Japan

Hepatocyte transplantation is one of the most attractive approaches to rescue patients from liver failures, such as liver cirrhosis, hepatocellular carcinoma, and drug-induced liver injury. Because human induced pluripotent stem cell-derived hepatocyte-like cells (iPS-HLC) can be produced on a large scale and generated from a patient with liver failure, these cells are expected to be used in hepatocyte transplantation. Conventional transplantation methods, either intrasplenic or portal venous injection, have some difficulty in controlling engraftment efficacy and avoiding unexpected engraftment in the other organs because the transplanted cells are delivered into systemic circulation before engrafting into the liver. Therefore, we herein attempted to employ a cell sheet engineering technology for experimental hepatocyte transplantation. Delivering a large number of cells and localizing the cells at a desired area (liver surface) are main objectives of the present study. To compare a cell sheet transplantation with a direct cell injection method, we transplanted the human iPS-HLC to the two-thirds partially hepatectomized mice (Rag2$^{-/-}$Il2rg$^{-/-}$) by these two methods. First, we assessed the distribution of the transplanted cells in these recipient mice at 1 day after transplantation. Cell engraftment in organs other than the liver was decreased in the human iPS-HLC sheet transplantation group as compared with the intrasplenic transplantation group. Next, the serum human albumin

(ALB) levels in recipient mice were measured at 2 weeks for assessing cell engraftment levels. The human ALB levels in the human iPS-HLC sheet transplantation group showed significantly higher values than those in the intrasplenic transplantation group, suggesting high potential for cell engraftment of the sheet transplantation procedure. Finally, we validated therapeutic potentials of the human iPS-HLC sheet transplantation using a mouse model of lethal acute liver injury induced by carbon tetrachloride (CCl_4). The human iPS-HLC sheet transplantation successfully improved the survival rate of these mice compared with the human iPS-HLC intrasplenic transplantation (69.2% vs 36.4%, 7 days). Taken together, the present study demonstrated that the human iPS-HLC sheet transplantation offers efficient cell engraftment at a target site and provides therapeutic values toward CCl4-induced liver injuries. Thus, the human iPS-HLC sheet transplantation could be established as a reliable therapeutic approach for a patient with severe liver diseases.

T-1019
DOWN REGULATION OF SOX9 EXPRESSION ASSOCIATES WITH HEPATOGENIC DIFFERENTIATION OF HUMAN LIVER MESENCHYMAL STEM/PROGENITOR CELLS

Najimi, Mustapha[1], Paganelli, Massimilliano[2], Nyabi, Omar[1], Sid, Brice[3], Evraerts, Jonathan[1], Dollé, Laurent[4], Benton, Carley[5], van Grunsven, Leo[4], Heimberg, Harry[6], Sokal, Etienne[7]

[1]Labo of Pediatric Hepatology and Cell Therapy, Brussels, Belgium, [2]Pediatric Gastroenterology, Hepatology and Nutrition, Sainte-Justine University Hospital - Université de Montréal, Montreal, QC, Canada, [3]2Louvain Drug Research Institute, Toxicology and Cancer Biology Research Group, PMNT Unit, Université Catholique de Louvain, Brussels, Belgium, [4]Liver Cell Biology Lab, Vrije Universiteit Brussel, Brussels, Belgium, [5]Università Vita-Salute San Raffaele, Milan, Italy, [6]Beta Cell Biology Consortium, Brussels, Belgium, [7]Cliniques Universitaires St-Luc, Brussels, Belgium

Understanding the mechanisms triggering hepatogenic differentiation of stem/progenitor cells would be useful for studying post-natal liver regeneration and development of liver cell therapies. Many evidences support the involvement of Sox9 transcription factor in liver development. Here, we investigate the possibility of liver mesenchymal stem/progenitor cells to constitutively express Sox9 by using RT-qPCR, immuno-cytochemistry and western blotting. The involvement of Sox9 in hepatogenic differentiation was assessed by following its expression at different steps of the process, evaluating the impact of its altered expression and analyzing its expression in human liver disease specimen. Liver mesenchymal stem/progenitor cells constitutively express Sox9 both at the mRNA and protein levels. Upon hepatogenic differentiation, Sox9 expression is down regulated mainly in the maturation step after oncostatin M treatment. Induction of Sox9 expression using TGFβ is accompanied with a decrease of the quality of hepatogenic differentiation. Blunting Sox9 expression using specific ShRNA clearly alters the levels of several hepatic markers, an effect confirmed in HepG2 cells. In human liver disease specimen, Sox9 expression is enhanced both at the mRNA and protein levels as compared to healthy donors. The current data demonstrate that Sox9 may play a pivotal role in hepatocyte lineage development including adult liver mesenchymal stem/progenitor cells. Further studies on the identification of pathways regulated by or regulating Sox9 will certainly gain insight into the molecular networks controlling hepatogenic differentiation.

T-1020
MUTANT IDH BLOCKS MURINE PRIMARY LIVER PROGENITOR CELL DIFFERENTIATION TO DRIVE LIVER CANCER PATHOGENESIS

Parachoniak, Christine A.[1], Saha, Supriya K.[1], Ghanta, Krishna S.[1], Ross, Ken[1], Fitamant, Julien[1], Najem, Mortada S.[1], Gurumurthy, Sushma[1], Akbay, Esra A.[2], Deshpande, Vikram[1], Wong, Kwok-Kin[2], Ramaswamy, Sridhar[3], Bardeesy, Nabeel[1]

[1]Cancer Center, Massachuetts General Hospital, Boston, MA, USA, [2]Medical Oncology, Department of Medicine, Dana-Farber Cancer Institute, Boston, MA, USA, [3]Cancer Center, Massachusettes General Hospital, Boston, MA, USA

The liver is composed of two major epithelial cell types, namely hepatocytes and bile duct cells (cholangiocytes). Although normally a quiescent organ, the adult liver exhibits remarkable regenerative capacity and cellular plasticity. This process can involve transient cell cycle re-entry of hepatocytes and bile duct cells, activation of bipotential hepatic progenitors (oval cells), or transdifferentiation between the hepatocyte and biliary compartments. Importantly, this process can be activated under conditions of acute injury or carcinogenesis. Recently, mutations in Isocitrate dehydrogenase 1 (IDH1) and IDH2 were identified among the most common genetic alterations in intrahepatic cholangiocarcinoma (IHCC), a deadly primary liver cancer. Mutant IDH proteins in IHCC and other malignancies acquire an abnormal enzymatic activity allowing them to convert alpha-ketoglutarate (αKG) to 2-hydroxyglutarate (2HG). Furthermore, the neomorphic activity of mutant IDH has a role in cell differentiation, survival, and extracellular matrix maturation; however, the molecular pathways by which IDH mutations lead to tumor formation remain unclear. Here we show that mutant IDH blocks primary liver progenitor cells from undergoing hepatocyte differentiation through the production of 2HG and suppression of HNF4α, a master regulator of hepatocyte identity and quiescence. It has been shown that 2HG inhibits the activity of multiple αKG-dependent dioxygenases, such as the Tet family of enzymes. We observed that this inhibition results in alterations in DNA methylation at specific loci in tumor cell lines. Correspondingly, genetically engineered mouse models (GEMMs) expressing mutant IDH in the adult liver show aberrant response to hepatic injury, characterized by HNF4α silencing, impaired hepatocyte differentiation and markedly elevated levels of cell proliferation. Moreover, mutant IDH and activated Kras, genetic alterations that co-exist in a subset of human IHCCs, cooperate to drive the expansion of liver progenitor cells, development of premalignant biliary lesions, and progression to metastatic IHCC. These studies provide a functional link between IDH mutations, liver progenitor cell fate, and IHCC pathogenesis and present a novel GEMM of IDH-driven malignancy.

T-1021

UNDERSTANDING HUMAN LIVER TOXICITY USING STEM CELL DERIVED HEPATOCYTES

Szkolnicka, Dagmara[1], Farnworth, Sarah L.[2], Lucendo - Villarin, Baltasar[1], Storck, Christopher[3], Zhou, Wenli[4], Iredale, John P.[5], Flint, Oliver[6], Hay, David[1]

[1]Medical Research Council Centre for Regenerative Medicine, University Of Edinburgh, Edinburgh, United Kingdom, [2]FibromEd Products Ltd, Edinburgh, United Kingdom, [3]Discovery Toxicology, Bristol- Myers Squibb, Princeton, NJ, USA, [4]Department Of Oncology, Second Military Medical University, Shanghai, China, [5]Medical Research Council Centre For Inflammation, University Of Edinburgh, Edinburgh, United Kingdom, [6]Discovery Toxicology, Bristol - Myers Squibb, Princeton, NJ, USA

Introduction; Despite major progress in the knowledge and management of human liver injury, acute liver failure remains a serious medical condition. There are approximately 2000 cases per year of acute liver failure (ALF) in the United States and drug overdose plays a major factor, accounting for over 450 deaths per year. In particular, acetaminophen overdose is one of the predominant causes of acute liver failure in the United States. Although there are clinical interventions for ALF, for example, N-acetylcysteine treatment, the only cure for critically damaged and failing human liver function is donor organ transplantation. While this approach is highly successful it is severely limited by organ donation. Alternative approaches to restoring liver function have therefore been pursued, including the use of somatic and stem cell populations. Although such approaches are essential in developing scalable treatments, it is also an imperative to develop predictive human systems that more effectively study and/or prevent the onset of liver damage and failure. Methods: We used a renewable human stem cell resource, from defined genetic backgrounds, and drove them through developmental intermediates to yield highly active, drug-inducible, and predictive human hepatocyte populations. To model hepatotoxicity, cells were exposed to a number of compounds, compared to human hepatocytes and cell ATP was measured using The CellTiter - Glo Luminescent Cell Viability Assay (Promega TM) and used to calculate IC50. Metabolic pathway gene expression was examined using RT2 Profiler PCR Arrays. Intact metabolic pathways were predicted by pharmacogenomics knowledge base (PharmGKB; www.pharmgkb.org). Results: Human embryonic stem cell (hESC) populations were differentiated to hepatocytes using a serum-free system. Upon hepatic specification, ~ 94% of cells expressed HNF4a and exhibited both basal and drug-induced metabolic activity. Subsequently, hESC derived hepatocytes were tested for their ability to predict human drug induced liver injury (DILI) and displayed equivalence to primary adult hepatocytes. Following validation, we compared major metabolic gene expression with freshly isolated human hepatocytes. Encouragingly, hepatocyte gene expression was similar for 50 % of the major metabolic genes. Through the use of PharmGKB, we determined that the acetaminophen pathway was intact and studied in detail. Conclusions: In summary, we have developed a serum-free and scalable cell-based model that faithfully predicts the potential for human liver injury. Such a resource has direct application in human modeling, in particular acetaminophen induced hepatotoxicity.

INTESTINAL / GUT CELLS

T-1022

RNA EXPRESSION PROFILING OF DROSOPHILA INTESTINAL CELL POPULATIONS REVEAL COMPARTMENTALIZATION AS A CONSEQUENCE OF DISTINCT REGIONAL STEM CELL SIGNATURES IN THE DROSOPHILA MIDGUT

Dutta, Devanjali[1], Buchon, Nicolas[2], Edgar, Bruce A.[1]

[1]Cell growth and proliferation, DKFZ-ZMBH Alliance, Heidelberg, Germany, [2]Department of Entomology, Cornell University, Ithaca, NY, USA

Drosophila midgut is a rapidly proliferating tissue and is maintained by a continuous supply of differentiated cells that originate from intestinal stem cells (ISCs). ISCs proliferate throughout life, renewing and generating transient cells called enteroblasts (EBs), which differentiate into enterocytes (ECs) or enteroendocrine cells (EEs). The proper regulation of intestinal stem cell maintenance, proliferation and differentiation is critical for maintaining gut homeostasis. Signaling pathways like JNK, JAK-STAT, EGFR/ERK, PDGF/VEGF pathways are involved in ISC proliferation in the midgut. As our understanding of stem cell development and function in vivo becomes more sophisticated, it has become increasingly important to profile the intestinal cell types at the transcriptome level so that they can be analyzed extensively and manipulated in various ways. Profiling various cell types would help identify novel cell type specific markers and unravel yet unknown mechanisms of intestinal stem cell maintenance and development by identifying differentially expressed genes and gene pathways. We developed a method for isolating the midgut cell types using Fluorescent activated cell sorting (FACS) and RNA amplification. Using the same, we generated the expression profiles of ISCs, EBs, ESG+ cells, ECs, EEs and Visceral muscle cells. Filtering for cell type specific genes by differential expression analysis led to the identification of novel stem cell markers, which were validated by using Gal4 enhancer traps. Notch activity is required for differentiation of progenitor cells (EBs) into ECs and EBs. Loss of Notch leads to over proliferation of progenitor cells forming 'Notch tumors' in the midgut. We profiled these tumors to understand their autonomous and niche requirements. Our RNASeq data showed EGFR, JAK STAT pathway ligands Spi, Upds to be the required autonomously for the growth of these tumors, which were further experimentally validated. We also studied the cell type specific responses in the Drosophila midgut upon infection by Pseudomonas entomophila bacteria. While the Drosophila midgut has been classically studied as a homogenous tissue, the identification of distinct functional compartments within the midgut abolished the idea of a centralized control mechanism and insinuated the presence of region specific factors, which uphold regionalization. Based on morphometric, gene expression and spatial differences; the midgut has been divided into 5 major regions or compartments. In order to appreciate the region specific variation in the cells of the intestine, we performed transcriptome profiling of all the 6 cell types from the 5 major regions of the Drosophila midgut. Using the robustness of RNA sequencing we identified atleast 5 different stem cell populations within the organism, which explains the basis of compartmentalization in the Drosophila midgut. Our comparison of the gene expression profiles supports the idea that cells of region 2,4 and 5 are very similar in their expression profile, with region 3 being the most distinct. Region 3 is a highly acidic region, also known as the 'Copper cell region' due to the presence of copper secretory cells, which are similar to mammalian gastric parietal cells. Finally, we conclude that within cell type differences override the regional differences in the midgut. We

believe that our study would elaborate a paradigm explaining intestinal homeostasis and explicate the cause of midgut regionalization in flies.

T-1023
WNT/BETA-CATENIN AGONISTS R-SPONDIN 1 AND SLIT2 INHIBIT P53-MEDIATED APOPTOSIS OF INTESTINAL STEM CELLS

Geng, Jian-Guo
University of Michigan, Ann Arbor, MI, USA

Proliferating adult stem cells found in the bone marrow, the skin bulge, the brain cortex and the small intestine are mysteriously resistant to chemoradiation. Given the importance of Wnt/beta-catenin activity in maintaining endogenous stem cells and the significance of p53 activity in guarding the stem cell genome, we hypothesized that canonical Wnt signaling could inhibit p53 expression in tissue-specific stem cells causing the observed chemoradiation resistance. Using ionizing radiation (IR) as an acute tissue injury and repair model, we found that IR up-regulated Slit2 and its single-span transmembrane cell-surface receptor Roundabout 1 (Robo1) in the IR-resistant intestinal crypts, but not in the IR-sensitive intestinal villi. R-spondin 1 (Rspo1) bound directly to the extracellular immunoglobulin-like 3 and 4 domains of Robo1, whereas the cytoplasmic CC3 motif of Robo1 bound directly to low-density lipoprotein receptor-related protein 6 (Lrp6) and promoted its phosphorylation and association with leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5). Formation of Robo1-Lrp6-Lgr5 complex induced by Rspo1 and Slit2 led to Wnt/beta-catenin activation and transcriptional suppression of p53 expression and its apoptotic activity. Activation of Wnt/beta-catenin pathway by Slit2 transgene or recombinant Rspo1 plus Slit2 delayed p53 expression and attenuated p53-mediated apoptosis in the intestinal crypts and the Lgr5-high intestinal stem cells (ISCs). In contrast, inactivation of Wnt/beta-catenin signaling by partial genetic deletion of Robo1/2 prompted and exaggerated them. Our results indicate that Rspo1 and Slit2 synergistically induce Wnt/beta-catenin activation for inhibition of p53-mediated ISC apoptosis, cumulating in accelerated repair of acute gut injury.

T-1024
AGED INTESTINAL STEM CELL NICHE IS LESS REGENERATIVE IN VITRO

Iqbal, Sharif, Pentinmikko, Nalle, Katajisto, Pekka
Institute of Biotechnology, University of Helsinki, Helsinki, Finland

Stem cell populations occupy a special microenvironment, called the 'niche', which plays an essential role in maintaining overall tissue homeostasis by regulating regeneration. However, the regenerative capacity of an organism is compromised during the aging process, leading to imbalances in tissue homeostasis. In order to investigate the functional significance of the stem cell niche in aging, we reconstituted 'young' and 'old' intestinal stem cell niches in vitro by co-culturing 4 or 26 month old CD24+ paneth cells with the rapidly cycling Lgr5+ stem cells from Lgr5-EGFP-IRES-CreERT2 knock-in mice. We found that organoid formation is reduced significantly when young Lgr5+ cells were co-cultured with old paneth cells or vice versa. However, the most dramatic reduction of organoid formation was observed when both Lgr5+ stem cells and paneth cells were old. These data indicate that age dependent changes in both the stem cell niche and stem cells themselves contributes to reduced regeneration of aging tissues.

T-1025
STROMAL WNTS SUPPORT ORGANOID CULTURE IN THE ABSENCE OF WNT AGONIST R-SPONDIN

Kabiri, Zahra[1], Greicius, Gediminas[2], Virshup, David M.[1]
[1]Program in Cancer and Stem Cell Biology, Duke-NUS, Singapore, Singapore, [2]National Cancer Center of Singapore, Singapore, Singapore

Ex vivo reconstitution of tissue stem cells with their physiologic niche can provide important insights into the requirements for self-renewal and differentiation. The intestinal epithelium can self-renew and expand ex vivo via a continuous process of stem cell proliferation and differentiation. Purified intestinal epithelium requires Wnt3 secreted from Paneth cells, as well as supplemental factors including recombinant R-spondin (RSPO). RSPO enhances Wnt responsiveness by inhibiting RNF43 and ZNRF3, ubiquitin ligases that otherwise target the Wnt receptor Frizzled for degradation. While this system has been useful to expand the epithelium ex vivo, its physiologic relevance has been questioned. We and others have found that global knockout of epithelial Wnt secretion, or loss of Paneth cells, does not disrupt intestinal homeostasis in mice. However, we find that epithelial cells from villin-cre/PorcnDel mice cannot form organoids ex vivo even in the present of RSPO, unless they are co-cultured with stromal cells. Additionally, we find that purified intestinal stromal cells fully support epithelial organoid formation without R-spondin supplementation. PorcnDel stromal cells can not support organoid formation, indicating that Wnt secretion from the stromal cells is required for the intestinal stem cell niche. We propose that in vivo, stromal-specific Wnts are both necessary and sufficient to support the epithelial stem cell niche. Ex vivo, Paneth cell Wnt3 secretion can compensate for the loss of stromal Wnt secretion but only when Wnt signaling is upregulated by exogenous RSPO. It will be of interest in the future to identify the specific stromal cell type(s) and their complement of secreted Wnts that are sufficient to form an intestinal stem cell niche.

T-1026
DYSREGULATION OF GLI SIGNALING UNDERLIES DEGECTIVE ENTERIC NERVOUS SYSTEM DEVELOPMENT

Liu, Jessica Ai-jia[1], Lau, Sin-Ting[1], Wong, John Kwong-Leong[2], Briscoe, James[3], Hui, Chi-chung[4], Ngan, Elly Sau-Wai[1]
[1]Surgery, The University of Hong Kong, Hong Kong, [2]Psychiatry, The University of Hong Kong, Hong Kong, [3]National Institute for Medical Research, London, United Kingdom, [4]SickKids, The Hospital for Sick Children Research Institute, Toronto, ON, Canada

During enteric nervous system (ENS) development, coordination of neurogenesis and gliogenesis require an appropriate balance between the proliferation and differentiation of enteric neural crest cells (NCCs). Defects in these processes may lead to intestinal aganglionosis, Hirschsprung (HSCR) disease in humans. We have previously shown that aberrant Hedgehog signaling interferes both proliferation and differentiation of NCCs and results in disease predisposition, but the underlying molecular and cellular events remain unclear. In this study, we further delineated the roles of the three key Hedgehog effector genes namely Gli1, Gli2 and Gli3 in ENS development. Western blot analysis with FACS-sorted NCCs revealed that Gli signaling is transiently activated during NCC differentiation. Using an in vivo reporter of Gli activity, we demonstrated that Gli activity is elevated in the early committed ENCCs. Transient high Gli signaling is required for the proper ENS development, as constitutively high Gli activity by conditional ablation of Suppressor of Fused (Sufu) in NCCs resulted in impaired neuronal differentiation, axonal fasciculation, gangliogenesis and early onset of glial lineage differentiation of NCCs. The ratio of Gli2 activator (Gli2A) and Gli3 repressor (Gli3R) was particularly

critical for ENS development, as they restrained the differentiation and cell cycle progression of NCCs. Aberrantly high Gli2A to Gli3R ratio in the NCCs resulted in dysregulation of cell cycle gene expression and an increase in S and G2/M phase, accompanied by early onset of NCC differentiation. Similarly, Gli3Δ699/ Δ699 mice, where a truncated form of Gli3 that mimics Gli3R was constitutively produced, exhibited reduced neuronal and glial differentiation, suggesting that forced expression of Gli3R was deleterious to NCC differentiation. Taken together, our findings suggest that transient activation of Gli activity promotes cell cycle progression and initiates NCC differentiation by regulating multiple proliferative signaling pathways. Aberrant Gli activity interferes NCC development and may contribute to HSCR pathogenesis.

T-1027
SOLUBLE FACTORS SECRETED BY HUMAN INTESTINAL SUBEPITHELIAL MYOFIBROBLASTS MEDIATES SPHEROID GROWTH OF HUMAN INTESTINAL EPITHELIAL STEM CELLS

Brinkley, Garrett, Kar, Upendra, Lei, Nanye, Khalil, Hassan, Jabaji, Ziyad, Wang, Jifang, Lewis, Michael, Stelzner, Matthias, Dunn, James, **Martin, Martin G.**
University Of California, Los Angeles School of Medicine, Los Angeles, CA, USA

Human intestinal stem cells (hISCs) reside at the crypt base, and generate both absorptive and secretory cell lineages (e.g., enterocytes, goblet, Paneth, and enteroendocrine cells) that constitute the epithelial lining of the gut. Recently, intestinal stem cell biology has been an area of intense study and has provided new insights into active and quiescent ISCs, and lineage specification. Though most of the studies are conducted in Drosophila and murine models, we and few other groups have published methods to propagate and differentiate hISCs. Intestinal subepithelial myofibroblasts (ISEMFs) are an important component of the ISC niche, and supports ISC growth and differentiation during stress and homeostatic conditions. In this study, we characterized the soluble factor(s) released from human ISEMFs that control the hISC population in a 3D ex-vivo culture system. We found that PGE2 secreted by ISEMFs induced spheroid formation and supports the growth of undifferentiated epithelium. Indeed, PTGS2 is highly expressed in ISEMF as assessed by RNAseq and RT-PCR, and PGE2 levels were measured by ELISA and Mass Spec assays. Furthermore, PGE2 mediates expansion of the hISC population by a mitogen-activated protein (MAP)/ERK kinase dependent pathway. Spheroid structures generated with the use of ISEMF condition media can be cultured, propagated and reverted back to enteroid structures that contain all the cell lineages of the native small intestine. We also found that other soluble factors secreted by ISEMF's promote the expansion hISC, and the identity of these factors is currently under investigation. Our data suggests that factors secreted by the subepithelial stromal cells have a significant influence of the growth and differentiation of intestinal epithelial stem cell, and that these factors may mediate alternations of the human intestine during various forms of stress, including inflammation, infection and radiation-induced injury.

T-1028
ADULT STEM CELLS IN THE SMALL INTESTINE ARE INTRINSICALLY PROGRAMMED WITH THEIR LOCATION-SPECIFIC FUNCTION

Middendorp, Sabine[1], Schneeberger, Kerstin[1], Wiegerinck, Caroline L.[1], Mokry, Michal[1], Akkerman, Ronald D.L.[1], van Wijngaarden, Simone[1], Clevers, Hans[2], Nieuwenhuis, Edward E.S.[1]
[1]Pediatrics, UMC Utrecht, Wilhelmina Children's Hospital, Utrecht, Netherlands, [2]Hubrecht Institute, Utrecht, Netherlands

In mammals, the small intestinal epithelium is highly specialized along the cephalocaudal axis with different absorptive and digestive functions in duodenum, jejunum and ileum. Several transcription factors, like GATA4 and CDX2, have been described to regulate location-specific gene expression in the mouse small intestine. However, so far it is unknown whether location-specific functional properties are intrinsically programmed within stem cells or if continuous signalling from mesenchymal cells is necessary to maintain the location-specific identity of the small intestine. By using the pure epithelial organoid technique, we show that region-specific gene expression profiles are conserved throughout long-term cultures of both mouse and human intestinal stem cells and correlated with differential *Gata4* expression. Furthermore, the human organoid culture system demonstrates that Gata4-regulated gene expression is only allowed in absence of WNT signalling. We show that location-specific function is intrinsically programmed in the adult stem cells of the small intestine and that their differentiation fate is independent of location-specific extracellular signals. In light of the potential future clinical application of small intestine-derived organoids, our data imply that it is important to generate GATA4-positive and GATA4-negative cultures to regenerate all essential functions of the small intestine.

LUNG CELLS

T-1029
GUIDED IN VITRO LUNG CELL DIFFERENTIATION BY UTILIZING HUMAN ESC AND IPSC NKX2-1 KNOCK IN REPORTER LINES

Kramer, Philipp Martin[1], Hawkins, Finn J.[2], Crane, Ana M.[3], Kotton, Darrell N.[4], Davis, Brian R.[5]
[1]Center for Stem Cell and Regenerative Medicine, University of Texas Health Science Center at Houston, Houston, TX, USA, [2]Boston University, Boston, MA, USA, [3]Center for Stem Cell and Regenerative Medicine Brown Institute of Molecular Medicine, Houston, TX, USA, [4]Boston University School of Medicine, Boston, MA, USA, [5]UTHealth, Houston, TX, USA

Transplantation of corrected, patient-specific, iPSC-derived lung stem/progenitor cells represents a potential therapeutic approach for various inherited monogenic lung diseases. Although our laboratory has demonstrated correction of such mutant iPSCs (e.g. Cystic fibrosis), there remains the significant challenge to derive lung-specific stem/progenitor cells suitable either to generate lung epithelial tissue in vitro or for transplantation in vivo. The lack of specific cell-surface markers to identify and enrich for lung progenitors reinforces the importance of generating reliable tools to monitor the emergence of early lung cell types. NKX2-1, also known as TTF-1 is an essential transcription factor active during embryonic development and organogenesis of lung, thyroid and forebrain; importantly a murine ESC NKX2-1 knock-in reporter has previously enabled purification of lung/thyroid progenitors capable of subsequent development of lung tissue (Longmire 2012). NKX2-1 is a haploinsufficient gene and mutations

can cause brain-lung-thyroid disease, characterized by neurological, pulmonary and thyroidal abnormalities. We utilized transcription activator-like effector nuclease (TALEN) technology for site-specific targeting of the NKX2-1 locus in human ESC/ iPSC lines and subsequently introduced a GFP containing donor matrix by homology directed recombination. The objective was to obtain NKX2-1 specific reporter expression without creating haploinsufficiency. We have verified that expression of GFP by the targeted reporter lines accurately recapitulates NKX2-1 expression in the development of ventral forebrain neurons. Furthermore, in a step-wise differentiation assay designed to derive anterior foregut endoderm, we were able to generate cells co-expressing NKX2-1 and GFP. By immunofluorescence staining we confirmed that all NKX2-1 containing cells co-express FOXA2 and are exclusively GFP positive. These sorted GFP positive cells show gene expression of early lung markers (P63, SOX9) and have the capacity to express SP-B upon further differentiation after cell sorting. Low levels of thyroid marker PAX8 are also detectable in GFP sorted cells, but neuroectodermal markers TUJ1 or PAX6 are completely absent. ESC/ iPSC 'knock-in' reporter lines engineered with TALEN-based methods to express GFP under regulatory control of the NKX2-1 gene locus provides a powerful platform for the potential tracking and purification of human progenitors undergoing the earliest stages of lineage specification to lung, thyroid, or forebrain.

T-1030
MOUSE MODEL OF THE COMMON HUMAN ALDEHYDE DEHYDROGENASE (ALDH)-2 MUTATION EXHIBITS ABERRANT TRACHEAL EPITHELIAL PHENOTYPES

Kuroda, Aoi[1], Hegab, Ahmed E.M.[1], Arai, Daisuke[1], Gao, Jingtao[1], Yasuda, Hiroyuki[1], Sano, Motoaki[2], Betsuyaku, Tomoko[1]
[1]Division of Pulmonary Medicine, Department of Medicine, Keio University School of Medicine, Tokyo, Japan, [2]Department of Cardiology, Keio University Hospital, Tokyo, Japan

Aldehyde dehydrogenases (ALDHs) are a group of enzymes that play a major role in detoxification of aldehydes. High levels of ALDH expression are observed in stem/progenitor cells of many tissues and organs. Airway epithelium is continuously exposed to both endogenous and exogenous aldehydes such as lipid peroxidation, oxidative stress, and airborne pollutants. Among ALDHs, ALDH2 is preferentially expressed in airway basal stem cells (Hegab AE et al. Stem Cells Dev 2013). ALDH2 gene mutation (ALDH2*2), which is found in a large number of Asians, causes near complete loss of ALDH2 function and is associated with alcohol flushing syndrome and an increased risk for Alzheimer's disease, cardiovascular diseases, and some cancers. However, the influence of ALDH2 loss of function on airway epithelium has yet to be clarified. ALDH2*2 transgenic (Tg) mice with disturbed function of normal ALDH2 due to overproduction of the non-functional enzyme and ALDH2 deficient (+/-: heterozygous and -/-: homozygous) mice with a C57BL/6 background were obtained, and 3 to 4 mice per group aged 2 to 6 months were used in this study. Age-matched wild-type C57BL/6 mice were used as controls. At sacrifice, lungs and tracheas were harvested and prepared for H and E and immunostaining for various cellular markers (cytokeratin 5 as a marker for basal cells, CC10 for Clara cells, anti-acetylated alpha tubulin for ciliated cells, and prosurfactant protein C for alveolar type II cells). We measured the thickness of epithelia and nuclear density of the upper, middle, and lower parts of trachea in both longitudinal and horizontal sections on high-power digital images. The lung epithelium looked normal in ALDH2*2 Tg, ALDH2+/- and -/- mice. However, ALDH2*2 Tg and ALDH2+/- and -/- mice exhibited significantly lower nuclear density (28.6 nuclei/100 um basement membrane in wild-type down to 23.4, 23, and 25 nuclei/100 um in ALDH2*2 Tg, ALDH2-/- and ALDH2+/-, respectively; $p<0.0001$) and thinner epithelia (16.8 um in wild-type down to 15.7, 15.5, and 14.1 um in ALDH2*2 Tg, ALDH2-/- and ALDH2+/-, respectively; $p<0.05$) in trachea compared to those of wild-type mice. Both ALDH2*2 Tg and ALDH2+/- and -/- mice showed a decrease in basal cells in trachea, and the basal cell marker cytokeratin 5 staining was consistently of lower intensity, especially in ALDH2-/- mice. On top of that/In addition, a proportional decrease of Clara cells and an increase in ciliated cells were observed in both ALDH2*2 Tg and ALDH2+/- and -/- mice compared to wild-type controls. The percentage of intermediate cells (cells that are not basal cells, Clara cells, or ciliated cells) was also increased. Interestingly, ALDH1A1 staining, which is expressed mainly in non-basal cells, was significantly less in ALDH2*2 Tg and ALDH2+/- mice, while it was completely undetectable in ALDH2-/- mice, suggesting that the loss of ALDH2 function also disturbs the function of other ALDHs. Deficiency of functional ALDH2 causes aberrant phenotypes in tracheal epithelial structures, cellular composition, and rates of turnover in adult mice. It also causes functional disturbance in other ALDHs.

T-1031
IN VITRO GENERATION OF 3D MULTICELLULAR LUNG TISSUE FROM HUMAN PLURIPOTENT STEM CELLS

Nadkarni, Rohan[1], Pilquil, Carlos[2]
[1]Stem Cell and Cancer Research Institute (SCC-RI), McMaster University, Hamilton, ON, Canada, [2]McMaster SCCRI, Hamilton, ON, Canada

Pluripotent stem cells (PSCs) can differentiate into all specialized cell types of the body, providing suitable material for regenerative medicine applications, as well as allowing the study of development and disease. The production of 3D lung tissue in vitro from human PSCs has not been reported in the literature. Successful generation of such tissue may be used in modeling lung diseases outside the body. We have developed a novel multi-stage protocol for differentiating human PSCs into lung "organoids," 3D organ-like structures consisting of tissue sharing cellular organization and lineage-restricted marker expression with that of native lung tissue. Initial stages of the protocol mimicked key differentiation steps during embryonic development en route to lung organogenesis, which included definitive endoderm differentiation, followed by anterior foregut specification and production of lung progenitor cells co-expressing NKX2.1 and FOXA2. Next, cell aggregates were transferred to a 3D culture system to promote further differentiation and maturation. During 3D culture, we observed the development of individual lung bud-like structures and larger organoids consisting of multiple buds. These buds resembled mouse embryonic lung buds during E11.5-E14.5. Through immunohistochemistry of organoid sections, we observed airway-like epithelial tubules that expressed E-cadherin and airway markers Clara cell secretory protein (CC10), Mucin-5B (MUC5B), SOX2, SOX9 and sonic hedgehog (SHH). In addition, clusters of cells were found to express alveolar markers surfactant protein C (SFTPC) and aquaporin 5 (AQP5). Haematoxylin and eosin (H and E) staining of organoid sections showed epithelial cell types having properties of Clara, basal, ciliated and goblet cells within tubular structures, whose organization was similar to that of the native proximal and distal airways. We also observed mesenchymal cells in H and E-stained organoid sections. Ultrastructures captured by transmission electron microscopy (TEM) showed that epithelium lining the airway-like tubules was polarized and contained microvilli and cilia-like protrusions on their luminal surface. Overall, we have generated 3D lung tissue entirely in vitro from human PSCs for the first time, containing pulmonary cells from both the conducting and gas-exchange zones of the native lung including epithelium and mesenchyme. Importantly, our work provides material that may be used in studying respiratory disorders in vitro.

EPITHELIAL CELLS (NOT SKIN)

T-1032
AMNION CELL THERAPY INCREASES BRONCHIOALVEOLAR STEM CELL NUMBERS FOLLOWING BLEOMYCIN CHALLENGE

Lim, Rebecca, Chan, Siow Teng, Wallace, Euan M.
The Ritchie Centre, Monash University, Clayton VIC, Australia

Human amnion epithelial cells (hAECs) have demonstrated great promise for regenerative medicine, possessing multipotent differentiation ability, low immunogenicity and anti-inflammatory properties. Using the bleomycin lung injury mouse model, we have shown for the first time that hAECs can prevent and reduce lung injury. However, the exact regenerative and reparative mechanisms of hAECs have yet to be evaluated. In this study, we investigated the potential of hAECs to affect endogenous bronchioalveolvar stem cells (BASCs) following bleomcycin-induced lung injury. Immunocompetent mice (C57Bl6) were given a single intranasal dose of bleomycin. After 24 hours, saline (vehicle) or 4x106 hAECS were administered via an intraperitoneal injection. These groups were named: B+S and B+hAEC. Mice were sacrificed at Day 3 or Day 7; lungs were collected and digested into a single cell suspension. BASCs were identified as CD45neg CD31neg Sca-1pos CD34pos and collected for an in vitro colony-forming assay. Compared to saline treated animals, administration of hAECs resulted in a slight increase in BASC numbers at Day 3. This increase was significant by Day 7. BASCs isolated from B+S and B+hAEC animals on Day 3, gave rise to colonies and formed three-dimensional structures similar to embryoid bodies. BASCs isolated from B+hAEC animals on Day 7 gave rise to colonies but failed to form three-dimensional 'embryoid bodies'. Treatment with hAECs following bleomycin challenge appeared to trigger the expansion of endogenous BASCs as early as Day 3. BASCs isolated from B+hAEC animals on Day 7 appear to display reduced stem cell properties. These preliminary findings, in combination with our published work, suggests that that administration of hAECs may recruit endogenous lung stem cells to aid in lung repair as well as promotes their differentiation.

T-1033
DEVELOPMENTAL CHANGES IN MAMMARY EPITHELIAL CELL PROPERTIES RELEVANT TO BREAST CANCER TREATMENT

Balani, Sneha, Kannan, Nagarajan, Makarem, Maisam, Eaves, Connie
Terry Fox Laboratory, BC Cancer Research Centre, Vancouver, BC, Canada

Breast cancers originate through a multi-step process in which mutations and epigenomic changes progressively accumulate in clonally expanding cells. Current evidence suggests that these changes are superimposed on a normally hierarchical differentiation process in which rare mammary cells with long-term self-maintenance ability are responsible for generating expanding numbers of cells with progressively restricted proliferative potential and differentiation options. Thus mutations may occur at different "normal" stages of mammary cell differentiation giving rise to biologically as well as genetically complex tumours that are maintained by a minority of cells that continue to generate progeny with limited proliferative ability. These sources of biologic heterogeneity within individual tumours pose major challenges to the rational design of more effective and less toxic therapies. They also underscore the importance of gaining a better understanding of key molecular pathways operative in different types of normal breast epithelial cells, particularly those that regulate their DNA integrity and stability. We have found that normal human mammary luminal clonogenic progenitor cells are more resistant (~1.5-fold) than the basal clonogenic cells to in vitro exposure to ionizing radiation, the continuing mainstay of localized human breast cancer treatment. However, this difference is not obtained when the responses of luminal and basal clonogenic progenitors from normal adult mouse mammary epithelial cells are similarly compared using the same treatment conditions, regardless of donor age, and both mouse mammary progenitor types are ~1.5-fold more resistant than human luminal progenitors. We have now begun to investigate whether any difference is seen between neonatal and adult mouse mammary progenitors, since recent data would predict that the neonatal cells would more closely resemble malignant mammary cells. These results will establish the utility of the mouse system to examine factors that regulate normal and transformed mammary progenitor cell responses to ionizing radiation and set the stage for future mechanistic investigations of differentiation stage-specific changes in these responses.

T-1034
CELL HIERARCHY AND LINEAGE COMMITMENT OF BOVINE MAMMARY EPITHELIAL CELL POPULATIONS AND THE UNIQUE RECOVERY PATTERN OF BOVINE MORPHOLOGY IN THE MOUSE STROMA

Barash, Itamar[1], Rauner, Gat[2]
[1]*Institute of Animal Science, ARO The Volcani Center, Bet-Dagan, Israel,* [2]*Institute of Animal Science, ARO, The Volcani Center and The Hebrew University of Jerusalem, Bet-Dagan/Jerusalem, Israel*

The bovine mammary gland is a favorable organ for studying mammary cell hierarchy due to the adaptation of its cell populations to extensive expansion and differentiation. It also shares basic characteristics with the human breast. Here, we aimed at: (i) characterizing bovine mammary epithelial cell hierarchy and establishing multipotency and regenerative capabilities of an individual stem-like cell population; (ii) recapitulating the bovine mammary development in the mouse stroma. Lin^- bovine mammary epithelial cells (bMEC) from Holstein heifers were sorted according the expression of CD24 and CD49f into four populations. The $CD24^{med}CD49f^{pos}$ enriched population maintained high expression of basal markers. In culture, it generated both luminal and basal clones and had high floating sphere formation and growth rate. Upon transplantation into the cleared mammary fat pad of immunocompromized mice, it gave rise to multi-layered outgrowths .This population was, therefore, positioned at the top of the cell hierarchy and referred to as stem cell-enriched population. A more committed, bi-potent basal ($CD24^{neg}CD49f^{pos}$) population generated both luminal and basal clones in vitro, but was almost completely restricted to generating basal unilayered outgrowths upon transplantation into the mouse stroma. Together with the luminally restricted progenitor population ($CD24^{high}CD49f^{neg}$), it may serve as reservoir for the highly differentiated $CD24^{med}CD49f^{neg}$ luminal cells. To analyze the development of bovine mammary morphology in the mouse mammary stroma, bMECs were transplanted into the cleared mammary fat pad of immunodeficient mice. Multilayered hollow spheres developed within fibrotic areas, but in contrast to mice, no epithelial organization was formed between adipocytes. The multilayered spheres shared morphological and immunohestochemical characteristics with the heifer gland's epithelium including lumen size, cell proliferation, cytokeratin orientation, estrogen/progesterone receptor expression and localization, and milk protein synthesis. However, they did not extend into the mouse fat pad via ductal morphology. Nevertheless, a single case of terminal ductal lobuloalveolar unit (TDLU) development was recorded in mice treated with estrogen and progesterone, implying the feasibility of this representative bovine morphology's development.

In vitro, paracrine inhibition of bovine epithelial mammosphere development by adipocytes, was generalized to breast epithelial mammosphere formation. It was antagonized by FGF administration, indicating an active equilibrium between inhibitory and supportive effects exerted by the adipose and fibrotic regions of the stroma, respectively. Taken together, these findings imply that unique bovine mammary cell properties are integrated within a conserved cell hierarchy paradigm delineated in their mouse and human counterparts. The "bovinized" mouse model may allow better understanding of the bovine mammary gland development, and may serve as a useful tool for studies that are difficult to perform with farm animals.

T-1035
HS3ST3-MODIFIED HEPARAN SULFATE CONTROLS KIT-POSITIVE PROGENITOR EXPANSION BY REGULATING 3-O-SULFOTRANSFERASES

Patel, Vaishali N.[1], Lombaert, Isabelle[2], Shworak, Nicholas W.[3], Yongmei, Xu[4], Liu, Jian[5], **Hoffman, Matthew P.**[1]
[1]NIDCR, NIH, Bethesda, MD, USA, [2]NIH, Bethesda, MD, USA, [3]Dartmouth-Hitchcock Medical Center, Dartmouth University, Lebanon, NH, USA, [4]University of North Carolina, Chapel Hill, NC, USA, [5]Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, NC, USA

The exquisite control of growth factor function by heparan sulfate (HS) is dictated by the tremendous structural heterogeneity of sulfated modifications. It is not known how specific HS structures control growth factor-dependent progenitor expansion during organogenesis. We isolated KIT+ progenitors from fetal salivary glands during a stage of rapid progenitor expansion and profiled expression of HS biosynthetic enzymes. Enzymes generating a specific type of 3-O-sulfated-HS (3-O-HS) are enriched, and FGF10/FGFR2b signaling directly regulates their expression. Bioengineered 3-O-HS binds FGFR2b and stabilizes FGF10/FGFR2b complexes in a receptor- and growth factor-specific manner. Rapid autocrine feedback increases 3-O-HS, KIT and progenitor expansion. Knockdown of multiple Hs3st isoforms limits fetal progenitor expansion, but is rescued with bioengineered 3-O-HS, which also increases adult progenitor expansion. Rapidly altering a specific 3-O-sulfated epitope provides a cellular mechanism to modulate the response to FGFR2b signaling and control progenitor expansion. 3-O-HS may expand KIT+ progenitors in vitro for regenerative therapy.

T-1036
PNEUMACULT™: AN INTEGRATED CULTURE MEDIUM SYSTEM FOR IN VITRO HUMAN AIRWAY MODELING

Hou, Juan[1], Riedel, Michael J.[2], Thomas, Terry E.[2], Eaves, Allen C.[2], Louis, Sharon A.[2]
[1]STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]STEMCELL Technologies Inc, Vancouver, BC, Canada

In vitro modeling of the human airway can be used for the study of viral infection, drug transport and toxicity, and disease modeling. Primary human bronchial epithelial cells (HBECs) can be cultured at the air-liquid interface (ALI) using specialized media, resulting in differentiated cells having morphological and functional characteristics that mimic the in vivo airway. HBECs can also be expanded in monolayer culture for several passages whilst retaining the ability to differentiate at the ALI. Traditional formulations for expansion and differentiation of primary HBECs have typically contained undefined components such as Bovine Pituitary Extract (BPE) which may be subject to lot variability thus leading to inconsistent medium performance. PneumaCult™-ALI, a defined, BPE-free medium, promotes efficient mucociliary differentiation of primary HBECs in ALI cultures. Here, we report the development of PneumaCult™-EX, a defined and BPE-free medium that supports the rapid expansion of primary HBECs for subsequent mucociliary differentiation in PneumaCult™-ALI medium. Commercially available primary normal human airway epithelial cells such as Lonza's HBECs (P1; Cat#: CC-2540s) were thawed and seeded directly into T-25 cm2 culture flasks containing PneumaCult™-EX or control medium (BEGM™; Lonza) at a density of 3.5 x 103 cells/cm2. Culture media were fully replenished every other day and cultures were passaged once cells reached approximately 80% confluence. At each passage, cells were enzymatically dissociated using Trypsin-EDTA (0.05%) and then re-plated at a density of 1 x 104 cells/cm2 in PneumaCult™-EX or control medium. To test the differentiation potential of culture-expanded HBECs at each passage, air-liquid interface cultures were prepared using aliquots of HBECs that had been expanding using either PneumaCult™-EX or control medium, and differentiation was performed using PneumaCult™-ALI. Fold expansion was measured over 4 passages and the differentiation potential was assessed during each of the first 3 passages after which HBECs typically lose the ability to differentiate at the ALI. In 7 independent donor samples, the average fold expansion over 4 passages was not significantly different between cells cultured in PneumaCult™-EX and cells cultured in the less defined, BPE-containing control medium (7.1 ± 1.4 vs. 7.2 ± 1.9; mean ± SD, n=7, p = 0.9 in paired t-test). Similar to cells cultured in control medium, cells cultured in the absence of BPE using PneumaCult™-EX showed cobblestone morphology and uniformly expressed the basal cell marker p63 at least until passage 4 as assessed by immunocytochemistry. Furthermore, cells cultured in either PneumaCult™-EX or control medium could be successfully differentiated at each passage during early passages (passage 1 - 3) using PneumaCult™-ALI to generate a pseudostratified mucociliary epithelium, as indicated by the qualitative observation of beating cilia under brightfield microscopy and by positive Periodic acid-Schiff (PAS) staining at 28 days post air-lift. PneumaCult™-EX, a novel defined and BPE-free expansion medium, together with PneumaCult™-ALI create a fully integrated tissue culture system for in vitro human airway modeling. By promoting efficient expansion of primary HBECs in monolayer culture, PneumaCult™-EX allows the user to generate large numbers of cells for use in assays related to basic respiratory research, toxicity testing and drug development.

T-1037
PURIFICATION AND CHARACTERIZATION OF PRIMITIVE SUBSETS OF SURFACE AND CRYPT EPITHELIAL CELLS OF HUMAN TONSILS

Kang, Catherine[1], Kannan, Nagarajan[2], Zhang, Lewei[3], Eaves, Connie[2,3], Rosin, Miriam[1,2]
[1]Simon Fraser University, Burnaby, BC, Canada, [2]BC Cancer Agency, Vancouver, BC, Canada, [3]University of British Columbia, Vancouver, BC, Canada

Cancer of the oropharynx (including the tonsils and base of the tongue) is a global health problem with an increasing incidence and prevalence worldwide especially in developed countries. Human Papillomavirus DNA is present in up to 90% of oropharyngeal cancers, and initial HPV integration has been localized to the epithelium of tonsillar crypts. This suggests that a primitive epithelial cell in this region may be the cell of origin of HPV-associated oral cancers. Numerous studies have demonstrated the presence of stem cells in other human epithelia but those responsible for sustaining the tonsillar crypts have not been identified. We first used *in situ* immunohistochemical analysis to identify differentially expressed markers on surface of tonsillar epithelial cells and now show how these can be used to obtain highly

purified suspensions of both surface and crypt epithelial cells with *in vitro* clonogenic ability (CFCs). The results show that prospectively isolated $CD45^-CD31^-CD44^+NGFR^+$ cells from either surface or crypt epithelial tissue, when cultured in 5% O_2 at low cell numbers on an irradiated NIH3T3 cell feeder layer in medium containing a ROCK inhibitor generate colonies at a frequency of up to 31% (15.8±11.7% for surface CFCs and 21.7±9.96% for crypt CFCs, n=5, representing enrichments of 83 and 544-fold, respectively). These cells were also able to form multilayered epithelial sheets when seeded onto collagen matrix mixed with fibroblasts in 3D organotypic cultures. In order to examine the self-renewal ability of these progenitors, we performed serial replating experiments. The results of these show that crypt-derived $CD45^-CD31^-CD44^{high}NGFR^{high}$ cells not only contained more CFCs than their surface counterparts, but also had superior ability to generate daughter CFCs. These experiments set the stage for studying intrinsic properties regulating this clonogenic subset of tonsillar epithelial cells, and their response to HPV infection, as a first step towards elucidating the pathogenesis of HPV-associated oral cancers

T-1038
HUMAN MAMMARY STEM AND PROGENITORS SHOW LINEAGE-SPECIFIC DIVERGENT MECHANISMS OF ROS CONTROL AND ASSOCIATED SENSITIVITY TO OXIDATIVE DNA DAMAGE

Kannan, Nagarajan, Nguyen, Long Viet, Makarem, Maisam, Dong, Jeff, Shih, Kingsley, Eirew, Peter, Eaves, Connie
Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Genomic instability and DNA damage are cardinal features of most human breast cancers, although little is known about the mechanisms that normally guard against these features in normal human mammary cells or their component subsets. The control of intracellular reactive oxygen species (ROS) is an important one of these, since excess ROS can directly produce structural changes in DNA and, if not immediately 'corrected', lead to lasting mutations. To investigate ROS control in different freshly isolated subsets of normal human mammary cells, we used FACS to obtain a highly luminal progenitor (LP)-enriched subset ($EPCAM^+CD49f^+$, ~20% pure *in vitro* clonogenic cells) and a basal cell (BC) subset ($EPCAM^{-/low}CD49f^+$) that is highly enriched in mammary stem cells (~0.1%) and bipotent/myoepithelial progenitor cells with *in vitro* clonogenic potential (also ~20% pure). We found the LPs have higher levels of membrane-intact mitochondria, consume significantly higher levels of oxygen and constantly produce higher levels of superoxide anion radicals (O_2*) and H_2O_2 than BCs. The LPs also contained higher anti-oxidant superoxide dismutase (SOD; reduce O_2* to H_2O_2) and glutathione peroxidase (GPX; reduces H_2O_2 to water) activities associated with strikingly higher levels of all 3 SODs and most of the glutathione-dependent and -independent peroxidases except GPX-2, a mitochondrial anti-oxidant enzyme. In contrast, GPX-2 was almost only expressed in BCs where it was consistently detected at higher levels than in LPs. We also found that human LPs differ from matched sources of BCs in their greater resistance to glutathione depletion obtained by adding the glutathione inhibitor, BSO, or by transducing the cells with a GPX-2 shRNA lentivirus. Notably, the BSO-induced killing of BCs could be readily rescued by pretreatment with exogenous anti-oxidants such as N-acetyl cysteine or Trolox. Mammary stem cells (as quantified by a sub-renal xeno-transplant assay) demonstrated the same sensitivity to BSO as BCs assessed by their clonogenic activity *in vitro.* This suggests ROS control mechanisms are shared by all BC elements in striking contrast to their LP derivatives. LPs also displayed greater resistance to direct oxidative insults (exposure to H_2O_2 and X-radiation), LPs expressed higher levels of key oxidative DNA damage control enzymes (namely OGG1, MUT, MUTYH), and demonstrated significantly increased accrual of oxidative damage-induced (genomic 8-oxo-dGTP) mutations predicted by their ability to survive high levels of intracellular ROS. Taken together, our findings reveal a major difference in the molecular machinery that produces and controls ROS levels in primitive normal human mammary basal and luminal cells. These differences correlate with an ability of the LPs to maintain and tolerate elevated levels of ROS accompanied by, and possibly a major cause of, a continuous acquisition of unrepaired ROS-induced DNA damage, resulting in a previously unanticipated mechanism of promoting their mutagenesis, telomere attrition and associated oncogenic transformation.

EPIDERMAL CELLS

T-1041
CLINICAL USE OF AUTOLOGOUS EPIDERMAL CELLS IN WOUND HEALING - THE POTENTIAL ROLE OF VSEL CELLS IN SKIN REGENERATION

Borowczyk, Julia, Zimolag, Eliza, Zuba-Surma, Ewa K., Madeja, Zbigniew, **Drukala, Justyna**
Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

Wound closure is determined by the epidermis forming a specialized protective barrier. The characteristic feature of the epidermis is a fast regeneration conditioned by a large number of stem cells. Isolation of epidermal stem and progenitor cells residing in the germinal layer enables the reconstruction of epidermis in vitro and its application in large wound healing. The possibility of having large-scale cultures of proliferating cells from small tissue biopsy and permanent covering of the wound, without a risk of graft rejection, is an important advantage of skin reconstruction with use of autologous cells. Our clinical experience (clinical trial approved by the ethics committee of the Jagiellonian University, Cracow, Poland; No. KBET/480/B/2003) indicated that autologous keratinocyte transplantation as a cell suspension is very effective in case of chronic leg ulcers and burn healing. This procedure significantly improves and accelerates epidermal regeneration, but this technic has some limitations and can be used only when granular tissue is well developed. It does not result in hair follicles and sebaceous glands restoration. Epidermal unipotent stem cells are committed to only one kind of tissue and have limited regenerative abilities. Thus, pluripotent stem cells (PSCs) are considered as the best candidate for medical applications, as they would be able to differentiate into cells from all three germ layers (ecto-, meso-, and endoderm). Nevertheless, the acquisition of pluripotent human cells (i.e. from human embryos) evokes many ethical and technical issues therefore, other sources of this kind of cells are needed. Few years ago Ratajczak et al. described a rare population of pluripotent stem cells in adults called VSELs characterized by small size and expression of specific markers (CD133, SSEA-4, Oct-4, CXCR4). Our previous data showed that burn triggers the mobilization of VSELs and progenitor cells expressing early epidermal markers into peripheral blood but is still too early to determine the potential role of these cells in skin regeneration. The aim of our study was to establish the presence of VSELs and their possible localization in human skin. Several previous studies demonstrated that cells with multipotent character reside in the bulge region of hair follicles and contribute not only to interfollicular epidermis but also to hair follicle and sebaceous gland. Nowadays, keratin 15 (K15) and CD200 are considered as the most specific biomarkers for identifying human hair follicular stem cells. We hypothesize, that maybe this region is a niche for more primitive cells as well. Our results from flow cytometry analysis indicates that there is a very rare (<0,01%)

population of CD133+/SSEA4+ cells in human epidermis and dermis. With ImageStream system we confirmed that these cells are very small (4±1.8 μm in diameter) and express the stemness gene Oct-4. So far, using confocal microscopy we managed to localize single small cells (5 μm diameter) expressing CXCR4 in a sub-bulge area. The obtained data indicate that there is a rare population of cells deposited in human skin demonstrating characteristic features of VSELs. However, it is necessary to show cells expressing markers of pluripotency and their co-localization.

T-1042

LGR6+ CELLS CONSTITUTE TWO INDEPENDENT STEM CELL POPULATIONS IN MURINE EPIDERMIS AND GIVE RISE TO MULTICLONAL SKIN TUMORS

Füllgrabe, Anja[1], Are, Alexandra[1], Joost, Simon[1], Haegebarth, Andrea[2], Clevers, Hans C.[2], Toftgård, Rune[1], Kasper, Maria[1]
[1]Karolinska Institute, Stockholm, Sweden, [2]Hubrecht Institute, Utrecht, Netherlands

The murine skin with its appendages, as hair follicles and sebaceous glands, is an excellent model system to study tissue stem cells. In order to investigate how Lgr6+ keratinocytes produce epidermis in addition to hair follicle structures, we looked for Lgr6-expressing cells in the interfollicular epidermis (IFE). We found that a sub-population of the IFE expresses the hair follicle stem cell marker Lgr6. Genetic lineage tracing using a multi-color fluorescent reporter allele revealed that Lgr6+ IFE cells represent a stem cell population, able to self-renew and maintain the epidermis without contribution from the Lgr6+ hair follicle stem cells. Gene expression profiling of the Lgr6+ hair follicle and IFE populations by RNA-sequencing confirmed distinct hair follicle and IFE signatures of the two Lgr6+ populations. Furthermore, we investigated the ability of Lgr6+ cells to give rise to K-Ras-driven tumors. Spontaneous papilloma development was observed on the lips and paws. In dorsal skin, Lgr6+ cells formed papillomas only in combination with wounding. Multi-color single-cell tracing revealed that these papillomas are of multiclonal origin and the overall tumor growth is promoted by monoclonal expansion of individual cells.

T-1043

TRANSIT AMPLIFYING CELLS ORCHESTRATE STEM CELL ACTIVITY AND TISSUE REGENERATION

Hsu, Ya-Chieh, Li, Lishi, Fuchs, Elaine
Rockefeller University, New York, NY, USA

Transit-amplifying cells (TACs) are an early intermediate in tissue regeneration. Here, using hair follicles (HFs) as paradigm, we show that emerging TACs constitute a signaling center that orchestrates tissue growth. While primed stem cells (SCs) generate TACs, quiescent-SCs only proliferate after TACs form and begin expressing Sonic Hedgehog (SHH). TAC generation is independent of autocrine SHH, but their pool wanes if they can't produce it. We trace this paradox to two direct actions of SHH: promoting quiescent-SC proliferation and regulating dermal factors that stoke TAC expansion. Ingrained within quiescent-SC's special sensitivity to SHH signaling is their high expression of GAS1. Without sufficient input from quiescent-SCs, replenishment of primed-SCs for the next hair cycle is compromised, delaying regeneration and eventually leading to regeneration failure. Our findings unveil TACs as transient but indispensable integrator of SC niche components and reveal an intriguing interdependency of primed and quiescent SC populations on tissue regeneration.

T-1044

SPATIAL IDENTITY CONTROLS MOLECULAR PROPERTIES AND STEM CELL BEHAVIOUR IN MURINE EPIDERMIS

Page, Mahalia E.[1], Andersen, Marianne S.[2], Sendrup, Sarah[2], **Jensen, Kim B.**[2]
[1]Wellcome Trust and Medical Research Council Cambridge Stem Cell Institute, University of Cambridge, Cambridge, United Kingdom, [2]BRIC - Biotech Research and Innovation Centre, University of Copenhagen, Copenhagen N, Denmark

The epidermis constitutes the outer layer of our largest organ the skin and its life-long integrity is vital for our existence. Maintenance of the epidermis is governed by stem cells located in distinct cellular niches. We recently reported that murine epidermis is compartmentalised into autonomously maintained functional units. It is however not clear how the distinct stem cell compartments are maintained, how cell behaviour is controlled and whether there is functional heterogeneity within identified stem cell populations. We hypothesise that cellular behaviour is controlled by spatial identity. Using a combination of fate mapping studies with single cell analysis of purified stem cell populations, we now extend the current understanding of the mechanisms that governs epidermal maintenance. The analysis focuses on the stem cell compartment responsible for the maintenance of the upper hair follicle including the sebaceous gland. Here we observe extensive heterogeneity within the purified stem cell populations and identify distinct subsets of stem cells with unique behavioural and molecular properties. The observed stem cell heterogeneity provides an explanation for the observed tissue compartmentalisation that can potentially be extrapolated to other epithelial tissues such as the intestine. When extending the study to tumour formation evidence supports that compartmentalisation of stem cell functions provides a context, whereby a significant proportion of stem cells becomes refractory to oncogenic transformation in vivo.

T-1045

DYNAMICS OF LGR6+ HAIR FOLLICLE STEM CELL PROGENY DURING BASAL CELL CARCINOMA FORMATION

Are, Alexandra, Füllgrabe, Anja, Toftgård, Rune, **Kasper, Maria**
Biosciences and Nutrition, Karolinska Institute, Stockholm, Sweden

Skin is an excellent tool to study the dynamics of stem cells during homeostasis, wound repair and cancer formation. Epidermal stem cells are located in distinct niches of the hair follicle (HF) and the interfollicular epidermis, and their respective progeny are normally restricted to defined areas. However, injuries like acute wounds allow stem cell progeny to repopulate new areas. What happens during cancer formation? Can stem cell progeny, carrying an oncogenic mutation, also colonize new areas and thereby perhaps speed up tumor growth? To address this question we specifically manipulated a stem cell population in the HF isthmus, marked by Lgr6 expression (Lgr6IST). During normal skin homeostasis, Lgr6IST stem cells and their progeny contribute to the isthmus and the sebaceous gland and only rarely participate in anagen growth and bulge stem cell maintenance (Snippert et al., 2010). To test whether Lgr6IST stem cells have the potential to induce basal cell carcinoma (BCC)-like tumors, we introduced a homozygous deletion of Ptch1 into Lgr6IST stem cells. To this end, we crossed Lgr6-EGFP-IRES-CreERT2 mice with Ptch1fl/fl mice - carrying conditionally floxed (fl) Ptch1 alleles - and induced deletion of Ptch1 via administration of tamoxifen in the HF resting phase. For simultaneous tumor tracing, we used an R26Tomatofl (hereafter, Tom) reporter line. As expected, BCC formation started in the Lgr6IST cells. However, unexpectedly, tumor growth eventually

affected the entire HF below the sebaceous gland. By studying the temporal dynamics of Lgr6IST/Tom/ Ptch1fl/fl cells we found that upon Ptch1 deletion, Lgr6IST cell progeny expanded during anagen growth from the isthmus to the outer root sheath of the HF, survived the subsequent regression phase and thereby could colonize the bulge and hair germ area. Consequently, tumor growth could establish over the entire HF. In sum, our study proposes a model, in which tumor-initiating cells can take advantage of a normal homeostatic process (here the hair cycle) to expand into new areas and thereby promote tumor growth.

T-1046

CHEMOTATIC EFFECTS OF IL-8 AND MIP-3 ALPHA IN HUMAN KERATINOCYTES, DERMAL FIBROBLASTS, AND MESENCHYMAL STEM CELLS

Kim, Yun Hee, Jeon, Saewha
Cutigen Research Institute, Tego Science Inc., Seoul, Republic of Korea

Wound healing is a complex process involving the orchestrated interaction of multiple growth factors, cytokines, chemokines, and cell types. It has been reported that several chemokines expressed in wounds can recruit several cells such as leukocytes, fibroblasts, endothelial cells and mesenchymal stem cells (hBMSC), possibly involved in cutaneous wound healing. In order to address the roles of chemokines in wound healing, chemokine receptor expression and chemotatic effect of IL-8 and MIP-3α were confirmed in three different cell types: human keratinocytes, dermal fibroblasts, and bone marrow-derived mesenchymal stem cells. In addition, the wound healing effects were investigated with either IL-8 or MIP-3α was in mice. The mRNA expressions of IL-8 and MIP-3α receptors, CXCR1/CXCR2 and CCR6, were detected in all three cell types, with the level of expression in keratinocytes and hBMSC higher than dermal fibroblasts. Consistent with these observations, IL-8 and MIP-3α induced *in vitro* chemotaxis of both keratinocytes and hBMSC. In mice, the topical treatment of IL-8 or MIP-3α promoted the re-epithelialization and collagen synthesis without inflammation. Our observations suggest that the interactions either between CXCR1 and IL-8, or between CCR6 and MIP-3α can play an important role in wound healing. Therefore, a therapy using chemokines such as IL-8 and MIP-3α could become an alternative to the treatment strategies using growth factors and skin substitutes for wound healing.

ENDOTHELIAL CELLS / HEMANGIOBLASTS

T-1049

DEFINING THE TEMPORAL EMERGENCE OF HEMATOPOIETIC REPOPULATING ACTIVITY DURING THE IN VITRO DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

Kouskoff, Valerie[1], Pearson, Stella[1], Lacaud, Georges[2]
[1]*Cancer Research UK Manchester Institute, Manchester, United Kingdom,*
[2]*Paterson Institute for Cancer Research, Manchester, United Kingdom*

The generation of in vivo repopulating hematopoietic cells from in vitro differentiating embryonic stem cells has remained a long-standing challenge. To date hematopoietic engraftment has been mostly achieved artificially through the enforced expression of ectopic transcription factors. Here, we will present data demonstrating that ES cells differentiated in serum-free culture conditions give rise to in vivo repopulating hematopoietic cells in the absence of ectopically expressed factors. These ES-derived repopulating cells confer multi-lineages and long-term engraftment in conditioned recipients. Analysis of the temporal emergence of this activity reveals that engrafting hematopoietic cells arise immediately upon the commitment of FLK1+ mesoderm precursors to the blood program and before the acquisition of the hematopoietic marker CD41. Furthermore, we establish that the formation of these progenitors endowed with in vivo engrafting ability is exquisitely sensitive to the cytokine milieu. Collectively, our findings reveal that a transient wave of in vivo repopulating hematopoietic cells is generated during the in vitro differentiation of embryonic stem cells and is critically dependent on differentiating culture conditions.

T-1050

LEPTIN ENHANCES ENDOTHELIAL CELL DIFFERENTIATION AND ANGIOGENESIS THROUGH STAT3 ACTIVATION IN MURINE EMBRYONIC STEM CELLS

Kurtovic, Silvia[1], Ng, Tina[1], Gupta, Ankur[1], Arumugaswami, Vaithilingaraja[1,2], Chaiboonma, Kira L.[1,2], Aminzadeh, Mohammad Amin[3], Makkar, Raj[3], Dafoe, Donald C.[1,2], Talavera-Adame, Dodanim[1,2]
[1]*Comprehensive Transplant Center, Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA, Regenerative Medicine Insititute,*
[3]*The Heart Institute,Cedars-Sinai Medical Center, Los Angeles, CA, USA,*

The metabolic regulation of leptin and its angiogenic effects have been well characterized in adult mammals. However, the role of leptin in the differentiation of endothelium and angiogenesis during embryo development has not been completely characterized. We hypothesize that leptin promotes differentiation of embryonic stem cells (ESCs) to endothelial cells (ECs) through activation of the canonical JAK/STAT pathway and, in this way, stimulates angiogenesis. We used an *in vitro* model consisting of murine ESCs aggregated into embryoid bodies (EBs). Vascular density was quantified in binarized images from leptin-treated EBs compared to untreated controls. EC and angiogenesis marker expression was analyzed by qRT-PCR. CD31 expression was also quantified by FACS and co-expression of CD31 and phosphoSTAT3 (pSTAT3) was analyzed by ICC. CD31 positive cells were isolated by magnetic sorting and expanded *in vitro*. Upregulation of endothelial markers (CD31, eNOS, vWF, CD34) and angiogenic markers (FLK1, TIE1, TIE2, ANG1, and ANG2) except FLT-1was found in leptin-treated EBs compared to controls. In addition, a significant increase in vessel density and CD31 (65% vs. 17%) expression was observed in leptin-treated EBs compared to controls. Interestingly, CD31positive cell clusters were also composed of cardiotin positive cells (a specific marker for cardiac cells). However, only CD31 positive cells derived from leptin-treated EBs co-expressed the nuclear transcription factor pSTAT3 and supported up to five passages *in vitro*. Therefore, leptin promotes ECs differentiation in mouse EBs through upregulation of EC and angiogenesis markers and the canonical JAK/STAT pathway activation plays a central role in this differentiation process.

T-1051

DIPEPTIDYL PEPTIDASE-4 INHIBITION AGGRAVATES VASCULAR LEAKAGE THROUGH THE PARADOXICAL EFFECT

Lee, Choon-Soo[1], Kim, Yun Gi[2], Cho, Hyun-Jai[2], Kim, Ju-Young[2], Hur, Jin[2], Lee, Sae-Won[2], Lee, Eun Ju[2], Kwon, Yoo-Wook[2], Yang, Han-Mo[2], Oh, Byung-Hee[2], Park, Young-Bae[2], Kim, Hyo-Soo[2]

[1]Department of Molecular Medicine and Biopharmaceutical Sciences, Department of Molecular Medicine and Biopharmaceutical Sciences,Graduate School of Convergence Science and Technology, and College of Medicine or College of Pharmacy, Seoul National University, Seoul, Republic of Korea, [2]Seoul National University Hospital, Seoul, Republic of Korea

Background: DPP-4 inhibitors are widely used as anti-diabetic medicine. It is also known to accumulate SDF-1α, which stimulates angiogenesis through SDF-1α/CXCR-4 signaling pathway. There is possibility, however, that DPP-4 inhibition may induce vascular leakage and neovascularization, a fundamental pathophysiology of diabetic retinopathy, through accumulation of SDF-1α. Methods and Results: We first checked the presence of CXCR-4 (receptor for SDF-1α) and DPP-4 (antigenic enzyme which cleaves SDF-1α) in human umbilical vein endothelial cells (HUVECs) and smooth muscle cells (SMCs) before evaluating the change in SDF-1α and phosphorylated Src/VE-cadherin in hypoxia/reoxygenation condition. CXCR-4 and DPP-4 were present in both the surface of HUVECs and SMCs and the expression levels were not changed significantly after inducing hypoxia/reoxygenation condition. Under hypoxia/reoxygenation condition, SDF-1α mRNA level was substantially increased in both HUVECs and SMCs. SDF-1α protein was also significantly increased at hypoxia/reoxygenation condition in both HUVECs and SMCs and SMCs showed significantly higher level of SDF-1α protein concentration than HUVECs. Phosphorylated Src and VE-cadherin was also significantly increased at hypoxia/reoxygenation condition in HUVECs. We subsequently performed immuno-fluorescent staining for VE-cadherin and SDF-1α in HUVECs in both normoxia and hypoxia/reoxygenation conditions. Interestingly, after merging the separate immuno-fluorescent images of SDF-1α and VE-cadherin, VE-cadherin cell-cell junction disruption mostly occurred where SDF-1α was strongly stained. To test the hypothesis whether DPP-4 inhibition would increases vascular permeability, we treated HUVECs with Diprotin A (DipA, a DPP-4 chemical inhibitor), and assessed the phosphorylation of Src and VE-cadherin using westernblot assay. Treatment of serum-starved HUVECs with 100 μM of DipA resulted in a significant increase in phosphorylation of Src and VE-cadherin in both normoxia and hypoxia/reoxygenation conditions. DipA significantly increased VE-cadherin phosphorylation in HUVECs, which was reversed by AMD3100 (SDF-1α/CXCR-4 antagonist), and PP2 (Src blocker). Immune-fluorescence staining for VE-cadherin showed that DipA aggravated breakdown of cell junctions, which was prevented by AMD3100 or PP2. On transwell endothelial permeability assay, DipA significantly enhanced SDF-1α mediated endothelial permeability, which was reversed by AMD3100 or PP2. Miles assay demonstrated that systemic injection of DipA for 5 days aggravated SDF-1α induced vascular leakage and neovascularization at the ears of mice, which was also prevented by AMD3100 or PP2. Finally, in the murine retinopathy of prematurity model where retinal vasculopathy is induced by the sequential exposure to normoxia and hyperoxia, systemic injection of DipA aggravated vascular leakage of retinal vessel, which was prevented by AMD3100. Conclusions: Our study raises the hypothesis that DPP-4 inhibitors might aggravate vascular leakage, the fundamental pathophysiology of diabetic retinopathy, by increasing vascular permeability through SDF-1α/CXCR-4 axis followed by Src activation and phosphorylation of VE-cadherin. These data bring forth the safety issue of therapeutic agents based on DPP-4 inhibition, which should be confirmed in clinical data.

T-1052

MOLECULAR INSIGHTS INTO THE HEMANGIOGENIC PROGRAM REVEALED BY GENOME-WIDE ANALYSIS OF ER71/ETV2 CHROMATIN OCCUPANCY

Liu, Fang[1], Li, Daofeng[2], Cha, Minji[1], Kang, Inyoung[1], Yu, Lawrence[1], Wang, Ting[2], Choi, Kyunghee[1]

[1]Washington University School of Medicine in st. Louis, St. Louis, MO, USA, [2]Genetics, Washington University School of Medicine in st. Louis, St. Louis, MO, USA

Blood and vascular systems need to be established prior to functional organogenesis for successful embryogenesis. Effective formation of blood and vascular system requires coordinated expression of genes that control blood and endothelial cell lineage development. However, how the hematopoietic and endothelial cell lineage genes are initiated and maintained during development remains unclear. Previous studies have established that an ETS transcription factor ER71/ETV2 is necessary and sufficient to initiate the hematopoietic and endothelial cell program. Here, we performed genome-wide analysis of ER71/ETV2 chromatin occupancy to understand how hematopoietic and endothelial gene expression patterns are coordinated during the mesodermal transition to hematopoietic and endothelial cell lineages. We discover that ER71/ETV2 is crucial for the generation of Flk-1-expressing hemangiogenic cells through VEGF-Flk1 signaling. A receptor tyrosine kinase, Flk-1+ hemangiogenic mesoderm, hemangioblast, is a common precursor of primitive and definitive blood, and endothelial cells. Using ER71 ChIP-Sequencing combined with microarray analysis of Flk-1+ hemangioblast during ES cell differentiation, we found that the binding of ER71 on the regulatory regions of genes related to VEGF signaling, such as Flk1/Kdr, Flt1, Flt4, Nrp1 and Mapk, were highly enriched. The Flk-1-expressing hemangiogenic cells were missing in ER71 knockout mouse embryos from E7.5 to E8.5, in which VEGF signaling pathway was downregulated. We further demonstrated that the activation of VEGF pathway during hemangioblast was dependent on the ER71 expression in differentiated ES cells (EBs). We further identify that ER71/ETV2 establishes an ETS hierarchy in the blood and endothelial cell formation. The expression kinetics of ETS factors in mouse embryos and in EBs revealed that Er71 gene was transiently expressed and preceded that of other ETS factors, including Fli1, Erg, Elk3, Ets1, Ets2. These ETS genes were up-regulated by enforced ER71 expression, but down-regulated by ER71 deficiency. ETS genes were also bound and activated by ER71 as shown in ChIP-Seq, ChIP-PCR and luciferase reporter assay, suggesting the positive regulation of ER71 on the other ETS factors. Importantly, the blood and vessel defects were also observed in Fli1 or Erg knockout embryos, although they were not as severe as ER71 deficiency. While Flk-1 expressing hemangiogenic cells were missing in the absence of ER71, Fli1 knockout or overexpression showed no effects on the generation of these cells, which indicates that Fli1 is unable to initiate the hematopoietic and endothelial program. Most importantly, hematopoietic genes (Tal1/Scl, Gata2, Lmo2) and endothelial genes (Cdh5/Vecad, Tek/Tie2) were activated by ER71 at the hemangioblast stage. As differentiation progressed, when ER71 expression was no longer detected, these gene loci were alternately occupied by Fli1, suggesting the switching of ETS factors in maintaining the hematopoietic and endothelial cell development. In conclusion, the hematopoietic and endothelial cell program is initiated and maintained by successive ETS factor coordination. These data provide insights into the regulation of hemangioblast, blood and endothelial cell development, which is critical for understanding mesoderm cell fate

determination and the subsequent differentiation of blood and vessel.

T-1053
UNDER-EXPRESSION OF RETINALDEHYDE DEHYDRORENASE 2 CONTRIBUETS TO THE DEFECTIVE FUNCTION OF CIRCULATING ENDOTHELIAL PROGENITAL CELLS IN PEDIATRIC MOYAMOYA DISEASE

Moon, Youn joo[1], Lee, Ji Yeoun[1], Choi, Seung Ah[1], Park, Woong-Yang[2], Wang, Kyu-Chang[1], Joung, Je Gun[3], Hyun Seung, Kang[4], Kim, Jeong Eun[4], Phi, Ji Hoon[1], Kim, Seung-Ki[1]
[1]Seoul National University Children's Hospital, Seoul, Republic of Korea, [2]Sungkyunkwan University School of Medicine, Suwon, Republic of Korea, [3]Samsung Medical Center, Seoul, Republic of Korea, [4]Seoul National University College of Medicine, Seoul, Republic of Korea

Moyamoya disease (MMD) is a common etiology of childhood stroke. Recently, endothelial progenitor cells (EPCs) have been shown to play a key role in the pathogenesis of MMD. This study was performed to identify differentially expressed genes (DEGs) and role of retinoic acid (RA) in EPCs from MMD patients. We performed gene expression profiling of EPCs from MMD patients and normal controls. The tube formation capability was assessed after treatment with all-*trans* RA (atRA) in MMD EPCs and after transfection with retinaldehyde dehydrogenase 2 (Raldh2) with or without of atRA in normal EPCs. Multiplex ELISA assay in the supernatants of MMD EPCs was performed. A total of 396 DEGs were identified. Among the DEGs, Raldh2 was significantly down-regulated in MMD EPCs. Defective tube formation capabilities in MMD EPCs were restored after atRA treatment. Normal EPCs showed decreased *in vitro* capillary formation after knock down of Raldh2 and recovery from this functional impairment after atRA treatment. Multiplex ELISA assays demonstrated decreased level of TGF-β1, MMP-9, and VCAM-1 after atRA treatment. EPCs of MMD patients showed specific DEGs compared to normal control. RA deficiencies caused by under-expression of Raldh2 in the MMD EPCs may play a key role in their functional impairments.

T-1054
GENERATION AND UTILIZATION OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS-DERIVED-IPS FOR STUDYING ENDOTHELIAL GENE REGULATION

Nakhaei-Nejad, Maryam
Medicine, University of Alberta, Edmonton, AB, Canada

Damage to endothelial cells (EC) that form the inner lining of blood vessels, leading to their dysfunction, is the core of various vascular diseases. Understanding molecular mechanisms that govern endothelial gene expression and function is crucial towards developing novel therapeutic approaches for vascular endothelial dysfunction-related complications. Induced pluripotent stem cells (iPS) provide a valuable in vitro system to study genetic modifications that contribute to physiological and pathophysiological function of specific cell types, including EC, without ethical and immunological hurdles associated with human embryonic stem cells (hESC). Thus, we aimed to study endothelial gene regulation by generating iPS from human umbilical vein EC (HUVECs) and differentiating iPS back into EC. This system provides a model to explore how endothelial specific genes are active, then repressed and subsequently reactivated in a homogenous system. Confounding genetic variations that exist when comparing gene expression in distinct EC and non-EC of different origins, are thus eliminated in this system. We have generated iPS using originally reported transcription factors. Colonies with hESC morphology were isolated and passaged. Quantitative RT-PCR and immunofluorescence analysis were used to characterize iPS and cell lineages derived from iPS. Isolated iPS colonies had a significant increase in embryonic genes expression while endothelial specific genes including von Willebrand factor (VWF), and vascular endothelial growth factor receptor 2 (VEGFR2) expressions were not detected. Pluripotency of the iPS were demonstrated by generation of embryoid bodies (EB) and detection of specific markers for the three germ layers, as well as direct differentiation to functional neurons and astrocytes. To study human vascular endothelial development, EBs generated from iPS were transferred to fibronectin-coated dishes in medium containing established endothelial growth factors. VEGFR2+ cells were isolated using flow cytometry and expanded. The raised population (EC-diff) had endothelial morphology and expressed highly endothelial specific markers including VWF. We have previously identified transacting factors that participate in its transcriptional regulation as activators and repressors. Thus, we explored the expression pattern of the identified transacting factors as a mean to determine whether expression pattern of transcription factors that target VWF regulation are associated with establishment of EC phenotype. We compared the expression of these transcription factors in HUVECs, iPS and EC-diff cells and demonstrated that the level of transacting factors that participate as repressors were modestly increased in iPS and EB compared to HUVEC and EC-diff. On the other hand, the activators expressions were almost undetectable in iPS and EB but detected to similar levels in HUVEC and EC-diff. We have been able to successfully generate human iPS and further differentiate them into EC, as well as non-EC types. Our analyses using VWF as a model for endothelial-specific gene suggested that activators have a more important role in regulation of endothelial gene expression compared to repressors. This system provides an invaluable opportunity for understanding the mechanisms responsible for endothelial gene regulation and consequently enables us to develop new therapeutic approaches for vascular diseases.

HEMATOPOIETIC CELLS

T-1055
MASS PRODUCTION OF FUNCTION NEUTROPHILS FROM HUMAN HEMATOPOIETIC STEM CELLS BY SERUM FREE CULTURE STRATEGY

Yao, Chao-Ling, Hu, Yu-Shou, Mu, Chia-Hsiang
Yuan Ze Universityv, Chung-Li City, Taiwan

In recent years, the number of people who receive chemotherapy increased due to the rising trend of malignancy. Hematopoietic stem cells (HSCs) from bone marrow, peripheral blood and cord blood are widely used in transplantation after cancer chemotherapy. Among them, there are several limitations of cord blood hematopoietic stem cell transplantation, such as slow recoveries of neutrophils and platelets. The aim of this study is to develop a neutrophil induction medium to increase the number of neutrophils in vitro, to accelerate the recovery of autoimmune system of patients after cord blood HSC transplantation and to overcome the limitation of clinical application. In this study, HSCs were isolated from cord blood and were expand by a serum-free HSC expansion system (SF-HSC medium) that was developed previously by our lab. Then we screened five of cytokines (SCF, G-CSF, GM-SCF, IL-3 and IL-1 beta) and optimized the concentration of cytokines that can effectively induce serum-free expanded HSCs into CD66+/MPO+ cells. In addition, we also checked CD66+/MPO+ cells by neutrophil-associated surface antigens, such as CD11b, CD13, CD15, CD16, CD33, CD64, TLR-2 and TLR-4, and neutrophil-specific functional assays, such as phagocytosis assay, chemotaxis assay and cellular reactive oxygen species (ROS) production assay, challenged

with Pseudomonas aeruginosa after transplanted neutrophils in NOG mices. Finally, we confirmed induced cells were functional neutrophils. In conclusion, we have successfully established a neutrophil induction medium that can effectively induce HSCs into mature and functional neutrophils in vitro. We believe that combination of SF-HSC medium and neutrophil induction medium can generate a large amount of functional neutrophils and provide a promising cell source for basic research and clinical application in the future.

T-1056
IN VIVO CLONAL DYNAMICS OF HEMATOPOIETIC CELLS UNDER HOMEOSTATIC AND STRESS CONDITIONS

Yu, Vionnie[1], Yusuf, Rushdia[1], Oki, Toshihiko[1], Wu, Juwell[2], Saez, Borja[1], Cook, Colleen[1], Lin, Charles[2], Kharchenko, Peter[3], Scadden, David T.[4]

[1]Center for Regenerative Medicine, Massachusetts General Hospital, Boston, MA, USA, [2]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, MA, USA, [3]Center for Biomedical Informatics, Harvard Medical School, Boston, MA, USA, [4]MGH/Harvard Stem Cell Institute, Boston, MA, USA

Heterogeneity among adult tissue stem cells has been documented in terms of proliferation and lineage bias. Here, we report endogenous fluorophore cell tagging to enable individualized marking of clones of cells, quantification of them and their descendents *in vivo* over time and isolation for molecular analysis. Measurements of clonal size and complexity at each stage of differentiation revealed prominent clonal instability under homeostatic conditions, but remarkably stereotypicbehavior within clones under transplantation, inflammation or genotoxic stress. Clonally related cells had strikingly similar patterns of response in terms of cell type and magnitude of cell production, cell loss or stem cell activation in response to stress. Yet between clones, these features were highly diverse. These data reveal cell intrinsic behaviors of hematopoietic stem/progenitor cellsthat are maintained with limited variation and can be hierarchically organized along multiple different behavioral types. Population responses therefore appear to be the integrated composite of a spectrum of individual clones with strong, autonomously enforced lineage, production and stress response biases. Modulating a tissue response to injury may be possible through select activity of specific clonal subsets.

T-1057
HEMATOPOIETIC STEM CELL QUIESCENCE ATTENUATES DNA DAMAGE REPAIR AND RESPONSE CONTRIBUTING TO AGE-DEPENDENT DNA DAMAGE ACCUMULATION

Beerman, Isabel[1], Seita, Jun[2], Inlay, Matthew A.[3], Weissman, Irving L.[4], Rossi, Derrick J.[5]

[1]Stem Cell and Regenerative Biology, Harvard Medical School, Boston, MA, USA, [2]Stem Cell Biology and Regenerative Medicine, Stanford Institute, Stanford, CA, USA, [3]Department of Molecular Biology and Biochemistry, University of California Irvine, Irvine, CA, USA, [4]Stanford University, Stanford, CA, USA, [5]Harvard Medical School, Boston, MA, USA

Hematopoietic stem cells (HSCs) maintain homeostasis and provide regenerative potential for the blood system throughout life. As such, it has been postulated that HSCs may be uniquely capable of preserving their genomic integrity throughout aging to ensure lifelong functional potential. To directly test this we quantified DNA damage in HSCs and downstream progenitors from young and old mice revealing that strand breaks significantly accrue in HSCs during aging, with the greatest extent of age-associated damage localized to the HSC compartment. DNA damage in HSCs was associated with broad attenuation of DNA repair and response pathways that was dependent upon HSC quiescence. Consistent with this, cycling fetal HSCs and adult HSCs driven into cycle exhibit up-regulation of these pathways leading to repair of accumulated strand breaks. Our results demonstrate that HSCs are not exquisitely geno-protected during aging. Rather, HSC quiescence and concomitant attenuation of DNA damage repair and response pathways underlies DNA damage accumulation providing a potential mechanism through which pre-malignant mutations can accrue in HSCs during aging.

T-1058
USE OF ZINC FINGER-MEDIATED CHROMATIN LOOPING AND GENE TRANSFER TO CORRECT HEMOGLOBINOPATHIES

Breda, Laura[1], Rupon, Jeremy[2], Motta, Irene[1], Deng, Wulan[3], Dong, Alisa[1], Blobel, Gerd[2], Rivella, Stefano[1]

[1]Pediatrics, Hematology-Oncology, Weill Cornell Medical College, New York, NY, USA, [2]Hematology, Children's Hospital of Philadelphia, Philadelphia, PA, USA, [3]Howard Hughes Medical Institute-Janelia Farm Research Campus, Ashburn, VA, USA

The hemoglobinopathies, such as β-thalassemia and sickle cell anemia (SCA), are characterized by mutations of the β-globin gene resulting in either decreased or functionally abnormal hemoglobin (Hb) production. As bone marrow transplant is the only curative option for these patients, there is a strong need for new therapeutic approaches. Both β-thalassemia and SCA represent ideal targets for gene therapy since introduction of a normal β-globin gene can ameliorate the phenotype, as we have shown previously. Overcoming the developmental silencing of the fetal γ-globin gene represents an additional approach for the treatment of hemoglobinopathies. Here, we explore a novel approach to activate the γ-globin gene using forced chromatin looping and compare this strategy with various know chemical inducers of γ-globin. The genes of the β-globin locus undergo developmental activation and silencing in part through competition for an upstream enhancer, the locus control region (LCR). The LCR physically contacts the developmentally active globin gene forming a chromatin loop at the expense of the other genes within the locus in a process which is dependent in part on the protein Ldb1. Previously, we have shown that tethering Ldb1 to the murine β-globin promoter with a custom designed zinc finger protein (ZF-Ldb1) can induce loop formation and β-globin transcription in an erythroid cell line lacking a master regulatory protein and therefore loop formation and transcription within the β-globin locus. We sought to apply this approach to the human locus by activating expression of the fetal γ-globin gene using a ZF-Ldb1 targeting the γ-globin promoters with the goal of re-directing the LCR away from the β-globin promoter. We have developed a protocol to isolate and expand hematopoietic stem cells derived from patients' blood that can be treated by gene transfer and then differentiated into erythroid cells. Using this approach we investigated whether lentiviral-mediated gene transfer of ZF-Ldb1 has the potential to reactivate the synthesis of fetal Hb (HbF) in SCA cells. We compared the efficacy and toxicity of ZF-Ldb1 to drugs that induce HbF. We also compared the ability of β-globin versus ZF-Ldb1 gene transfer to ameliorate the phenotype of β-thalassemia cells. Our preliminary data indicate that lentiviral-mediated ZF-Ldb1 gene transfer increased the synthesis of HbF in SCA erythroid cells up to 74% and, concurrently, reduced sickle Hb (HbS) down to 20%. This effect is particularly relevant, given the toxicity caused by HbS and the beneficial effects of HbF. In separate experiments, we observed that decitabine and pomalidomide increased HbF induction in SCA cells up to 61%, while roughly 1 copy of the ZF-Lbd1 vector raised HbF to 73%. However, while cell viability did not change after transduction with

ZF-Ldb1, cell viability decreased to 39% and 26% with decitabine and pomalidomide, respectively. Furthermore, preliminary experiments in β-thalassemia specimens indicate that the absolute amount of HbF or adult Hb (HbA) increased to similar levels after ZF-Ldb1 or β-globin gene transfer. In conclusions, lentiviral-mediated ZF-Ldb1 gene transfer holds great potential for the treatment of hemoglobinopathies. In SCA specimens this is associated with higher efficacy and lower toxicity compared to administration of pharmacological inducers. In thalassemic specimens, the level of HbF induction might be similar to that achieved by β-globin gene transfer.

T-1059
CELL CYCLE CHECKPOINTS CONTROL GENOME INTEGRITY IN HEMATOPOIETIC STEM AND PROGENITOR CELLS

Brown, Andreas[1], Ueberle, Bettina[1], Geiger, Hartmut[2]
[1]Institute of Molecular Medicine, University of Ulm, Ulm, Germany, [2]Cincinnati Children's Hospital Medical Center Experimental Hematology, Cincinnati, OH, USA

Checkpoints and subsequent DNA repair mechanisms in hematopoietic stem and progenitor cells (HSPCs) greatly differ from those in differentiated tissue in terms of activity, DNA repair efficiency and outcome. Our hypothesis is thus that distinct checkpoint mechanisms are required to maintain lifelong genomic stability and integrity of hematopoietic stem cells. A variety of DNA damage checkpoints are active during the course of cell cycle progression, such as the G1/S and the G2/M checkpoints as well as the spindle assembly checkpoint. The G1/S checkpoint is responsible for DNA damage induced cell cycle arrest during G1 phase and ensures that DNA damages, especially DNA double strand breaks are repaired in G1 and not carried into S phase. Similarly, the G2/M checkpoint response arrests cells in G2 phase as a reaction of serious DNA damage and prevents premature entry into mitosis. In contrast, the spindle assembly checkpoint induces a cell cycle arrest during prometaphase before all chromosomes are attached to the mitotic spindle apparatus thus preventing precocious anaphase onset. Strikingly, one single unattached sister chromatid is sufficient to trigger a "wait anaphase" signal which enables the cell to resolve false microtubule-kinetochore attachments. The mitotic checkpoint is an important mechanism preventing chromosomal instability and aneuploidy, two major hallmarks of tumorigenesis. It is currently unclear whether a counterpart of the spindle assembly checkpoint described for example for fibroblast exists in HSPCs and how checkpoint mechanisms contribute to maintenance, genomic integrity and quiescence of the hematopoietic stem cell pool. Our data demonstrate that hematopoietic stem and progenitor cells lack the G1/S checkpoint and do not arrest in G1 phase in response to irradiation induced DNA damage - in contrast to differentiated hematopoietic cells. Proteins, which are involved in checkpoint signalling, such as Chk2 and p21, are not activated in response to induction of DNA double strand breaks and accumulate at the centrosome. Furthermore, we present data that HSPCs provide an active G2/M checkpoint. Independent of checkpoint activity, HSPCs exhibit extensive levels of apoptosis when irradiated. Preliminary data further support that the spindle assembly checkpoint is activated in HSPCs as a reaction to treatment with microtubule-disturbing toxins. After prolonged checkpoint activity, cells undergo apoptosis or mitotic catastrophe in a p53-dependent manner. Especially long-term stem cells activate apoptosis to a much greater extent than less primitive progenitors or differentiated cells, indicating an important mechanism enabling damaged cells to be separated from the stem cell pool. By applying chemical inhibitors of mitotic kinases, such as the Aurora kinases or Mps1, the checkpoint can be overridden and cells prematurely exit mitosis without successful chromosome segregation and cytokinesis, which might be a critical mechanism contributing to genetic instability in stem cells.

T-1060
GENERATION OF "SEMI-UNIVERSAL DONOR STEM CELLS" THAT EXPRESS A SINGLE FUNCTIONAL HUMAN LEUKOCYTE ANTIGEN (HLA) CLASS I ALLELE

Gornalusse, German[1], Festag, Marvin[1], Hirata, Roli[1], Riolobos, Laura[1], Turtle, Cameron[2], Riddell, Stan[2], Russell, David W.[1]
[1]*Medicine/Hematology, University of Washington, Seattle, WA, USA,* [2]*Program in Immunology, FHCRC, Seattle, WA, USA*

Long-term engraftment of cellular products derived from human embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) is limited due to the rejection of cells with polymorphisms in the human leukocyte antigen (HLA) genes, especially the HLA class I genes. To sustain allograft survival, many strategies have been proposed including HLA-typed stem cell banks, the generation of patient specific iPSCs, or the creation of HLA-homozygous iPSCs. However, all these methods are costly and time-consuming because they require the preparation and approval of multiple cell lines. Our approach to this problem is to create HLA class I-deficient, universal donor stem cells by gene targeting at the β2-Microglobulin (B2M) locus, which encodes the common subunit of all HLA class I heterodimers. However, by chromium release assay, we found that HLA class I-negative CD45+ hematopoietic cells derived from B2M-/- ESCs were susceptible to Natural Killer (NK) cell-mediated lysis. In order to address this potential limitation of class I-negative cells, we expressed a unique HLA class I allele as a single chain dimer (SCD) on the surface of B2M-/- ESCs. These SCDs are fusion proteins with a B2M subunit covalently attached to a flexible linker and the coding sequence of a class I heavy chain. "Semi-universal" cell lines expressing a single SCD should be compatible with a significant percent of the population since they need only match one HLA class I allele. We designed SCDs of HLA-A*0201, which has a frequency of 48% and 46% among Caucasians and Hispanics respectively, as well as SCDs of the common B*0702 and C*0702 alleles. We created HLA class I deficient B2M-/- ESCs by AAV-mediated gene targeting and transduced these cells with an integrating foamy virus vector that expressed the SCD gene from different promoter sequences. By flow cytometry, we showed that in undifferentiated ESCs the EF1α promoter-containing vector produced long-term expression of the HLA-A*0201 SCD, that this expression persisted upon in vitro differentiation of ESCs into CD45+ hematopoietic cells, and that other class I alleles were not expressed in these cells. We also expressed SCDs as gene-targeted knockins by introducing the coding sequence of HLA-A*0201 into the third exon of the B2M gene, which allows for appropriate transcriptional regulation of the SCD. B2M-/-, HLA-A*0201 SCD+ cells were able to present a specific CMV pp65 peptide to HLA-A*0201-restricted CD8+ T-cells as effectively as B2M+/+ cells based on an intracellular IFN-γ expression assay. The effects of SCDs on NK-mediated lysis are currently under investigation. Our results suggested that cells derived from HLA-engineered ESCs could also be designed to load any clinically relevant antigen. To demonstrate this, we constructed single chain trimers (SCTs) in which antigenic peptides were included in the fusion proteins. One application of this strategy is to present a peptide derived from a non-polymorphic cellular protein and thereby prevent the loading of other peptides and limit unwanted reactivity to donor cell antigens. We therefore expressed SCTs based on A*0201 and B*0702 that included peptides of cellular proteins that are commonly found in these HLA molecules. Another application of SCDs is to create highly specific cellular vaccines that present a unique peptide antigen; with this in mind we also created SCTs based on A*0201 that contained the

CMV pp65 peptide antigen. The immune responses produced by these SCTs will be presented at the meeting.

T-1061
ROLE OF CONNEXIN 32 IN HEMATOPOIESIS: MAINTAINING THE BONE-MARROW RECONSTITUTION CAPABILITY IN SECONDARY RECIPIENTS

Hirabayashi, Yoko[1], Tsuboi, Isao[2], Yoon, Byung-Il[3], Kanno, Jun[1], Trosko, James E.[4], Inoue, Tohru[2]
[1]Center for Biological Safety and Research, Div. of Cellular and Molecular Toxicology, National Institute of Health Sciences, Tokyo, Japan, [2]Department of Functional Morphology, Nihon University School of Medicine, Tokyo, Japan, [3]Laboratory of Histology and Molecular Pathogenesis, Kangwon National University, School of Veterinaly Medicine, Chuncheon, Republic of Korea, [4]Michigan State University, East Lansing, MI, USA

Our studies on the role of connexin (Cx) 32 in steady-state hematopoiesis and leukemogenesis were reported previously. Namely, in wild-type mice, Cx32 expression was solely detected in primitive hematopoietic stem/progenitor cells (HSCs/HPCs). In addition, Cx32-knockout (KO) mice showed the following characteristics: first, a prominent decrease in the number of peripheral mononuclear cells (PMCs) associated with various HPCs and a significant increase in the number of primitive HSCs/HPCs in an early stage; second, these trends seemed to be the opposite in aged mice, *i.e.,* a larger number of mature HPCs and a smaller number of immature HSCs/HPCs than in age-matched wild-type mice; and third, an apparently delayed regeneration of HPCs after chemical abrasion. Furthermore, the incidence of leukemogenicity induced by methylnitrosourea increased prominently, as shown by both whole-body studies using wild-type and Cx32-KO mice and bone-marrow transplantation studies using wild-type mice separately reconstituted with unfractionated bone marrow cells from wild-type and Cx32-KO mice. In this study, we examined the bone marrow reconstitution capability of HSCs in Cx32-KO mice by serial transplantation. With $2x10^5$ bone marrow cells freshly isolated from wild-type F1(Ly5.1/Ly5.2) mice as rescuing cells, which prevent acute radiation injury, 500 cells in the lineage-negative, c-kit-positive, and Sca1-positive (LKS) fraction isolated from Ly5.2 mice, wild-type or Cx32-KO were separately transplanted into lethally irradiated Ly5.1 mice as the primary recipients. Two months after transplantation, the primary recipients in both reconstituted groups, i.e., the wild-type and Cx32-KO groups, showed hematopoiesis without significant differences in various hematopoietic parameters. However, mice in Cx32-KO group showed a higher average percentage of PMCs originating from the donor cells but a lower average percentage of cells in the LKS fraction originating from the donor cells than those in the wild-type group (with Cx32). Namely, the average percentages of Ly5.2 PMCs with respect to the total ± standard deviations (s.d.) were 34.7±14.3% and 50.7± 9.9% (p=0.006), whereas those in the LKS fraction were 20.8±5.2% and 14.2±5.3% in the wild-type group and Cx32-KO group, respectively (p=0.110). Then, Ly5.2 donor cells in the LKS fraction were separately isolated from the primary recipients of both groups and 400 cells each were transferred into secondary recipient mice (Ly5.1) with $2x10^5$ rescuing cells from F1(Ly5.1/Ly5.2) mice. Four months after reconstitution, similarly to the primary recipients, secondary recipients in both groups showed reconstituted hematopoiesis without any significant differences in various hematopoietic parameters. However, cells of donor origin in the LKS fraction could be detected only in the wild-type group. Namely, four out of seven recipients in the wild-type group showed over 0.5% donor cells and the average percentage with s.d. for 4 mice was 25.1±27.9%, whereas none of the recipients out of five in the Cx32-KO group showed more than 0.5% donor cells. The above-mentioned findings in the present study, in addition to the previous findings, imply that Cx32 plays an essential role in maintaining self-renewal proliferation of primitive hematopoietic stem cells to prevent their exhaustion, and also in simultaneously suppressing neoplastic alterations.

T-1062
CHARACTERIZATION OF GATA2 VE ENHANCER ACTIVITY IN MOUSE HEMATOPOIETIC STEM CELLS

Hosoya, Tomonori, Lim, Kim-Chew, KU, CHIA, Engel, James Douglas
Cell and Developmental Biology, University of Michigan, Ann Arbor, MI, USA

Hematopoietic stem cells (HSCs) support life-long replenishment of all hematopoietic cell lineages. We previously identified a vascular endothelial (VE) enhancer in the fourth Gata2 intron. We recently discovered that CreERT2 placed under the regulatory control of the VE enhancer ablates GATA2 in fetal and adult HSC, and abolished definitive HSC activity in conditional Gata2 knock-out mice. Flow cytometric analysis of an mCherry (mCh) reporter gene placed under the control of the VE enhancer revealed that mCh expression (VE enhancer activity) is exclusively confined to immature Lin- Sca1+ cKit+ (LSK) cells, but is not expressed in mature cells expressing hematopoietic cell surface markers TER119, Mac1, Gr1, B220 or CD19. Since the LSK population contains not only HSCs, but also multi-lineage progenitors, we further examined a more pure HSC population as defined by the SLAM cell surface molecules, CD150 and CD48 (long-term repopulating HSC frequency in LSK CD150+ CD48- (LSKS) cells is 47%, but about 4% in LSK). The frequency of mCh+ cells was approximately 60% among mouse adult bone marrow LSKS cells. We found that both the mCh+ and mCh- LSKS cells retained long-term reconstitution potential in lethally irradiated mice. However, while the mCh+ LSKS cells regenerated both mCh+ and mCh- LSKS cells sixteen weeks post-transplantation, the mCh- LSKS cells regenerated only mCh- LSKS cells. In contrast to their abundance in the adult bone marrow, almost all LSKS were mCh+ in the e14.5 fetal liver. These data indicate that LSKS mCh- HSC arise from LSKS mCh+ HSC. Thus, it appears that LSKS mCh+ HSC are positioned at the pinnacle of the hematopoietic cell hierarchy and that the Gata2 VE enhancer is selectively active in a subset of adult bone marrow HSC.

T-1063
ABERRANT ACTIVATION OF THE MIR145-TIRAP-IFN-GAMMA AXIS MEDIATES MDS-LIKE MARROW FAILURE

Ibrahim, Rawa, Wegrzyn-Woltosz, Joanna, Umlandt, Patricia, Fuller, Megan, Karsan, Aly
Genome Sciences Centre, BC Cancer Research Centre, Vancouver, BC, Canada

The Myelodysplastic Syndromes (MDS) are characterized by ineffective hematopoiesis in one or more lineage of the bone marrow, resulting in peripheral cytopenias and the propensity to develop into either acute myeloid leukemia (AML) or bone marrow failure (BMF). The molecular and cellular events involved in MDS development are yet to be elucidated. Previous work from our lab has shown that haploinsufficiency of miR-145 and miR146a recapitulates some of the characteristics associated with del(5q) MDS. Pathway analysis predicts that innate immune signaling is among the most highly deregulated pathways following loss of these two miRNAs. The innate immune signaling moleculesTRAF6 and TIRAP are targets of miR-146a and miR-145 respectively. While the role of TRAF6 in del(5q) MDS has been investigated, little is known about the role of TIRAP in MDS pathogenesis. To investigate the role of TIRAP in aberrant

hematopoiesis, we transplanted lethally irradiated mice with marrow cells expressing TIRAP or vector control. Similar to low risk MDS patients, the marrows of TIRAP transplanted mice are characterized by increased apoptosis as measured by Annezin V staining. Furthermore, they develop BMF characterized by pancytopenia as early as 4 weeks post-transplant (unlike TRAF6 transplanted mice which succumb to BMF or AML approximately 4 months post-transplant). In MDS, normal hematopoiesis is blocked in the normal fraction of the marrow by autoimmunity associated cytokines. We performed expression profiling to identify factors responsible for suppression of normal hematopoiesis in our BMF model. Q-RT-PCR showed increased expression of both IL-10 and IFNγ in TIRAP expressing marrow compared to vector control. This increase in cytokine expression occurs in a TRAF6 independent manner suggesting the involvement of TIRAP in a non-canonical signaling pathway. Interestingly, IFNγ and IL-10 have been implicated in MDS and other BMF conditions. Using IL-10 and IFNγ KO mice, we found that loss of IFNγ but not IL-10 rescues the pancytopenia phenotype and prevents early death due to BMF. Interestingly, mice transplanted with TIRAP expressing IFNγ KO bone marrow succumb to a myeloproliferative disorder at later time points, suggesting that TIRAP activates both myelosuppressive pathways mediated by IFNγ as well as myeloproliferative pathways.

T-1064
NOVEL CULTURE MEDIUM USING A SMALL-MOLECULE AGONIST OF THROMBOPOIETIN RECEPTOR

Honda, Makoto[1], Nishino, Taito[2], **Inamura, Mitsuru**[1]
[1]ReproCELL Inc, Kanagawa, Japan, [2]Nissan chemical industries, LTD, Tokyo, Japan

Hematopoietic stem cells (HSCs) are defined by their capacity to self-renew, to differentiate into all blood cell lineages and can be applied for transplantation therapy. Since a large number of HSCs are required for clinical use, the improvement of techniques for expansion of HSCs ex vivo is a challengeable issue. Several cytokines are available for this purpose. Thrombopoietin (TPO) is an essential cytokine that regulates megakaryocyte production, signaling through its receptor c-MPL. We have developed a small-molecule agonist (NR-101) of c-MPL and reported that human HSCs were expanded efficiently ex vivo with NR-101. Using a new small-molecule agonist related to NR-101, we produced a novel culture medium, ReproHSCTM, which includes a TPOR agonist. Here we demonstrated that ReproHSCTM efficiently expands human CD34+CD38- primitive hematopoietic cells in culture and thereby enhances repopulating capacity of HSCs in NOD/SCID mice. Human blood cord CD34+ cells were cultured with ReproHSCTM supplemented with only SCF for 7 days. The total cell number was increased about 40-fold during culture. CD34+ cells and CD34+CD38- cells were expanded 12-fold and 8.5-fold, respectively. We then transplanted expanded cells with ReproHSCTM supplemented with SCF and flt3 ligand for 14 days into NOD/SCID mice and analyzed the SCID-repopulating CD45+ cells with flow cytometry. The expanded cells established engraftment better than the fresh CD34+ cells did. These results show that ReproHSCTM is a novel medium suitable for the expansion of HSCs ex vivo.

T-1066
IMPROVING THE ANGIOGENIC ABILITIES OF MOBILIZED PERIPHERAL BLOOD STEM CELLS ACHIEVED BY PRIMING WITH ACTIVATED PLATELET SUPERNATANT FOR REGENERATIVE CELL THERAPY

Kang, Jin-A[1], Yun, Ji-Yeon[2], Hur, Jin[3], Lee, Hwan[1], Jin, Yunjung[1], Choi, Jae-Il[4], Kang, Jeehoon[5], Choi, Young-Eun[1], Lee, Hyun-Chae[1], Yang, Han-Mo[6], Park, Young-Bae[6], Kim, Hyo-Soo[7]
[1]Seoul National University Hospital, Seoul, Republic of Korea, [2]Seoul National University, Seoul, Republic of Korea, [3]Seoul Natl University Hospital Division of Cardiology Department of Internal Medicine, Seoul, Republic of Korea, [4]Seoul Natl University Hospital, Seoul, Korea, Democratic People's Republic of, [5]Seoul National University Hospital, Seoul, Republic of Korea, [6]Seoul National University Hospital, Seoul, Republic of Korea, [7]Seoul National University Hospital Department of Internal Medicine, Seoul, Republic of Korea

Platelets play a critical role in hemostasis but also have the ability to promote angiogenesis and tissue repair by secreting of numerous cytokine and forming an angiogenic milieu. We investigated whether autologous 'activated platelet supernatant (APS)' can prime peripheral blood stem cells and enhance the pro-angiogenic potential for stem cell-based therapy in ischemic diseases. Granulocyte-colony stimulating factor mobilized peripheral blood stem cells (^{mob}PBSC) were isolated from healthy volunteers, while APS was collected from platelet rich plasma by thrombin activation. ^{mob}PBSCs were primed with APS for 6 hours, and the characteristics of APS-primed ^{mob}PBSCs were checked. For safety analysis, we estimated the thrombogenicity of platelets in whole blood mixed with $^{APS\ primed\ mob}$PBSCs by expression of glycoprotein IIb and IIIa on platelets. APS had a higher level of various cytokines, such as IL8, IL17, PDGF and VEGF than naïve platelet supernatants. Through APS priming APS-primed ^{mob}PBSCs had more expression of angiogenic factors, surface markers (i.e. CD34, CD31, and CXCR4) and integrins (integrin α5, β1 and β2) than $^{Veh\ primed}$ and $^{Pre\ primed\ mob}$PBSC. Also APS-primed ^{mob}PBSCs were polarized toward CD14++/CD16+ pro-angiogenic monocytes. As a result, adhesion to endothelial cells and fibronectin which represents cell to cell and cell to extracellular matrix adhesion respectively, was increased The culture supernatant of APS-primed ^{mob}PBSCs contained high levels of IL8, IL10, IL17 and TNFα, thereby augmented proliferation and capillary network formation of endothelial cells. In-vivo transplantation of APS-primed ^{mob}PBSC into athymic mice ischemic hindlimbs and Matrigel plugs elicited vessel differentiation and improvement in tissue repair. In thrombogenicity test, platelet activity increased after mixing whole blood with ^{mob}PBSC regardless of the priming agent. However, this was reduced by pretreatment of aspirin, which is an antiplatelet agent prescribed to patients with ischemic diseases. Collectively, our data demonstrate that ^{mob}PBSCs primed with APS have improved angiogenic potential, and this can be an adjunctive strategy to enhance the efficiency of stem cell therapy for ischemic diseases.

T-1067

REPROGRAMMING OF HUMAN ERYTHROID PROGENITORS TO MAGAKARYOCYTES BY FLI1 AND ERG1 TRANSCRIPTION FACTORS

Kheolamai, Pakpoom[1], Siripin, Darin[2], U-Pratya, Yaowalak[3], Wattanapanitch, Methichit[4], Supokawej, Aungkura[2], Issaragrisil, Surapol[3]

[1]Division of Cell Biology, Faculty of Medicine, Thammasat University, Pathumthani, Thailand, [2]Faculty of Medical Technology, Mahidol University, Bangkok, Thailand, [3]Division of Hematology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, [4]Siriraj Center of Excellence for Stem Cell Research, Bangkok, Thailand

Megakaryocytes and erythrocytes are derived from common progenitors called megakaryocytic-erythroid progenitor cells (MEPs). However, megakaryocyte lineages follow a very different gene expression program and morphological transformation from that of erythrocytes. Although, previous studies suggest that transcription factors ERG and FLI1 might play roles in directing MEP differentiation toward megakaryocyte lineage, the functions of these transcription factors in directing and maintaining megakaryocyte gene expression program are still not fully elucidated. To study the roles of ERG and FLI1 in directing and maintaining megakaryocyte gene expression program, we overexpressed Erg and Fli1 genes in purified human erythroid progenitors which are derived from bone marrow and umbilical cord blood by magnetic activated cell sorting with the use of erythrocyte lineage markers, CD71 and glycophorin A (GPA). Phenotypic characteristics of the transformed erythroid progenitors (named GPA+/TEPs and CD71+/TEPs) were subsequently determined by cell morphology, the expression of megakaryocyte lineage markers, the ability to form megakaryocyte colonies under appropriate culture condition, as well as the ability to produce functional platelets in vitro. Our results showed that the percentages of GPA+/TEPs and CD71+/TEPs which express megakaryocyte-specific marker CD41 (also known as glycoprotein IIb/IIIa which is a fibrinogen receptor) after culture in megakaryocyte inducing medium were much higher than those of their non-transformed counterparts (41.97 ± 5.86 % and 56.80 ± 2.48% vs. 1.30 ± 0.60 %, $P < 0.05$). In addition, the morphology of GPA+/TEPs and CD71+/TEPs was changed from small cells with high nuclear/cytoplasmic ratio (which are typical for erythroid progenitors) to large multi-nucleated cells which express megakaryocyte specific protein CD41 and megakaryocyte specific genes β1-tubulin and c-MPL (a thrombopoietin receptor). Moreover, the GPA+/TEPs and CD71+/TEPs could generate several megakaryocyte colony-forming units (CFU-MK) which express glycoprotein IIb/IIIa after cultured in collagen-based megakaryocyte inducing medium. Furthermore, the GPA+/TEPs and CD71+/TEPs could produce functional platelets which are able to aggregate with normal human platelets in response to thrombin stimulation. In conclusion, our study demonstrates that the over-expressions of megakaryocyte-enriched transcription factor FLI1 and ERG are sufficient to reprogram committed erythroid progenitors to megakaryocytes which have an ability to produce functional platelets in vitro. The generation of induced megakaryocytes from erythroid progenitors will increase our understanding of the highly sophisticated processes of megakaryopoiesis and thrombopoiesis and might lead to the development of a novel source of platelets for future clinical applications.

T-1068

CANONICAL WNT SIGNALING ALTERATIONS AND DELAYED T-CELL DIFFERENTIATION IN HUMAN HEMATOPOIETIC STEM CELL AGING

Khoo, Melissa L.M., Carlin, Stephen M., Lutherborrow, Mark A., Ma, David D.F., Moore, John J.

Blood Stem Cells and Cancer Research, St Vincent's Centre for Applied Medical Research and UNSW, Sydney, NSW, Australia

Somatic stem cells maintain tissue homeostasis and play a key role in the regenerative potential of organ systems. During aging, there is a decline in the functional potential of somatic stem cells, which may contribute to pathophysiology in the elderly. Age-related defects in the hematopoietic stem cell (HSC) compartment can culminate in clinically relevant consequences, such as reduced regenerative potential, decreased competence of the adaptive immune system, and increased incidence of myeloid diseases, including myelodysplastic syndromes and leukemia. In addition, difficulties associated with clinical allogeneic hematopoietic cell transplantation may be compounded, since the risk of life-threatening complications is increased with prolonged periods of T-cell deficiency. The ability to promote T-cell generation from mobilized adult peripheral blood (PB) could provide improved immune reconstitution following transplantation, and as a result, improved patient outcomes. Although the decline in HSC function during aging has been well-documented, the underlying mechanisms remain largely undetermined. In addition, the majority of studies have investigated the effects of HSC aging in animal models, and there remains a paucity of studies in human HSC aging. In the present study, we have endeavoured to explore the mechanisms involved in human HSC aging through examining the differential expression profiles of young and aged HSCs and the capacity for differentiation into hematopoietic lineages. We found that genes involved in Wnt signaling are differentially expressed in young (cord blood) and aged (mobilized adult PB) human HSCs, with reduced Wnt signaling activation in aged HSCs. Up-regulation of key Wnt signaling mediator β-catenin was found to be critical for the promotion of T-lineage differentiation in the OP9-DL1 co-culture system; while moderate levels of β-catenin were sufficient for myeloid differentiation. Examination of Wnt target gene activation during T-lineage differentiation confirmed the association between reduced expression of Wnt target genes, increased age, and impaired/delayed T-cell differentiation. Immunofluorescence staining analysis revealed the localization of β-catenin in the nuclei of HSCs during T-lineage differentiation, indicative of active canonical Wnt signaling. In addition, we identified that the defect in Wnt signal activation of aged human HSCs occurs in the early T-progenitor (CD34+CD7+) subset. Our results suggest that manipulation of Wnt signaling activity in HSCs could improve T-cell generation and immune reconstitution in clinical hematopoietic transplantation, and may have the potential to reduce pathophysiology in the elderly. Further investigations are warranted to allow a complete understanding of the multifactorial mechanisms underlying age-associated deficiencies in hematopoiesis.

T-1069

ASSESSMENT OF THE ENGRAFTMENT POTENTIAL OF HEMATOPOIETIC REPOPULATING CELLS EXPANDED WITH A NOVEL SMALL MOLECULE

Gori, Jennifer[1], Chandrasekaran, Devikha[1], Adair, Jennifer Eileen[1], Sauvageau, Guy[2], **Kiem, Hans-Peter**[1]
[1]*Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA,* [2]*Institute for Research in Immunology and Cancer, Montreal, QC, Canada*

Small molecules that expand hematopoietic stem/progenitor cells (HSPCs) while preserving multipotency has the potential to increase the cell dose available for transplantation thus improving clinical outcome in patients transplanted with gene-modified autologous or allogeneic umbilical cord blood (UCB) $CD34^+$ cells, especially when cell dose is a limiting factor. While some of these studies can be done ex vivo and/or in immunodeficient mice, clinically relevant large animal studies are critical for assessment of long-term multilineage repopulation. We established a stem cell competitive repopulation assay in the nonhuman primate model that allows us to study multiple different ex vivo expansion conditions in a single animal. Here we studied the novel small molecules UM729 and UM171 that synergize with StemRegenin1 (SR1) to expand human HSPCs capable of long-term engraftment in immunodeficient mice. Toward translation for clinical use, we hypothesized that UM171 would expand macaque (*Macaca nemestrina, Mn*) $CD34^+$ HSPCs obtained from UCB, steady state and mobilized bone marrow, to increase the cell dose available for engraftment. We first evaluated engraftment of Mn UCB $CD34^+$ cells expanded with cytokines ± SR1, UM729, or UM171 in NSG mice. While all conditions expanded $CD34^+$ cells 44-fold, the UM171-expanded cells had had significantly higher 10-week engraftment compared to other cohorts (41% primate $CD45^+$ cells, $p<0.05$). We next tested the effect of UM171 on expansion of bone marrow-derived $CD34^+$ cells. Combination SR1/UM171 expanded $CD34^+CD38^-$ cells from mobilized and steady state marrow 9-fold and 3-fold, respectively. To determine whether inclusion of UM171 during transduction improved gene-modified cell survival, marrow $CD34^+$ cells were transduced with GFP expressing lentivirus with or without UM171. Co-culture with UM171 increased $CD34^+$ cell transduction and expansion 3-fold compared to cells cultured with vehicle. To determine engraftment potential of UM171-expanded autologous gene-modified $CD34^+$ cells, we use the competitive repopulation assay in the clinically relevant macaque model. In this study, mobilized bone marrow $CD34^+$ cells were divided into two fractions and transduced with lentivirus vectors expressing the P140K variant of methylguanine methyltransferase (MGMT) with GFP or mCherry. Cells transduced/expanded with the combination SR1/UM171 (GFP) had 2-fold higher gene transfer (66% vs. 33%) and expanded 2-fold more compared to SR1 alone (mCherry). The original $CD34^+$ cell donor was preconditioned with myeloablative irradiation (1020 cGy) and then co-infused with the GFP (UM171/SR1) and mCherry (SR1) transduced cell fractions (infusion product ratio of GFP^+CD34^+ to $mCherry^+CD34^+$ >2:1). In vivo gene marking analysis revealed 5-fold higher GFP^+ granulocytes compared to $mCherry^+$ granulocytes (20% GFP^+ vs. 4% $mCherry^+$) 60 days after transplantation. Marking was also detected lymphocytes (11% GFP^+, 5% $mCherry^+$). These findings show that UM171 enhances transduction/expansion of HSPCs and marks an important step toward clinical translation of small molecules for HSPC therapeutics for use in UCB and gene modified autologous HSPC transplantation.

T-1070

EBF2 COORDINATES AN OSTEOBLASTIC NICHE FOR HEMATOPOIETIC STEM CELLS AND PREVENTS THEIR APOPTOSIS VIA ANGIOGENIN1

Hinzen, Christoph, Wang, Qiongman, Zimber-Strobl, Ursula, **Kieslinger, Matthias**
Helmholtz Center Munich, Munich, Germany

Hematopoietic stem cells (HSC) are supported by a specialised microenvironment that regulates their behaviour. Within the bone marrow, the transcription factor Ebf2 is expressed by adipocytes and a subset of immature osteoblastic cells (IEO). Ebf2 is required for the maintenance of HSC, but the contribution of osteoblastic and adipocytic cells to Ebf2-mediated HSC support is unclear. Here, we identify virtually all supporting activity of Ebf2 within Osterix-positive IEO cells, whereas its expression in adipocytes does not contribute. IEO cells express many genes already implicated in the support of HSC and several of those are de-regulated in absence of Ebf2, identifying Ebf2 as a central regulator of an osteoblastic HSC niche. Angiogenin1 (Ang1) is a novel Ebf2 target whose expression is diminished in Ebf2-deficient IEO cells. Ang1-expressing IEO cells are in contact with immature hematopoietic cells and Ang1 prevents apoptosis of HSC under steady-state conditions. Thus, Osx+ IEO cells are niche cells for HSC and Ebf2 coordinates the expression of supporting genes. Ang1 is a novel factor in the communication between the HSC and its niche and is required for HSC survival.

T-1073

THE ROLE OF NEUROTROPHIN SIGNALING IN MEGAKARYOCYTE DIFFERENTIATION AND PLATELET PRODUCTION

Kizilyer, Ayse[1], Singh, Vir[2], Jones, Letitia[2], Singh, Meera[2], Davidson, Donna[2], Kiebala, Michelle[2], Maggirwar, Sanjay[2]
[1]*Department of Pathology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA,* [2]*Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA*

Platelets are small, anucleate blood cells that play a role in hemostasis, thrombosis, and inflammation. Disturbances in platelet production (thrombopoiesis) and/or platelet turnover give rise to aberrant platelet counts and pose a health risk due to severe hemorrhages, thrombus formation or impaired immune response. Current therapies for managing these abnormalities involve platelet transfusions and intervention in the platelet production process. However, these methods are not time- or cost-effective, and other conditions such as productive infections and alloimmunization limits their efficacy. Developing more effective therapies requires a better understanding of the molecular mechanisms underlying thrombopoiesis. Platelet production is a multistage process requiring megakaryocyte maturation and fragmentation in the bone marrow and it is triggered by an array of growth factors and cytokines. Neurotrophins are one of those growth factors that exist in the bone marrow. While their roles in cell proliferation, differentiation and survival have been extensively characterized in the central nervous system, neurotrophins have also thought to elicit effects on both hematopoietic and stromal cells of the bone marrow. In this study, we demonstrate the presence of neurotrophin receptors, tropomyosin receptor kinases (Trks) and/or low affinity p75NTR, in human megakaryocytic cell lines and primary megakaryocytes. Treatment of human megakaryocytic cell lines with a potent Trk inhibitor, K252a, increases formation of platelet-like particles. In line with this, mice treated with K252a intraperitoneally for 15 days were found to posses higher platelet counts above the

baseline. Furthermore, K252a is also able to accelerate platelet recovery after antibody-mediated platelet depletion in mice. These results suggest that neurotrophins are naturally occurring negative regulators of thrombopoiesis and further investigation of neurotrophin signaling is expected to yield improved strategies for proper control of platelet numbers.

T-1074
DIFFERENT GROWTH FACTOR-REGULATED CELL CYCLE AND FATE CHANGES OF PURIFIED HUMAN HEMATOPOIETIC STEM CELLS REVEALED IN MICROFLUIDIC CULTURES

Knapp, David JHF[1], Rabu, Gabrielle M.[1], Ricicova, Marketa[2], Lecault, Véronique[2], Aghaeepour, Nima[1], van Loenhout, Marijn[2], Miller, Paul Harry[1], Beer, Philip Anthony[1], Da Costa, Daniel[2], VanInsberghe, Michael[2], Heyries, Kevin[2], Hansen, Carl[2], Piret, James[2], Eaves, Connie[1]
[1]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada, [2]University of British Columbia, Vancouver, BC, Canada

Background: Human hematopoietic stem cells (HSCs) have been historically difficult to obtain at sufficient purity to examine directly their responses to specific growth factors (GFs) alone or in combination. We have taken advantage of the recently described 10% purity of human HSCs in the CD49f$^+$ subset of CD34$^+$CD38$^-$CD45RA$^-$CD90$^+$ cord blood cells (CD49f$^+$ CB cells) to test the ability of a 5 GF combination to maintain HSC numbers in 3 wk-bulk cultures and to investigate the effect of component GFs on the initial kinetics of their survival, division, and differentiation imaged at high resolution in 4-day single-cell cultures in microfluidic arrays. Methods: Human CD49f$^+$ CB cells were isolated by FACS and HSCs quantified by limiting dilution transplants in NOD/SCID-IL2Rγc$^{-/-}$ (NSG) mice assessed for human chimerism in the marrow 30 wk later. Human CD34$^+$CD38$^-$ cells present at that time were isolated and injected into secondary NSG recipients which were then similarly assessed for another 30 wks. "Bulk" cultures were initiated with10^3 CD49f$^+$ CB cells in serum-free medium (SFM) containing 100 ng/mL FLT3L and Steel factor (SF) and 20 ng/mL IL3, IL6, and GCSF (5 GFs). Microfluidics cultures were established in multi-layer poly(dimethylsiloxane) chips containing 4032 wells each. CD49f$^+$ cells were loaded into the wells in SFM and perfused with defined GFs and a non-toxic concentration of fluorescent anti-CD45RA. Chips were maintained with temperature, CO_2 and humidity control with bright-field images taken every 20-30 min and fluorescent images daily. Results: Limiting dilution transplant assessment showed HSCs (cells able to reconstitute mice for 30 wk) constituted 1/9 CD49f$^+$ cells (95% CI = 1/3 to 1/22). After 21 days in culture in 5 GFs, the total number of HSCs present was not significantly changed (49% of input,95% CI = 8-310%, p=0.3). Secondary 30-wk transplants confirmed the equivalent continuing regenerative activity of the cells cultured in 5 GFs with a trend towards their superior chimerism. In the microfluidic cultures, ~90% of input CD49f$^+$ cells survived in 5 GFs over the first 4 days and, of these, 85% executed a division within that time (n=190, 3 expts). ~80% of 1st divisions produced both daughter cells expressing the differentiation marker CD45RA, with only ~20% giving progeny that retained a CD45RA$^-$ phenotype. When exposed only to FLT3, IL3, SF, IL6, or GCSF, survival was increasingly reduced from incubation in all 5 GFs together (Holm-corrected, pair-wise p<0.001). However, selective removal of SF did not have this effect (p=0.9), although it significantly reduced the number of cells that divided in 4 days (p<0.001). IL3 and SF alone supported rare cell divisions and FLT3L, IL6, and GCSF alone did not support any. Conclusions: The 5 GF cocktail used here promotes the full initial survival and rapid division of most input CD49f$^+$ CB cells but most of these show immediate evidence of differentiation. Interestingly, the fraction that does not is numerically similar to the input frequency of HSCs with >1 year *in vivo* regenerative activity and that are also maintained through the multiple cell divisions that occur in more prolonged (3-wk) cultures. Analysis of the initial survival, division and "differentiation" responses of single input CD49f$^+$ cells exposed to different combinations of these 5 GFs suggest that the individual GFs support overlapping but mechanistically separable programs that must be activated in a combinatorial fashion to optimize HSC outputs.

T-1075
MATERNAL SMOKING EFFECTS ON CORD BLOOD ENDOTHELIAL PROGENITOR CELLS: EVIDENCE FOR OXIDATIVE STRESS AND APOPTOSIS

Korraa, Soheir S.M.[1], Shatla, Rania Hamid[2]
[1]Radiation Health Research, National Centre for Radiation Research and Tech, Cairo, Egypt, [2]Radiation Health Research, Faculty of Medicine - Ain Shams University, Cairo, Egypt

Genotoxic/carcinogenic substances or metabolites in cigarette smoke are capable of passing through the placenta and harming a newborn's health. Smoking is also known as a factor in the formation of oxidative damage and is involved in vascular inflammation and impairment of endothelial progenitor cells (EPCs). This study aim was to evaluate the redox balance, apoptosis of circulating mononuclear cells and the number of EPCs in maternal blood of smokers compared to nonsmokers. Cord blood from 26 maternal smokers and 36 non-smokers was obtained. EPCs in terms of CD34, CD133 and KDR were identified by flow cytometry. Oxidative stress was identified in terms of plasma lipid peroxidation and nitrite/nitrate levels. Apoptosis was identified in terms of DNA fragmentation, Bax mRNA and Bcl-2 protein cell expression Cord Blood from maternal smokers showed lower levels of EPCs per 105 mononuclear cells surface markers [CD34 (53.4 ± 6.7 vs. 59.6 ± 6.3), CD133 (51.7 ± 4.1 vs. 55.3 ± 3.5) and KDR (50.2 ± 5.2 vs. 53.9 ± 5.6)] compared to non-smokers. Lipid peroxidation measured in terms of plasma malondialdehyde (14.5 ± 2.3 vs. 10 ± 3.1 nmole/ml) and nitrite/nitrate levels (18 ± 1.5 vs. 12.5 ± 1.2 µg/ml) were significantly higher among smokers compared to non-smokers. Regarding apoptosis: DNA fragmentation (0.5 ± 0.2 vs. 1.4 ± 0.3 %) and Bax mRNA relative expression (0.14 ± 0.01 vs. 0.04 ± 0.01) were significantly higher, while Bcl-2 protein was significantly lower (6.7 ± 1.2 vs. 8.9 ± 2.1 nmole/ml) in cord blood leucocytes of maternal smokers fibrinogen and lower levels of HDL-C. ROS and MnSOD were higher (p<0.001), while catalase and GPx-1 were lower (p<0.001) as was EPC number (p<0.001) in smokers. CPC and EPC correlated with HDL-C, CRP, ROS and enzyme expression and activity. These results suggest that maternal smoking effects involves and oxidative stress that induces apoptosis of EPCs and that maternal smoking plays an important role in impairing progenitor cells.

T-1076
PROCESSING AND CRYOPRESERVING CORD BLOOD DERIVED HEMATOPOIETIC STEM CELLS USING THE SYNGENX™-1000 PLATFORM

Kumar, Vijay, Perea, John
Scientific Affair, SynGen Inc., Sacramento, CA, USA

Background: The SynGenX™-1000 Platform has been developed for automated processing of cord blood (CB). It consists of two companion products: the SynGenXTM-1000 System for harvesting buffy coat (BC) from CB and the CryoPRO-2 cryopreservation/storage bag set for mixing the BC with cryoprotectant and storage at cryogenic temperatures. The SynGenXTM-1000 system consists of a control module (CM), docking station (DS), functionally closed

sterile disposable cartridge (DC) and supporting DataTrak application software that is run on a PC. Cord blood is transferred under sterile conditions to the DC, which is then snapped into the control module and placed in the centrifuge cup. After high-speed (2,000xg) stratification, RBCs and BC are transferred to separate compartments within the DC during low speed centrifugation (50xg and 100xg, respectively) and the control module is transferred to the docking station to download the run data. Materials and Methods: A total of 21 non-clinical cord blood units (donated to NCBP-NYBC by mothers, pursuant to IRB-approved Informed Consent) with volumes ranging from 76-138 mL were utilized for this study. All units were processed with HESPAN at a ratio of 1 part HESPAN to 5 parts of cord blood prior to processing. The 20 ml BC harvest was transferred to CryoPRO-2 cryopreservation/storage bag and, after mixing with 5 ml cryoprotectant, the 25 ml sample in the freezing bag was frozen at a controlled rate and stored in the BioArchive® System. Pre-cryopreservation and post-thaw samples were analyzed for TNC, MNC, CD34+, and CD45+ cell recovery and viability. Results: Results are presented as the Mean ± S.D. for all values. The SynGenX-1000 system processed the BC into a final volume of 20.0 ± 0.1 mL, with a final average hematocrit of 26.6 ± 0.9%. The SynGenX-1000 recovered 86.0 ± 6.0 of TNCs, 93.7 ± 5.1% of MNCs, 100.1 ± 2.0% of CD34+ cells, and 89.5 ± 7.6% of CD45+ cells. Post- thaw recovery of MNCs was 86.4 ± 5.8% and of CD34+ cells was 82.9 ±12.9 %. Post-thaw CD34+ cells retained 94.7 ± 3.32% viability. Conclusions: The SynGenX™-1000 platform consistently yields TNC, MNC, CD34+ and CD45+ cell recoveries of greater than 75% after cryopreservation of cord blood samples with starting CB volumes of 76-138 mL. The results from this study demonstrate that the SynGenX™-1000 Platform achieved automated volume reduction of cord blood with highly efficient cell recovery and excellent viability after cryopreservation.

T-1077
11,12 EPOXY-EICOSATRIENOIC ACID SIGNALING IN HEMATOPOIETIC STEM CELL TRANSPLANT AND ENGRAFTMENT

Lahvic, Jamie L.[1], Li, Pulin[1], Binder, Vera[2], Riley, Elizabeth B.[2], Pugach, Emily K.[2], Tamplin, Owen J.[2], Zon, Leonard I.[2]
[1]*Harvard Medical School/Boston Children's Hospital, Boston, MA, USA,* [2]*Boston Children's Hospital, Boston, MA, USA*

11,12 epoxyeicosatrienoic acid (EET) is an endogenous mediator of inflammation and angiogenesis that is derived by cytochrome P450 epoxygenation of arachadonic acid. This lipid binds to an as yet unidentified G-protein coupled receptor to activate a variety of cell-type specific downstream signaling pathways, including PI3K, MAPK, and cAMP. We conducted a chemical screen for enhancers of competitive whole kidney marrow transplantation in the adult zebrafish, and found EET significantly enhances transplantation. Mouse whole bone marrow transplants using competitive long term transplantation techniques confirmed the positive role of EET. This effect is lost when EET treatment is combined with the PI3K inhibitor LY294002, demonstrating that EET signals via PI3K to enhance transplantation. To better interrogate the mechanism of EET signaling, we studied EET's role in zebrafish hematopoietic development. As in transplant conditions, during normal development hematopoietic stem and progenitor cells (HSPC) travel to and engraft multiple embryonic niches. Early EET treatment increased the number of HSPC born in the aorta-gonad-mesonephros (AGM) of the embryo. Morpholino knockdown of zebrafish Gα and PI3K catalytic subunits found that this phenotype is dependent on signaling of Gα12/13 subunits to activate PI3Kγ signaling. Chemical inhibition of PI3Kγ confirmed the importance of this signaling pathway. Akt phosphorylation leads to an upregulation of AP-1 family transcription factors in the endothelial cells of the AGM which give rise to the HSPC. AP-1 upregulation leads to increased transcription of runx1, a transcription factor necessary for the specification of HSPC. Our lab has developed a transgenic line that labels Runx1+ HSPCs, which we can image as they engraft in the caudal hematopoietic territory (CHT), the zebrafish equivalent of the mammalian fetal liver. Later embryo treatment with EET, after HSPC have already been born, results in enhanced engraftment of HSPC in this developmental niche. Chemical inhibition demonstrated that this later phenotype is also dependent on PI3Kγ signaling. EET induced PI3Kγ signaling can thus regulate both the birth of zebrafish HSPCs and their migration to and engraftment of the CHT. This signaling pathway has a conserved role enhancing the transplantation of adult zebrafish and mammalian HSPC.

T-1078
LOSS OF MIR-145 INHIBITS HSC SELF RENEWAL IN MDS VIA DYSREGULATED TGF-BETA SIGNALING

Lam, Jeffrey, Wegrzyn Woltosz, Joanna, Ibrahim, Rawa, Slowski, Kate, Umlandt, Patricia, Fuller, Megan, Karsan, Aly
BCCRC, Vancouver, BC, Canada

Myelodysplastic syndromes are a collection of hematopoietic malignancies in which genomic abnormalities within the HSC compartment leads to pancytopenia and eventual bone marrow failure. The most common karyotypic abnormality in MDS is the interstitial deletion of chromosome 5q. MicroRNA-145 is located within the commonly deleted region of del(5q) MDS and mice lacking miR-145 show a decrease in HSCs and myeloid progenitors. We identified members of the TGFβ-signaling pathway as a common target of miR-145. Analysis of del(5q) MDS patient bone marrow show an enriched TGFβ-signature compared to healthy controls and we show that TGFβ signaling is activated upon loss of miR-145 or enforced expression of a key target, Disabled-2 (DAB2). Loss of miR-143/145 or overexpression of DAB2 in mouse marrow resulted in a significant decrease in HSPC frequency, self-renewal, and CFC activity. In competitive transplants, vector-transduced marrow was able to outcompete DAB2 marrow in both the primary transplant as well as in secondary limiting dilution assays. However, a subset of mice with enforced DAB2 expression alone developed a transplantable MPD phenotype. In conclusion, our data identifies a role for miR-145 haploinsufficiency in the inappropriate activation of TGFβ signaling through DAB2 and establishes a role for DAB2 in HSC self-renewal in del(5q) MDS.

T-1079
EXPRESSION OF MFG-E8 IN FETAL HEMATOPOIETIC STEM CELLS DURING MOUSE EMBRYOGENESIS

Lee, Jaehun[1], Han, Jiyou[1], Choi, Byung-il[2], An, Su Yoen[1], Kim, Jonghyun[1], Jang, Yu Jin[1], Son, Jeong Sang[1], Lee, Gyunggyu[1], Park, Ji Young[1], Kim, Jong-Hoon[1]
[1]*Laboratory of Stem Cells and Tissue Regeneration, Korea university, Seoul, Republic of Korea,* [2]*Department of Pathology, National Cancer Center, Goyang, Republic of Korea*

Milk Fat Globule-EGF Factor 8 protein (MFG-E8) has been identified in various tissues and plays an important role in intercellular interactions, cellular migration, and neovascularization. Although previous studies have shown the expression of MFG-E8 in different immune or inflammatory cells under normal or pathophysiological conditions in mice and human, its expression in hematopoietic stem cells (HSCs) during early hematopoiesis has not been reported yet. In this study, we investigate the expression of MFG-E8 in multiple hematopoietic tissues at different stages of mouse embryogenesis. Immunohistochemial studies showed that MFG-E8 was specifically expressed in CD34+

HSCs in all hematopoietic sites, including yolk sac, aorta-gonad-mesonephros region, placenta, and fetal liver during embryogenesis. We also found that MFG-E8 was not expressed in c-kit+ HSCs, but become confined to F4/80+ macrophages in adult mouse bone marrow. This study demonstrates for the first time the expression of MFG-8 in fetal HSC populations and suggests the potential role of MFG-E8 in early embryonic hematopoiesis. This research was supported by the Bio and Medical Technology Development program of the National Research Foundation (NRF) funded by the Korean government (MEST) (No. 2012M3A9C7050139 and No. 2012M3A9B4028636 for JHK).

T-1080

ASSESSING INFLAMMATORY TRANSCRIPT EXPRESSION BY BLOOD MONOCYTES AND MICROGLIA DURING EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Lewis, Coral-Ann Brangwen[1], Thavaneetharajah, Preetheeban[1], Krieger, Charles[2], Rossi, Fabio M.V.[3]

[1]Biomedical Research Centre - University of British Columbia, Vancouver, BC, Canada, [2]Department of Biomedical Physiology and Kinesiology, Simon Fraser University, Burnaby, BC, Canada, [3]University of British Columbia, Vancouver, BC, Canada

Inflammatory monocyte infiltration from the blood into the spinal cord is essential for the progression of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (MS), from developmental to peak stages. This project aims to assess and compare the expression of inflammatory mediators by microglia, the resident macrophage population within the central nervous system, and infiltrating monocytes in the spinal cord of mice induced with EAE. Extravasated monocytes within the spinal cord eventually acquire macrophage markers that render them indistinguishable from microglia yet significant functional differences are known to persist. To this end, a parabiosis/irradiation/separation strategy recently developed was employed to create bone marrow (BM) chimeric mice in which blood cells express green fluorescent protein (GFP), while CNS resident cells do not. BM chimeric mice were then induced with EAE, spinal cords harvested, and macrophages and microglia collected and separated using fluorescence activated cell sorting. Using digital droplet PCR, transcript expression profiles were assessed at the single cell level and compared between microglia and blood-derived macrophages. The analysis of the transcriptome at the single cell level demonstrates the heterogeneity amongst and between myelomonocytic cells and microglia that exists during neuroinflammation. The results of this investigation will provide insight into the different roles that microglia and blood monocytes play during the development and progression of EAE, specifically identifying the pathogenic mechanism by which infiltrating monocytes operate and potential targets for therapeutic intervention in the treatment of MS.

T-1081

N-CADHERIN MARKED RESERVE STEM CELLS SUPPORT LONG-TERM HEMATOPOIESIS

He, XI (CiCi)[1], Sugimura, Ryohichi[1], Arai, Fumio[2], Zhao, Meng[1], Li, Zhenrui[1], Chen, Jie[3], Hembree, Mark[1], Haug, Jeffrey S.[1], Radice, Glenn[4], Suda, Toshio[5], **Li, Linheng**[1]

[1]Stowers Institute for Medical Research, Kansas City, MO, USA, [2]Keio University, Tokyo, Japan, [3]University of Missouri, Kansas City, MO, USA, [4]University of Pennsylvania, Philadelphia, PA, USA, [5]Keio Univ, Tokyo, Japan

N-cadherin (N-cad) was detected in the endosteal niche and also in its adjacent quiescent (BrdU-label retaining cells) hematopoietic stem cells (HSCs)1,2. However, N-cad expression at mRNA level was extremely low and enriched mainly in a subpopulation (reserve or 'back up') of HSCs3,4. This led to controversial reports regarding its expression and functionality in HSCs5-8. The biological significance of reserve HSCs has been proposed but not yet demonstrated3,9. Here we show that N-cad protein was detected in 7-10% of Lin-Sca1+c-Kit+ (LSK) cells as analyzed in the N-cad-mCherry knock-in mice. N-cad and CD150 expression were exclusive, but the former was 2.5-fold more enriched than the latter in the primitive (CD49bloCD34-Flk2-LSK)10,11 HSCs. We confirmed that N-cad enriched in Scl-H2B-GFP LRCs, a dormant or reserve state of HSCs3,10, which was located mainly in the endosteal niche of the trabecular bone region. In the transplantation experiment, N-cad+ HSCs had 30% higher engraftment rate than N-cad- HSCs. In contrast, bone marrow (BM) with Mx1-Cre+N-cadfl/fl (N-cadmut) compared to Cre-N-cadfl/fl (N-cadcont) showed an engraftment reduction, which statistically was significant in only a large, but a small number (20-30) of recipients. Flamingo (Fmi), as an atypical cadherin molecule12, was suggested to have an overlapping role with N-cad in facilitating the HSC-niche interaction13. We indeed found that Fmi+/-:N-cad double mutant HSCs significantly reduced the reconstitution capacity compared to the Fmi+/- HSCs, particularly in the secondary (59%) repopulation assay. Furthermore, in the serial transplantation experiment, N-cadmut donor BM cells led to a reduced survival ability of the tertiary recipients compared to the N-cad control BM. Thus, we demonstrated that N-cad is expressed and functions in reserve HSCs, loss of which affects long-term but not short-term hematopoiesis.

T-1082

EPIGENETIC MODIFIERS PROMOTE EXPANSION OF TRANSPLANTABLE HUMAN CORD BLOOD STEM/PROGENITOR CELLS LIKELY THROUGH ACTIVATION OF INFLAMMATORY/STRESS RESPONSE SIGNALING PATHWAYS

Mahmud, Nadim, Sidani, Amer, Koca, Emre, Kim, Alexander, Petro, Benjamin

Division of Hematology/Oncology, Department of Medicine, University of Illinois at Chicago, Chicago, IL, USA

Recent studies indicate that inflammatory molecules, including interferon, possess stimulatory effects which trigger excessive proliferation leading to hematopoietic stem cell (HSC) exhaustion and bone marrow failure. We hypothesized that transient activation of inflammatory molecules by chromatin modifying agents (CMA) stimulates proliferation of transplantable HSC in culture. We have previously demonstrated that sequential addition of a hypomethylating drug, 5-aza-2'-deoxycytidine (5azaD), followed by a histone deacetylase (HDAC) inhibitor, trichostatin A (TSA), during human umbilical cord blood CD34+ cell culture results in expansion of serially transplantable HSC. However, treatment with valproic acid (VPA), another HDAC inhibitor, results in only maintenance of HSC numbers. Our global microarray data reveals a set of 113 HSC expansion genes and 278 HSC maintenance genes preferentially detectable in 5azaD/TSA and VPA-expanded CD34+ cells respectively. Intriguingly, functional pathway analyses of the 113 HSC expansion genes indicate the importance of inflammation signaling genes such as S100A8 and Alox5. Furthermore, global genomic DNA methylation analysis of enriched CD34+ cells by LINE-1 methylation assay indicates transient hypomethylation of 5azaD/TSA-expanded cells followed by concomitant recovery of DNMT1 expression. Notably, demethylation of CpG sites of the promoters of individual inflammatory/stress response genes correspond with higher expression of genes including genes encoding calcium binding protein S100A8 and cytochrome P450 gene Cyp11A1 in 5azaD/TSA-expanded cultures. S100A8 is also a known agonist

of toll-like receptor 4, which plays a role in the maintenance and proliferation of endothelial progenitors. The transcript levels of inflammatory cytokines including IL-8 and TNFα and Alox 5 which is involved in leukotriene production were also found to be higher in 5azaD/TSA-expanded cells by qPCR. Consistently, ELISA assays of conditioned medium from 5azaD/TSA-expanded cells reveals that the levels of inflammatory mediators/cytokines including leukotriene B4, TNFα and IL-8 (10.95 ± 11.67, 426.87 ± 101.7; 346.8 ± 138.86; 0, 40 ± 3, 0; 43.77 ± 2.15, 323.28 ± 5.84, 122.26 ± 15.36 pg/ml; in control, 5azaD/TSA and VPA cultures respectively) were significantly greater than in control cultures. Interestingly reactive oxygen species (ROS), a mediator of inflammation/stress associated with potentially DNA damage repair response was lower in both 5azaD/TSA and VPA treated CD34+ cells in contrast to control. The lower intracellular ROS levels in CMA treated CD34+ cells corresponded with relatively higher transcript levels of ROS scavenger genes including GPX1, GPX3 and SOD1 in contrast to controls. Whether possible scavenging of ROS by these antioxidant gene products mitigates or the effects of initial trigger of ROS following addition of CMA in culture promotes expansion of transplantable HSC is currently under investigation. Activation of inflammatory signaling molecule NFκB p65 was detectable in 5azaD/TSA and VPA expanded cells in comparison to control culture by western blot. Notably, expansion of primitive CD34+CD90+ cells in the presence of 5azaD/TSA is reduced by 47.6% by addition of dexamethasone, an anti-inflammatory agent. Our studies, for the first time, identify inflammatory molecules regulated by epigenetic mechanisms that are potentially important in governing HSC fate choices.

T-1084
THE IN VIVO ROLE OF POLYCOMB-LIKE 2 (PCL2)

Manias Rothberg, Janet[1], Maganti, Harinad[2], Porter, Christopher[2], Pawlidor, Gareth[2], Perkins, Theodore[2], Paulson, Robert[3], Ito, Caryn Y.[2], Stanford, William L.[2]

[1]Ottawa Hospital Research Institute and The Graduate Program in Cellular and Molecular Medicine, Ottawa, ON, Canada, [2]Ottawa Hospital Research Institute, Ottawa, ON, Canada, [3]Penn State University, University Park, PA, USA

Polycomb genes are epigenetic repressors critical in cell fate decisions. We identified Polycomb-like 2 (PCL2) as a critical regulator of embryonic stem cell (ESC) self-renewal via its role in pluripotency feed-forward networks. Knockdown of Pcl2 in ESCs causes defects in differentiation and increased self-renewal characteristics. Pcl2 is expressed at its highest level during development but is largely restricted in adult tissues to hematopoietic tissues. We have generated a Pcl2 knockout mouse model using gene targeted ES cells to study the role of Pcl2 in vivo. Mutant mice that lack Pcl2 die at e15.5 and exhibit growth defects, hemorrhage and anemia. Based on flow cytometric analysis and peripheral blood smears, Pcl2-/- mice have significantly fewer enucleated erythrocytes, suggesting Pcl2 is necessary for definitive erythropoiesis. Transcriptomic analysis in mouse erythroid progenitors revealed a role for Pcl2 in cell cycle and cellular migration. Using a colony-forming assay to assess progenitor cell function, Pcl2-/- mice formed more CFU-GEMM colonies, indicating that cells lacking in Pcl2 are in a more primitive/progenitor state. Pcl2 is also required for HSC self-renewal as Pcl2-/- fetal liver cells fail to reconstitute the hematopoietic system of recipient mice in secondary transplants. Thus, Pcl2 is required for erythroid development and self-renewal of hematopoietic stem cells.

T-1085
EXPRESSION OF LINEAGE-SPECIFIC MARKERS ON EARLY STAGE OF ERYTHROCYTES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Mao, Bin[1], Huang, Shu[1], Zhou, Ya[1], Lu, Xulin[1], Yu, Jinfeng[1], Pan, Xu[1], Zhou, Jiaxi[2], Chen, Bo[1], Nakahata, Tatsutoshi[3], Ma, Feng[1]

[1]Institute of Blood Transfusion, Chinese Academy of Medical Sciences, Chengdu, China, [2]Institute of Blood, Chinese Academy of Medical Sciences, Tianjin, China, [3]CiRA, Kyoto University, Kyoto, Japan

Erythrocytes are one of the first blood cells generated in embryonic hematopoiesis in mammal. hESC/hiPSCs provide excellent cellular platforms to better understand human erythropoiesis and develop cliniclly applicable cell-based therapies. It has been reported that in vitro cultured erythrocytes derived from hESCs/hiPSCs are more immature than those originated from adult $CD34^+$ hematopoietic cells. Phenotypic molecules are subtle markers for tracking cell developmental process. Although the expression of lineage-specific markers for adult $CD34^+$ cell-derived mature erythrocytes has been well recognized (GPA^+, $CD71^+$, $CD36^+$, $CD45^-$, $CD34^-$), there lacks a specific pattern for those derived from hPSC/hiPSCs, especially in early stage. By coculture with murine fetal liver or AGM stromal cells, we recently have established an efficient production system of erythrocytes from hESC/hiPSCs. This provid an ideal tool to investigate early development of human erythrocytes. Upon FACS assay, we found that the earlist erythroid cells (GPA^+CD34^+) appeared on day 8 (D8) in the coculture, followed by the gradual emergence of GPA^+CD34^- cells. However, on D10 GPA^+CD34^+ cells still showed mesodermal and endothelial properties (6.9% KDR^+, 91.1% $CD31^+$, 44.9% $CD144^+$, 95.7% $CD71^+$), while GPA^+CD34^- cells obtained more erythroid characteristics (1.9% KDR^+, 62.3% $CD31^+$, 17.3% $CD144^+$, 97.8% $CD71^+$). Cell sorting result revealed that GPA^+CD34^+ cells were larger than GPA^+CD34^- cells, showing the former were more primitive erythroid cells. GPA^-CD34^+ cells included HSC-like cells, macrophages and other myeloid cells, while GPA^-CD34^- cells contained rare hematopoietic cells. On D10 of the coculture, four sorted cell fractions were re-plated in suspension culture (20% FBS / IMDM + SCF, IL-6, IL-3, TPO, EPO, Flt-3, VEGF, G-SCF) respectively for 8 days. GPA^-CD34^- cells barely survived. GPA^+CD34^+ and GPA^+CD34^- fractions exclusively gave rise to erythrocytes, while GPA^-CD34^+ cells mainly showed myeloid cell, mast cell and macrophage potential. CD36 is an important marker expressed on human erythroid progenitor cells. However, in our coculture system, majority of hESC/hiPSC-produced GPA^+ cells were CD36 negative. On coculture D10 and D14, four cell subsets were sorted according to the expression of GPA and CD36, and Hb components of each fraction were detected by immunostaining assay. GPA^+CD36^+ cells (D10 100% ε^+, 78.1% γ^+, 27.6% β^+; D14 100% ε^+, 98.6% γ^+, 78.4% β^+) showed a higher maturity level than GPA^+CD36^- cells (D10 100% ε^+, 65.3% γ^+, 12.5% β^+; D14 100% ε^+, 93.0% γ^+, 32.4% β^+), while little Hb were detected in GPA^-CD36^+ and GPA^-CD36^- populations. Through our studies, we found that the early developmental process of hESC/hiPSC-derived erythrocytes can be traced by GPA/ CD34 and GPA/ CD36 expressions. Now we are comparing the gene expression profiles of cell subsets defined by the expression of these erythroid lineage-specific markers. Our research may benifit for discovering important mechanisms in the early differentiation and maturation regulation of hESC/hiPSC-derived erythrocytes.

T-1086

ANTI-OBESE HORMONE ADIPONECTIN REGULATES EMERGENCY HEMATOPOIESIS AND ANTIBACTERIAL RESPONSE THROUGH SUPPRESSION OF TNF-ALPHA PRODUCTION IN BONE MARROW AND DOWNREGULATION OF SOCS3 IN HEMATOPOIETIC STEM/PROGENITOR CELLS

Masamoto, Yosuke[1], Arai, Shunya[1], Sato, Tomohiko[1], Yoshimi, Akihide[1], Takamoto, Iseki[2], Kubota, Naoto[2], Kadowaki, Takashi[2], Kurokawa, Mineo[1]

[1]University of Tokyo Hospital, Department of Hematology/Oncology, Tokyo, Japan, [2]University of Tokyo Hospital, Department of Diabetes and Metabolic Diseases, Tokyo, Japan

Anti-Obesity countermeasures attract rising attention because obesity induces multiple complications including infections. One of the key features of obesity is a decreased serum level of adiponectin, a critical anti-diabetic hormone secreted by adipocytes to modulate a number of metabolic processes. Hematopoietic stem/progenitor cells (HSPCs) expand in number against infections, especially bacterial infections through immune response. In spite of the emerging clinical and basic reports of adiponectin, precise mechanisms of adiponectin for infectious control, especially for hematopoietic response, are largely unknown. As adipocytes are one of the major cellular components of bone marrow (BM) microenvironment, we hypothesized that obesity-related dysfunction of adipocytes in BM might cause a compromised hematopoietic response leading to increased susceptibility to infection. With the use of a well-known mouse model of diet-induced obesity, we revealed that obesity had no apparent impact on steady-state hematopoiesis. Upon G-CSF administration to mimic harmful infections in vivo, obese mice showed an impaired expansion of HSPCs in BM, along with the reduction of myeloid cells in peripheral circulation, i.e. a diminished "emergency hematopoiesis". In a Listeria monocytogenes peritonitis model, obese mice also confirmed a reduced expansion of HSPCs and myeloid cells in BM, an attenuated recruitment of leucocytes into the peritoneal cavity, resulting in a delayed bacterial clearance in vivo. The level of adiponectin in BM was significantly reduced in obese mice compared to control mice, possibly reflecting a high relevance of adiponectin to hematopoietic response. To assess the exact role of adiponectin in emergency hematopoiesis, we utilized adiponectin-deficient (adipo-/-) mice and showed that genetic ablation of adiponectin caused no change in steady-state hematopoiesis whereas G-CSF administration in adipo-/- mice showed an attenuated HSPC expansion in BM, consistent with a series of obese mice. As was similar to obese mice, adipo-/- mice were more vulnerable to L. monocytogenes infection than wild type mice. Importantly, intravenous administration of recombinant adiponectin restored the responsiveness to G-CSF and the bacterial clearance on Listeria infection, both in adipo-/- and obese mice, affirming the importance of adiponectin treatment against infection. In HSPCs and myeloid cells from adipo-/- mice or obese mice, activation of Stat3, a key component for emergency hematopoiesis, was suppressed, and HSPCs from obese or adipo-/- mice showed aberrant Socs3 expression which could exert its Stat3-inhibitory role. These alterations in adipo-/- or obese mice were reverted by adiponectin treatment, underscoring the possible molecular role of adiponectin in this process. Additionally, TNF-α production was higher in BM of obese mice than that of wild type mice and genetic ablation of TNF-α almost abrogated overexpression of Socs3 seen in HSPCs from obese mice, which could reveal the involvement of TNF-α in Socs3 regulation by adiponectin. These data revealed that adiponectin regulates hematopoietic response against infections through TNF-α/Socs3/Stat3 axis, highlighting adiponectin as a legitimate target against infectious diseases in obese patients. Our findings could propose an auxiliary usage of adiponectin to patients with severe bacterial infections in addition to antibiotics.

T-1087

COMBINED INTRAUTERINE AND POSTNATAL CELL THERAPY USING FETAL LIVER AND ADULT BONE MARROW CELLS FACILITATES SUSTAINED DONOR CELL CHIMERISM IN A MURINE THALASSAEMIA MODEL

Chan, Jerry[1], Dighe, Niraja[2], Tan, Lay Geok[2], Sandikin, Dedy[2], Choolani, Mahesh[2], **Mattar, Citra Nurfarah**[2]

[1]Obstetrics and Gynecology, Kendang Kerbau Women and Children's Hospital, Singapore, Singapore, [2]Obstetrics and Gynecology, National University of Singapore, Singapore, Singapore

The potential of intrauterine haemopoietic stem cell therapy (IUHSCT) in early treatment of major thalassaemias is unrealised due to difficulty achieving clinically useful cell engraftment. Clinical attempts at IUSCT have generally failed to achieve sufficient engraftment, and low chimerism has similarly plagued animal models. This may be caused by competition from host cells, host immunity or donor cell source. Here we examine the hypothesis that IUHSCT will induce an immune-tolerant fetal environment that facilitates cell engraftment after postnatal boosting, using allogeneic and congeneic donor cells to define a strategy that can lead to long-term chimerism at therapeutic levels in a murine thalassaemia model. Fetal livers were harvested at E14-17 from pure-bred B6.129P(Cg)-Ptprca Cx3cr1tm1Litt/LittJ -GFP and C57BL6-CD45.1 mice on the day of IUHSCT. Liver mononuclear cells were treated with Diprotein A for CD26 inhibition just prior to transplantation. HbbTh3+/- and C57BL6-CD45.2 mice were mated and resulting litters injected with 2E+6 GFP or CD45.1 cells per pup via the intraperitoneal route at E14-17. A second batch of pups received 5E+6 GFP cells in utero. Surviving pups were examined serially for chimerism after weaning. Chimeric mice were treated with IV busulphan prior to postnatal challenge with donor cells from the same strain. GFP-BL6 chimeric mice were challenged with 1E+7 and 5E+6 fetal liver GFP cells at six and 12 postnatal weeks respectively. A third challenge with 3E+7 CD3 depleted adult bone marrow (BM) cells was performed at 20 weeks. Chimeric CD45.1-BL6 mice were boosted with 4E+7 CD3 depleted BM cells at eight postnatal weeks. All surviving offspring were wild-type (HbbTh3+/+). Following an initial dose of 2E+6 fetal liver cells in utero, median engraftment was 2.0% (range 0.6-5.6%) among GFP recipients at three weeks, and 2.9% (2.8-4.5%) among CD45.1 recipients at 8 weeks. In comparison, recipients of 5E+6 GFP cells showed 5.5% engraftment at three weeks (2.0-7.2%, p=0.12). Transient decreases in white cell and platelet counts were observed after busulphan. The first GFP cell boost resulted in chimerism of 2.5% (1.0-4.7%) by eight weeks, which steadily declined to 1.4% (0.4-2.8%) by 12 weeks. The second boost had no effect and chimerism declined to 0.3% (0-2.1%) by 20 weeks. Chimerism increased to 4.0% (2.3-6.0%) following the third cell boost before falling to undetectable levels by 32 weeks. Postnatal challenge in treated offspring with <1% chimerism and untreated WT pups had no effect. Following postnatal boost, median CD45.1 chimerism increased from 3.4 to 4.2% by 12 weeks (range 3.0-5.2%), eventually declining to 1.7% by 16 weeks. Unchallenged chimeric pups (<2%) showed a steady decline to 0.3% by 16 weeks (p=0.1). Clinically-relevant donor cell chimerism of >2% was achieved with high-dose IUHSCT and high-dose postnatal boost. A high intrauterine cell dose may be required to overcome host immunity and allow modest intrauterine engraftment, which in turn facilitates continued tolerance towards donor cells. A high postnatal cell dose may be necessary to overcome host competition for haemopoietic niches. Sub-therapeutic chimerism following IUHSCT predicts engraftment failure despite postnatal boost, possibly reflecting a non-

tolerant environment. The donor cell source is likely to be important. Though further investigation is needed, this could be a useful clinical strategy for early treatment of thalassaemia.

T-1088
STUDY OF T CELL DIFFERENTIATION OF CIRCULATING CD34+ HEMATOPOIETIC PROGENITORS DURING HIV INFECTION

Menkova, Inna, Benne, Clarisse, Levy, Yves, Leleivre, Jean-Daniel
Institut Mondor de Recherche Biomédicale, Creteil, France

Background. The goal of our study was to explore the role of de novo T cell lymphopoiesis from CD34+ hematopoietic progenitors (HP) in the context of the poor CD4+ cell restoration in HIV+ infected patients under effective combined antiretroviral therapy (c-ART). While several factors may explain this failure of the immune system, the impact of the dysfunction of T cell differentiation from CD34+ in this process has not been so far studied. We hypothesize that impaired T cell differentiation from HP could, at least partially, explain low CD4+ levels in treated HIV+ patients. Methods. PBMC were obtained from HIV-negative donors (n=14, Etablissement Français du Sang, Creteil) and HIV+ patients with Poor T cell Recovery under c-ART (PR, n=9, CD4=371/mm3, CD4/CD8=0.53) followed in CHU Henri Mondor (Creteil, France) and as a control from HIV+ Good Responder Patients presenting similar immunological characteristics to HIV- individuals (GR, n=14, CD4=1115/mm3, CD4/CD8=1.7). HIV+ patients and HIV-controls were matched for age and sex. GR and PR patients were matched for age, sex, HIV infection and c-ART durations. Groups were compared using Mann-Whitney test. Analysis of HP frequency in blood was performed on LSRII cytometer (FlowJo v8.2). T-cell precursor's frequency was determined using limiting dilution assay (LDA) on coculture of HP on OP9-delta1 stromal cell line in the presence of Fms-related tyrosine kinase 3 ligand (FLT3L) and Interleukine-7 (IL-7, both at 5 ng/mL), results were generated using ELDA webtool. Results. We found: 1) the same frequency of circulating CD34+ cells among GR, PR patients and HIV- donors (0.07; 0.09; 0.13 respectively, $p>0.05$); 2) a decrease in T-cell precursor's frequency in PR patients (1/241) as compared to GR patients (1/86; $p<0.05$) and HIV- controls (1/75; $p<0.05$) while T cell precursor's frequency in GR patients was not significantly different from HIV-controls. Moreover HP from GR and PR retain the similar ability to generate B cells, but not NK cells; 3) among studied parameters known to influence T cell recovery in HIV-infected individuals (e.g. immune activation and inflammation, IL7R genetic polymorphisms), none was significantly associated to low CD4+ T cell count in PR, except T cell differentiation potential of HP. By analyzing the frequency of Recent Thymic Emigrants (RTE: CD31highCD4+ or CD8+) in the blood of HIV-, GR and PR, we highlighted the marked decrease of these cells only in PR ($p<0.01$), probably as a direct consequence of impaired T cell lymphopoiesis in vivo. Conclusions. Our results strongly suggest that CD4+ cell restoration in HIV+ under c-ART depends on the T/NK cell differentiation potential of HP. Currently we are evaluating genetic networks involved in T/NK versus B lymphopoiesis using unsupervised transcriptomic approach, studying epigenetic marks of this balance, and investigate in gene-candidate driven fashion whether altered T cell differentiation potential is dependent on proapoptotic signals through dysregulated response to IL-7. In summary, we showed that poor CD4+ restoration under c-ART involved pre-thymic alteration, which could imply new therapeutic approaches, as IL-7 administration, aiming to restore T cell potential in bone marrow progenitors.

T-1089
MYELOFIBROSIS-RELATED STEM CELLS IN THE SPLEEN ARISE FROM AN INDUCTION BY THEIR MEGAKARYOCYTIC PROGENY

Zingariello, Maria[1], Martelli, Fabrizio[2], Bosco, Domenico[3], Rana, Rosa Alba[3], Whitsett, Carolyn F.[4], Lewandowski, Daniel[5], Spangrude, Gerald J.[6], **Migliaccio, Anna Rita**[7]
[1]Departments of Medicine, Campus Biomedico, Rome, Italy, [2]Hematology, Oncology and Molecular Medicine, Istituto Superiore Di Sanità, Rome, Italy, [3]Biomorphology, University of Chieti G. D'Annunzio, Chieti, Italy, [4]Kings County Hospital and Downstate Medical Center, Brooklyn, NY, USA, [5]CEA/DSV/iRCM/LRTS, Inserm U967, Fontenay-aux-Roses cedex, France, [6]Department of Medicine, Division of Hematology and Hematologic Malignancies, University of Utah, Salt Lake City, UT, USA, [7]Tisch Cancer Institute, Mount Sinai School of Medicine, New York, NY, USA

It has been recently hypothesized that cancer stem cells may promote their growth by activating the formation of "cancer stem cell niches". In patients with primary myelofibrosis, the most severe of Philadelphia-negative myeloproliferative neoplasms, and in the Gata1^{low} mouse model of the disease, "myelofibrosis-related stem cells" are functional in spleen but not in marrow. While the mechanism(s) that hampers the function of the stem cell niches in the marrow is starting to emerge, little is known on the mechanism(s) that activates formation of niches specific for myelofibrosis-related stem cells in spleen. We report here that stem cells (LSK/CD48^{neg}/CD150^{pos}) are present in great numbers in spleen (4,000/10^6 cells) but not in marrow (<10/10^6 cells) of Gata1^{low} mice. This spleen-restricted lodging is not an autonomous property of Gata1^{low} stem cells. Transplantation studies coupled with in vivo imaging demonstrated that fluorescent-tagged Gata1^{low} LSK expand normally in marrow of recipient mice until day 5 but that their number decreases in this organ by day 9. By contrast with wild-type LSK, by day 5 Gata1^{low} LSK are detected also in spleen that by day 25 is the only site expressing Gata1^{low} hematopoiesis. Since megakaryocytopoiesis in spleen is the first hematopoietic activity expressed by transplanted stem cells, we hypothesized that Gata1^{low} megakaryocytes are programmed to establish myelofibrosis-related stem cell niches in this organ. This hypothesis was tested by electron-microscopy studies that discovered a novel interaction between Gata1^{low} megakaryocytes and activated dendritic cell-derived fibrocytes that leads to formation of "TGF-β-collagen hot spots". Loss-of-function experiments were performed to investigate whether the "TGF-β-collagen hot spots" formed in spleen due to interaction between megakaryocytes and fibrocytes supported myelofibrosis-related stem cells. Genetic ablation of the P-selectin receptor prevented interaction between megakaryocytes and fibrocytes, formation of "TGF-β-collagen hot spots" and rescued the phenotype of Gata1^{low} mice. Double Gata1^{low}/P-selectin^{null} mice expressed hematopoiesis in marrow, and not in spleen, survived 3-months ($p=0.009$) longer than their Gata1^{low}/P-selectin wild type littermates and survived splenectomy. Treatment of Gata1^{low} mice with the TGF-β inhibitor SB43542 reduced by 5-fold the numbers of LSK/CD48^{neg}/CD150^{pos} cells in spleen ($p=0.04$) and increased by 5-fold those in marrow ($p=9x10^{-5}$), restoring hematopoiesis in this organ. By day 5 post-transplantation, fluorescent tagged-LSK purified from both marrow and spleen of TGF-β-inhibitor treated Gata1^{low} mice had expanded in marrow at rates 2-fold higher than those expressed by fluorescent-tagged LSK purified from marrow of wild type mice. These TGF-β-inhibitor treated Gata1^{low} LSK were barely detectable in spleen 5 days post-transplantation but were found almost exclusively in this organ 25 days later. These results indicate that myelofibrosis-related stem cells in the spleen arise from an induction of their megakaryocyte progeny and lead to a paradigm shift in our concept of cell autonomous

properties of stem cells. In fact, although the ability of myelofibrosis-related stem cells to proliferate in the spleen is not cell autonomous , the presence of these cells in great numbers in this organ is induced by "specific niches" formed by their megakaryocytic progeny.

T-1090
ERYTHROPOIETIC POTENTIAL OF IPS CELLS DERIVED FROM SHWACHMAN-DIAMOND SYNDROME

Miller, Christine[1], Serwold, Thomas[2], Wagers, Amy J.[3]
[1]Developmental and Stem Cell Biology, Joslin Diabetes Center, Boston, MA, USA, [2]Joslin Diabetes Center, Boston, MA, USA, [3]Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

Null mutations in the Swachman-Bodian-Diamond Syndrome (SBDS) gene cause Swachman-Diamond syndrome (SDS), a rare ribosomopathy that affects the bone marrow, pancreas and skeletal system. Patients with SDS exhibit a predisposition for Acute Myeloid Leukemia (AML) as well as defects in erythropoiesis. Although the exact mechanism underlying defective erythropoiesis in these patients is unknown, previous studies suggest that erythrocyte formation from differentiated hematopoietic cells is not impaired; instead, erythrocytes of SDS patients appear to undergo Fas-mediated apoptosis at an elevated rate. In this study, we utilized induced pluripotent stem (iPS) cells from SDS patients and subjected them to erythroid differentiation protocols in order to obtain a more complete developmental view of SDS erythropoiesis. To stimulate hematopoietic and erythroid differentiation, we co-cultured iPS cells with a mono-layer of gene-modified mouse OP9 cells using differentiation media supplemented with hematopoietic cytokines. Flow cytometric analysis demonstrated the emergence of CD29-/TRA-1-85+, CD235a+/CD34+ and CD235a+/CD71+ fractions, suggesting erythroid differentiation, and Wright-Giemsa staining of these FACS-sorted cells confirmed the hematopoietic/erythroid lineage phenotype. We also evaluated the hematopoietic progenitor potential of these cells using in vitro colony-forming assay. These studies establish a useful method to recapitulate the complete erythropoietic differentiation pathway in SDS patients by exploiting the pluripotency of human iPS cells.

CANCER CELLS

T-1092
ESTROGEN SIGNALING REGULATES PROLIFERATION AND DIFFERENTIATION OF ER ALPHA POSITIVE LUMINAL PROGENITORS THROUGH H19 GENE

Basak, Pratima[1], Chatterjee, Sumanta[2], Badiani, Ketan[3], Murphy, Leigh C.[3], Raouf, Afshin[4]
[1]Biochemistry and Medical genetics and Regenerative Medicine Program, University of Manitoba, Winnipeg, MB, Canada, [2]Immunology and Regenerative Medicine Program, University of Manitoba, Winnipeg, MB, Canada, [3]Biochemistry and Medical genetics and Cancer Care Manitoba, University of Manitoba, Winnipeg, MB, Canada, [4]Immunology, Regenerative Medicine Program and Cancer Care Manitoba, University of Manitoba, Winnipeg, MB, Canada

Estrogen signaling is indispensible to mammary gland development. Interestingly however, estrogen signaling is thought to act indirectly on the estrogen receptor alpha positive cells (ERα+) to regulate their proliferation and differentiation. Because ERα+ is only expressed in less than 20% of cells in the human breast tissue, the direct study of estrogen signaling on these rare cells has proven to be very challenging. We recently demonstrated that luminal progenitors that can be isolated from the human breast reduction samples express high levels of ERα+ while the undifferentiated bipotential progenitors show high expression of progesterone receptor (PR). In this study we examined if estrogen signaling could directly regulated the proliferation and differentiation potential of these luminal progenitors. To this end, we used the robust colony forming cell assay. Interestingly we found that in the presence of 17-β estradiol (E2), the luminal progenitors formed more colonies, which was attenuated in the presence of an ERα+ specific blocker (ICI). Moreover, in the presence of ICI, the luminal progenitors formed smaller colonies suggesting that estrogen signaling also regulates the proliferation capacity of the luminal progenitors. Next we examined if classical targets of estrogen signaling that are important to breast cancer proliferation (PR and H19, a long non-coding RNA) are also under estrogen regulation in the normal ERα+ luminal progenitors. Interestingly, we found that while H19 expression was increased in the E2-stimulated luminal progenitors, the PR expression remained unchanged, suggesting that H19 might be involved in estrogen-induced proliferation of luminal progenitors. To examine this hypothesis, we observed the colony formation potential of luminal progenitors transduced with a lentivirus to knockdown the expression of H19. The H19- deficient progenitors showed reduced ability to form colonies suggesting that ERα+ might regulates the proliferation and differentiation of luminal progenitors through H19. We further show that ERα+ directly activates H19 transcription by binding to 5 estrogen responsive half-sites in its promoter. Finally, we show that H19 expression positively correlates with ERα positive expression in primary breast tumors. This study for the first time demonstrates that estrogen-ERα signaling network can directly regulate the proliferation and differentiation of the human luminal progenitors. Out data also suggests an important role for H19 in estrogen regulation of luminal progenitor cell functions. Our data therefore, provides a framework to develop hypothesis as to how alterations to estrogen signaling in healthy luminal progenitors can lead to the development of ER+ breast cancer tumors. Such an understanding then could be used to identify premalignant changes to estrogen signaling, some which may prove useful in prevention of ER+ breast tumor development.

T-1093
GLIOBLASTOMA STEM CELL HETEROGENEITY

Bayin, Nermin Sumru[1], Modrek, Aram S.[1], August, Dietrich[1], Abel, Tobias[2], Chao, Moses V.[3], Song, Hae-Ri[1], Zagzag, David[4], Buchholz, Christian J.[2], Hoang, Dung Minh[5], Wadghiri, Youssef Z.[5], Igor, Dolgalev[6], Baysan, Mehmet[6], Heguy, Adriana[6], Placantonakis, Dimitris G.[1]
[1]Neurosurgery, NYU School of Medicine, New York, NY, USA, [2]Paul-Ehrlich-Institut, Langen, Germany, [3]Skirball Institute, NYU School of Medicine, New York, NY, USA, [4]Pathology, NYU School of Medicine, New York, NY, USA, [5]Radiology, NYU School of Medicine, New York, NY, USA, [6]Genome Technology Center, NYU School of Medicine, New York, NY, USA

Glioblastoma Multiforme (GBM), a deadly brain malignancy, is highly heterogeneous at the histologic and molecular levels. Cellular heterogeneity is believed to be established by stem-like cells within GBM, namely glioblastoma stem cells (GSCs), which have the ability to self-renew and differentiate into tumor lineages, including tumor derived endothelium and pericytes. GSCs are highly tumorigenic compared to non stem-like tumor cells and resistant to current chemoradiotherapy. Therefore, they represent important therapeutic targets. Although functional criteria defining GSCs are well established, molecular characteristics of GSCs and universal markers identifying GSCs are yet to be discovered. Surface markers and signaling pathways that are important for neural stem cells, the normal counterparts of GSCs, have been a major focus in the field. One of these markers is

CD133, a cell surface glycoprotein known to mark embryonic neural stem cells, as well as adult stem cells and cancer stem cells in different tissues. CD133+ GSCs were shown to have higher tumorigenic ability compared to CD133- cells. However, the facts that CD133- cells can form tumors and that some GBM tumors do not have a CD133+ population indicate that CD133 is not a universal GSC marker. The Notch signaling pathway, which is important for neural stem cell self-renewal, is also essential to GSC self-renewal. Blockage of Notch signaling leads to depletion of GSCs, including CD133+ GSCs, and leads to reduced tumorigenicity. Surprisingly, using primary human GBM samples that are lentivirally modified to express a fluorescent protein upon activation of Notch signaling, we observed only partial overlap between CD133+ GSCs and cells with activated Notch cascade (Notch+), suggesting that Notch signaling may serve as a marker for a distinct type of CD133- GSCs. To investigate this possibility we have isolated CD133+/Notch-, CD133+/Notch+ and CD133-/Notch+ populations and compared their stem profile in vitro and in vivo with respect to tumor cells that have neither CD133 expression nor Notch activation. Our preliminary in vitro data suggest that CD133+/Notch-, CD133+/Notch+ and CD133-/Notch+ cells have equivalent tumorsphere forming ability. Interestingly, we have observed that Notch+/CD133- populations can generate both Notch+ and Notch- cells, including CD133+ cells, whereas FACS-isolated Notch- cells do not give rise to Notch+ cells over time. RNA-sequencing revealed that CD133+/Notch- and CD133-/Notch+ have different transcriptional signatures. These results raise the possibility that these cells have different roles in maintaining heterogeneity and establishing the cellular hierarchy within these tumors. They may also have different responses to therapies. We are currently testing these cell types' in vivo tumorigenicity in an intracranial mouse xenograft model. To further understand how distinct GSC populations interact, we have established a lineage tracing system where we can selectively target CD133+ GSCs (Anliker et al. 2010) and Notch+ GSCs and follow their progeny. Furthermore, this combinatorial system will help us understand the cellular hierarchy shaped by distinct GSC populations and how they contribute to tumorigenesis and response to chemoradiotherapy.

T-1094
MET SIGNALING IN COLORECTAL CANCER-INITIATING CELLS PROMOTES RESISTANCE TO EGFR-TARGETED THERAPY

Boccaccio, Carla, Luraghi, Paolo, Reato, Gigliola, Cipriano, Elia, Sassi, Francesco, Orzan, Francesca, Bigatto, Viola, De Bacco, Francesca, Menietti, Elena, Bertotti, Andrea, Trusolino, Livio, Comoglio, Paolo M.
Candiolo Cancer Institute - FPO (IRCCS), Candiolo, Italy

Colorectal cancer is believed to be supported by a subpopulation of stem-like cells termed colon cancer-initiating cells (CC-ICs), which may also confer therapeutic resistance. However, how CC-ICs respond to targeting agents such as anti-EGR receptor (anti-EGFR) antibodies has not been fully characterized. To explore this question, we systematically generated CC-ICs from patient-derived xenografts of metastatic colorectal cancer ("xenopatients"). These cultures, termed *"xenospheres"*, long-term self-propagated *in vitro* and formed phenocopies of original patient tumors *in vivo*, thus obeying the operational definition of cancer-initiating cells. CC-ICs retained the genetic determinants of response to anti-EGFR therapy: like original tumors, CC-ICs harboring a mutated *KRAS* gene were resistant, while those harboring wild-type *RAS* pathway genes (RAS^{wt}) were sensitive. However, in RAS^{wt} CC-ICs, EGFR inhibition could be efficiently counteracted by growth factors such as HGF, FGF, NRG1, TGF-α and HB-EGF. Among these, HGF is abundantly secreted by cancer-associated fibroblasts derived from the host.To assess the role of HGF *in vivo*, mice were genetically engineered to produce human HGF, so as to achieve endocrine and paracrine activation of human MET in xenografted CC-ICs. In this setting, MET inhibitors cooperated with anti-EGFR antibodies in enhancing (up to 90%) and prolonging (up to 6 months) tumor regression. These data show that RAS^{wt} CC-ICs rely on both EGFR and MET signaling, and provide proof of concept for concurrent targeting of the two pathways in the clinical setting.

T-1095
INHIBITORY EFFECTS OF ICARITIN ON TKI RESISTANT CML CELLS THROUGH DISRUPTION OF THE BCR-ABL-GRB2-RAS-MAPK SIGNALING PATHWAY

Chen, Min[1], Turhan, Ali G.[2], Zhang, Bo[3], Ding, Hongxia[3], Qingcong, Lin[3], Meng, Kun[3], Jiang, Xiaoyan[1]
[1]Terry Fox Laboratory, British Columbia Cancer Agency and Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada, [2]Department of Hematology, Institute Gustave Roussy, Villejuif Cedex, France, [3]Shenogen Pharma Group LTD, Beijing, China

ABL tyrosine kinase inhibitor (TKI) Imatinib Mesylate (IM) is effective at inducing clinical remission in early phase chronic myeloid leukemia (CML) patients but is not curative. Early relapses and acquired drug resistance remain significant issues in many IM-treated patients. In particular, relapses are frequently associated with point mutations in the BCR-ABL tyrosine kinase domain (TK, > 50%). Newer TKIs, dasatinib and nilotinib, have increased potency over IM and show a broader spectrum of activity against mutant forms of BCR-ABL. However, none of these agents is able to target a critical T315I gatekeeper mutation of BCR-ABL in TKI resistant patients. We have further demonstrated that CML stem cells are genetically unstable and generate many BCR-ABL TK mutations *in vitro* and *in vivo*. They are also less responsive to TKIs and are a critical target population for TKI resistance. Thus improved treatment approaches to target other key molecular elements active in CML stem cells and BCR-ABL-T315I resistant cells are clearly needed. It has recently been reported that estrogen receptor variant ERα36 is highly deregulated not only in breast cancer cells but also in liver cancer and leukemic cells, and that targeting this specific variant with a small molecular inhibitor (SNG162) inhibits CML cell growth. However, the underlying molecular mechanisms of these observations are not understood. Whether this inhibitor, alone or in combination with new ABL inhibitors, can target primary CML stem/progenitor cells and T315I-resistant cells have also not been investigated. In this study, we utilized two cell line model systems: K562 cells and IM-resistant K562 cells without BCR-ABL TK mutation and human UT7 cells expressing either wild type BCR-ABL or carrying BCR-ABL-T315I mutation. We have now demonstrated that protein expression of ERα36 is highly upregulated in both IM-resistant and T315I mutant cells as compared to control cells. Interestingly, the use of pre-clinically validated ERα36 inhibitors (SNG162 and SNG1153) alone inhibits cell growth and induces apoptosis of these cells. These effects can be further enhanced by combination treatment with a TKI. Importantly, we have discovered that treatment of IM-resistant and T315I mutant cells with SNG162 and SNG1153 inhibitors, alone or with a TKI, significantly reduced phosphorylation of BCR-ABL on tyrosine residue 177 (Tyr177), a residue essential for BCR-ABL induced leukemogenesis through its binding to GRB2 and activation of the downstream RAS-MAPK pathway. This new observation was supported by detection of a significant reduction in phosphorylation of MEK1/2 kinase, an important component of the RAS-MAPK pathway, in these cells. Most importantly, IP-Western analysis further demonstrated that the BCR-ABL-GRB2 protein interaction was markedly interrupted in cells treated with SNG inhibitors plus a TKI, which correlates with reduced phosphorylation of Tyr177 in these cells. Moreover, colony-forming cell

(CFC) assays showed that ERα36 inhibitors (SNG162 andSNG1153) in combination with a TKI are more effective at inhibiting growth of $CD34^+$ treatment-naïve IM-nonresponder cells as compared to single drug treatment (46% vs. 25%). We further demonstrated that SNG162 and SNG1153(up to 10 μM and 5 uM) are not toxic to $CD34^+$ normal bone marrow cells. ERα36 thus emerges as an attractive druggable target for combination therapies to target TKI insensitive CML stem/progenitor cells and T315I-resistant cells.

T-1096

THE ROLE OF BIN1 TUMOR SUPPRESSOR ISOFORMS IN REGULATION OF PROLIFERATION, APOPTOSIS AND TUMOR FORMATION OF HUMAN CUTANEOUS T-CELL LYMPHOMA CELLS IN VITRO AND IN VIVO

Esmailzadeh, Sharmin[1], Zhou, Youwen[2], Jiang, Xiaoyan[3]
[1]University of British Columbia, Vancouver, BC, Canada, [2]Dermatology and Skin Science, University of British Columbia, Vancouver, BC, Canada, [3]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Cutaneous T-cell lymphomas (CTCLs) represent a group of lymphoproliferative disorders that are characterized by homing of malignant T-cells to the surface of skin. There are two main types of CTCL: Mycosis Fungoides (MF) and its leukemic variant Sezary Syndrome (SS), which together represent about 65-70% of all CTCL cases. The precise genetic pathogenesis of these diseases remains largely undetermined. Recently, our research group has demonstrated that BIN1 expression is significantly lower in both MF and SS patients compared to controls. BIN1 is a nucleocytosolic adaptor protein with more than ten isoforms; BIN1 isoform (+10, +13) acts as tumor suppressor, whereas BIN1 (+12A) behaves as a cancer-related isoform in solid tumors. However, the role of BIN1 in regulation of normal hematopoiesis and lymphomagenesis remains unknown. To investigate the role of BIN1 in CTCL, BIN1 isoforms (+10, +13) and (+12A) lentiviral constructs were transduced into two CTCL cell lines, Hut78 and HH. Subcellular fractionation and confocal microscopy showed that BIN1 (+10, +13) is localized in both the cytoplasm and nucleus, whereas BIN1 (+12A) is mostly located in the nucleus. Overexpression of BIN1 isoforms led to a significant reduction in cell proliferation, as assessed by colony forming cell assays and 3H-Thymidine uptake assays (2-3 fold, $p<0.05$). Furthermore, a significant increase in spontaneous and specific apoptosis was observed in BIN1-transduced cells, with and without exogenous FAS-ligand (2-3 fold, $p<0.05$). A significant reduction in protein expression of c-FLIP (inhibitor of the FAS-mediated apoptosis pathway) and upregulation of downstream cleaved caspase-8, caspase-3 and PARP1 was also demonstrated in BIN1-transduced cells. Interestingly, re-introducing c-FLIP into BIN1-transduced cells resulted in a significant decrease in FAS-ligand-induced apoptosis ($p<0.05$), as well as a significant reduction in protein expression of cleaved caspase-8, caspase-3 and PARP1. Conversely, transient suppression of BIN1 in BIN1-transduced cells rescued its effects on cell proliferation, spontaneous apoptosis and FAS-ligand-induced apoptosis. Protein expression of c-FLIP was increased in BIN1-suppressed cells and consequently the expression of cleaved caspase-8, caspase-3 and PARP1 was decreased in these cells. These findings indicate anti-proliferative and pro-apoptotic roles for BIN1 in human CTCL and suggest that BIN1 isoforms induce apoptosis by downregulating the expression of c-FLIP, which leads to activation of the FAS-mediated apoptosis pathway. Furthermore, to investigate the effects of overexpression of BIN1 isoforms on the ability to induce tumors in vivo, we tested the leukemogenic potential of transduced HH cells by injecting them into non-obese diabetic/severe-combined immunodeficiency (NOD/SCID) mice. Mice injected subcutaneously with either parental HH or control empty vector cells (5 x106/per mouse) showed local tumor formation in 6 of 6 mice within 2 weeks post-injection. The local tumors enlarged progressively and were often 1.5-2 cm in diameter by 4 weeks post-injection. In contrast, no local tumors formed in mice given injections of equal numbers of BIN1-transduced HH cells, even after 8 weeks (12 mice). These findings further demonstrate that lentiviral transduction of BIN1 isoforms into HH cells results in tumor suppressor activity in NOD/SCID mice and can significantly decrease the leukemogenic potential of CTCL cells in vivo.

T-1097

DETECTING HUMAN GLIOBLASTOMA-DERIVED STEM LIKE CELLS USING AN OLIGOTHIOPHENE DERIVATIVE VERSUS CD133 AND CD44

Gracias, Aileen[1], Kavanagh, Edel[2], Bäck, Marcus[3], Nilsson, Peter[3], Joseph, Bertrand[2], Hermanson, Ola[1]
[1]Karolinska Institute Neuroscience, Stockholm, Sweden, [2]Oncology-Pathology, Karolinska Institute, Stockholm, Sweden, [3]IFM, Linköping University, Linköping, Sweden

Glioblastoma multiforme is one of the most aggressive, malignant brain tumors, and is prone to relapse. One of the reasons of the relapse has been attributed to the existence of so called cancer stem cells in glioblastoma. These glioblastoma-derived stem-like cells (GSCs), which also have features of tumour-initiating cells (TICS), possess the ability to self renew and form tumors. Previous studies from our labs have identified a Luminescent Conjugated oligothiophene (LCO) to be used as a molecular probe for the live detection of rat embryonic neural stem cells, but not differentiated cells, other stem cells such as ES cells, or various cancer cells. This LCO, referred to as p-HTMI, crosses the cell membrane freely, and within a few minutes illuminates autonomously in the cytoplasm of target cells. p-HTMI stained 1-2% of neural tumor cell cultures in a rat model of glioma (C6) but 100% of C6-derived stem-like cells. Here we report the use of this LCO to specifically detect the stem cell population in human glioma-derived stem like cells from three patients without triggering cell apoptosis or necrosis. The level of specificity was compared to other well-established stem cell markers such as CD133 and CD44. Preliminary results suggest that p-HTMI is a more specific yet more sensitive marker of GSCs than CD133 and significantly more selective than CD44. We propose that this LCO due to its unique optical property can be used for cell specification and potentially used for improved clinical therapy of glioblastoma.

T-1099

METFORMIN PROTECTS HEAD AND NECK CANCER STEM CELLS AGAINST DNA DAMAGE INDUCED APOPTOSIS

Honda, Thomas K.[1], Kuo, Selena[1], Ku, Jonjei[1], Abhold, Eric L.[1], Wang-Rodriguez, Jessica[1], Altuna, Xabier[2], Ongkeko, Weg M.[1]
[1]University of California, San Diego, La Jolla, CA, USA, [2]Hospital Donosita, San Sebastian, Spain

The cancer stem cell hypothesis posits that within a tumor exists a distinct subpopulation of cells responsible for tumor initiation, progression, and maintenance. These cancer stem cells (CSCs) tend to be resistant to conventional chemotherapy and, like normal stem cells, possess the ability to self-renew and differentiate, thereby effecting tumor recurrence. Metformin, the most widely prescribed drug for the treatment of diabetes, has received attention in recent years as a potential anticancer agent capable of targeting cancer stem cells through such means as inhibiting cell proliferation and abrogating chemo-resistance. In the current study, we sought to determine the effects of Metformin on a putative head and neck squamous cell carcinoma (HNSCC)

cancer stem cell culture. In contrast to the findings of previous studies, our data suggests that Metformin promotes properties of a cancer stem cell phenotype in HNSCC in vitro. Treatment with Metformin resulted in a dose-dependent induction of the stem cell genes CD44, BMI-1, Oct-4, and Nanog, as measured by qPCR. These results were supported by immunofluorescence data. Metformin treatment promoted self-renewal capacity of HNSCC stem cells, as demonstrated by the increase in size and number of tumorspheres formed in non-adherent and non-differentiating conditions. At various doses, treatment with Metformin alone had no effect on cell proliferation, as measured by MTS assay. Furthermore, when administered in combination with cisplatin, Metformin significantly protected against cisplatin-induced cell death, as demonstrated by MTS and TUNEL assays. This protective effect of Metformin was also shown by MTS in the ALDH+ population of a HNSCC cell line but not in its corresponding ALDH- population. Immunoblot experiments demonstrating a decrease in Akt phosphorylation upon treatment with Metformin suggests Metformin-mediated cytoprotection is independent of the Akt pathway. Other possible mechanisms are currently under investigation, including Metformin's ability to regulate autophagy. Although further in vivo studies are necessary, taken together, our findings suggest that Metformin may not be an effective therapeutic option for patients with HNSCC.

T-1100
BIOMARKERS OF RADIORESISTANCE AND THEIR RELATION TO CANCER STEM CELLS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

Ina, Kurth, Maebert, Katrin, Hein, Linda, Peitzsch, Claudia, Kunz-Schughart, Leoni, Baumann, Michael, Dubrovska, Anna
OncoRay-National Center for Radiation Research, TU Dresden, Dresden, Germany

Aim: Radiotherapy plays a key role in the management of early stage and locally advanced head and neck squamous cell carcinoma (HNSCC) either alone or combined with surgery and chemotherapy. Current anti-cancer therapy fails to achieve long-lasting cancer cures. The development and growth of a tumor have been attributed to the existence of cancer stem cells (CSCs), or tumor progenitor cells, which have been discovered for many types of cancer including HNSCC. The number and properties of CSCs and their radiosensitivity are intrinsically heterogeneous and vary between individual tumor entities, which affect tumor radiocurability. So far it has not been clarified, if CSCs indeed play a role in radioresistance. The objective of this study is the elucidation of novel biomarkers for radioresistance and their correlation to CSCs with respect to the early prediction of tumor relapse. The establishment of new biomarkers for the early detection and targeted treatment of radioresistant CSC would essentially optimize therapeutic strategies. Methods: In a first step we generated radioresistant cancer cell lines, which have been exposed to minimum of 40 Gy of X ray given in fractions of 2 or 4 Gy. Their radioresistance was verified in radiobiological 3D in vitro assays in comparison to the non-irradiated parental cell lines. Comparative analysis of stem cell marker expression and assessment of in vivo tumor growth were performed in order to elucidate putative CSC-related biomarkers predictive of radioresistance. We found the aldehyde dehydrogenase (ALDH) activity as an already known cancer- and stem cell feature to be up-regulated during the curse of fractionated ionizing radiation. Thus we further investigated and characterized the ALDH positive and ALDH negative populations within the above mentioned radioresistant and non-irradiated cancer cells in vitro and in vivo radiobiological assays. Our study has shown that irradiation of cancer cells increased the tumor progenitor populations that can be defined by classical stem cell markers including ALDH activity and CD133 with increasing number of radiation fractions, which also led to activation of PI3K/AKT signaling pathway and increased expression of stem cell transcription factors like Sox2 and BMI-1. Moreover, our in vivo studies suggest that high ALDH activity defines tumorigenic and radioresistant cell subsets by protecting cells from irradiation stress. Further comparative gene expression analysis reveals several transcriptional regulators involved in radioresistance, which are also known to be stem cell regulators. Thereby, our studies revealed ALDH activity as indicative of HNSCC progenitor cells with increased radioresistance. Further analysis of the signaling pathways regulating stemness and radioresistance may contribute to development of predictive biomarkers prediction of a tumor intrinsic radioresistance prior therapy and help to establish new molecular targets for the development of therapeutics to use in conjunction with radiotherapy.

T-1101
IDENTIFICATION OF ES CELL-SPECIFIC TRANSCRIPTION FACTOR ZFP57 AS A NOVEL ONCOGENE

Koide, Hiroshi[1], Tada, Yuhki[1], Yamaguchi, Yukari[2], Akagi, Tadayuki[1], Takamura, Hiroyuki[2], Ohta, Tetsuo[2], Yokota, Takashi[1]
[1]Department of Stem Cell Biology, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Ishikawa, Japan, [2]Department of Gastroenterologic Surgery, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Ishikawa, Japan

Several common biological properties between cancer cells and embryonic stem (ES) cells suggest the possibility that some genes expressed in ES cells might play important roles in cancer cell growth. To explore this possibility, in this study, we searched for an oncogene in self-renewing ES cells and identified a transcription factor ZFP57. We found that ZFP57 promotes the anchorage-independent growth of human fibrosarcoma HT1080 cells in soft agar. ZFP57 is also involved in HT1080 tumor formation in nude mice. Furthermore, we demonstrated that ZFP57 upregulates the expression of IGF2, which plays a critical role in ZFP57-induced anchorage-independent growth possibly through Akt activation. Interestingly, ZFP57 also promotes anchorage-independent growth in ES cells and immortal fibroblasts. Finally, immunohistochemical analysis revealed that ZFP57 is overexpressed in human cancer clinical specimens. Taken together, these results suggest that the ES-specific transcription factor ZFP57 is a novel oncogene.

T-1102
IDENTIFICATION OF KEY NON-CODING RNAS IMPLICATED IN HUMAN ORAL CANCER STEM CELLS

Ku, Jonjei[1], Zou, Angela[1], Kuo, Selena Z.[1], Wang-Rodriguez, Jessica[1], Ongkeko, Weg M.[2]
[1]University of California San Diego, La Jolla, CA, USA, [2]Surgery, University of California San Diego, La Jolla, CA, USA

Cancer stem cells (CSCs) are a small subpopulation of cells within a tumor which are responsible for tumor initiation, therapy resistance, metastasis, and relapse. Despite their importance in cancer progression, the molecular mechanism for how CSCs are regulated is not well understood. Recent attention has focused on microRNAs (miRNAs), which are small noncoding RNAs that regulate gene expression, and are involved in a variety of biological processes, including the maintenance of stem cell functions. In our study, we isolated putative CSCs within human head and neck squamous cell carcinoma (HNSCC) established cell lines using the markers ALDH and CD44, and profiled the differential expression of microRNAs between the ALDH-/CD44- and ALDH+/CD44+ cells. Flow cytometry analysis

and sorting of established HNSCC cell lines allowed us to isolate both populations within the same cell line. In three HNSCC cell lines, HN30, UMSCC-10B, and UMSCC-22B, we determined that they contained 48.0%, 2.7%, and 11.4% of ALDH+/CD44+ cells respectively. Using a microarray, 95 miRNAs implicated in stem cell function were profiled and large differences in several miRNA expression were discovered. Verified qPCR triplicate results revealed a 1900 fold upregulation of microRNA-126, 167 fold upregulation of microRNA-18a,118 fold downregulation of microRNA-302c, 15 fold upregulation of microRNA-let7b, and 20 fold downregulation of microRNA-181c. MicroRNA-181c may function as a tumor suppressor as studies have shown it to be downregulated in many cancers, as well as induce pluripotent cells from fibroblasts, and function as a critical player in cancer stem cells. Our results indicated that the expression patterns of individual miRNAs were differentially expressed between ALDH-/CD44- and ALDH+/CD44+ cells across multiple cell lines, suggesting a potential for these miRNAs to serve as epigenetic regulators of a stem-like phenotype for CSCs. Although further functional characterization of these miRNAs is necessary, our results identify key microRNAs that have the potential to serve as prognostic biomarkers and therapeutic targets for patients with HNSCC.

T-1103
TARGETING DRUG-INSENSITIVE CML STEM/PROGENITOR CELLS WITH COMBINATION TREATMENTS OF NEW ABL AND PP2A INHIBITORS

Lai, Damian[1], Liu, Xiaohu[1], Chen, Min[1], Rothe, Katharina[1], Hu, Kaiji[2], Dunn, Sandra E.[2], Jiang, Xiaoyan[1]

[1]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada, [2]Child And Family Research Institute, Vancouver, BC, Canada

Chronic myeloid leukemia (CML) is a lethal haematological malignancy resulting from transformation of a hematopoietic stem cell by the BCR-ABL oncogene. Imatinib Mesylate (IM) and other ABL tyrosine kinase inhibitors (TKIs) have had a major impact on treatment of early phase CML patients. However, TKI monotherapies are not curative and initial and acquired resistance, as well as relapse, remain challenges. We have demonstrated that CML stem cells are less responsive to TKIs and are a critical target population for TKI resistance. To prevent the development of resistant subclones, improved treatment approaches that target other elements active in CML stem cells are needed. One candidate is Abelson helper integration site 1 (AHI-1), an oncogene we identified that is upregulated in highly enriched populations of CML stem cells where BCR-ABL transcripts are also elevated. We have recently demonstrated that AHI-1 interacts with multiple kinases, including BCR-ABL and JAK2, and these complexes initiate BCR-ABL-transforming activity and mediate TKI response/resistance of CML stem/progenitor cells. Loss of these interactions significantly increases IM-sensitivity of CML stem/progenitor cells. These findings indicate that AHI-1 is a new therapeutic target in CML stem cells, but there are no specific small molecule inhibitors available that target AHI-1. By screening the Prestwick Chemical Library, we have recently identified a specific growth inhibitory compound that potentially targets AHI-1: Cantharidin (CAN), an inhibitor of protein phosphatase 2A (PP2A). PP2A is a family of serine/threonine phosphatases that regulate numerous cell signaling cascades involved in proliferation and cell cycle control of cancer cells. Using Immunoprecipitation/mass spectrometry we further identified the PP2A subunit B (PR55a) as a potential AHI-1 interacting protein. Interestingly, combination treatment with IM and CAN significantly prevents growth and induces apoptosis in CML K562 and IM-resistant K562 cells compared to single treatments (2-3 fold, $p<0.001$). The combination also greatly reduced colony formation (CFC) of CD34+ CML cells, but CAN also inhibited CFCs of CD34+ normal bone marrow (BM) cells. To overcome toxicity issues, we recently obtained new, pre-clinically validated PP2A inhibitors, LB1.0 and LB1.2. The combination of LB1.0 and LB1.2 (5μM) with IM (5μM) is more effective at selectively reducing CFCs generated from CD34+ treatment-naïve IM-nonresponder cells than single agents (56% vs. 13%). Importantly, our CFC data indicated that both LB1.0 and LB1.2 are much less toxic to CD34+ normal BM cells compared to CAN (2-3 fold, $p<0.005$). Cell cycle analysis in CML cells showed that treatment with LB1.0/LB1.2 alone was able to induce a shift from G1 to G2/M phase (3 fold, $p<0.05$). A similar shift in the cell population was also observed after combination treatment with IM, suggesting the G2/M phase arrest is solely due to PP2A inhibition. Confocal microscopy further showed the G2/M arrest leading to mitotic catastrophe in the treated cells. Interestingly, Western blot analysis further showed that the combination treatment significantly suppresses protein expression of AHI-1, BCR-ABL, JAK2, STAT5, AKT and Beta-Catenin compared to single agents. These results indicate that we have uncovered a new AHI-1-PP2A (PR55a) interaction and that simultaneously targeting both BCR-ABL and PP2A activities in CML stem/progenitor cells may improve outcomes in CML patients.

T-1104
CELL FATE DECISIONS IN MALIGNANT HEMATOPOIESIS: LEUKEMIA PHENOTYPE IS DETERMINED BY DISTINCT FUNCTIONAL DOMAINS OF THE MN1 ONCOGENE

Lai, Courteney K.[1], Moon, Yeonsook[2], Kuchenbauer, Florian[3], Starzcynowski, Daniel T.[4], Argiropoulos, Bob[5], Yung, Eric[1], Beer, Philip Anthony[1], Schwarzer, Adrian[6], Sharma, Amit[6], Park, Gyeongsin[7], Leung, Malina[1], Lin, Grace[1], Vollett, Sarah[1], Fung, Stephen[1], Eaves, Connie[1], Karsan, Aly[8], Weng, Andrew P.[1], Humphries, R. Keith[1], Heuser, Michael[9]

[1]Terry Fox Laboratory, BC Cancer Agency Research Centre, Vancouver, BC, Canada, [2]Inha University Hospital, Incheon, Republic of Korea, [3]Institute of Experimental Cancer Research, Comprehensive Cancer Centre, University Hospital of Ulm, Ulm, Germany, [4]Department of Pediatrics, Children's Hospital Medical Centre, Cincinnati, OH, USA, [5]Department of Medical Genetics, University of Calgary, Calgary, AB, Canada, [6]Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany, [7]Department of Hospital Pathology, Catholic University of Korea, Seoul, Republic of Korea, [8]Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada, [9]Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany

Acute leukemias span a vast array of distinct clinical entities and likely molecular determinants. It is not well understood how the cell of origin, accompanying mutations, extracelluar signals, alterations in the expression level, and/or structural differences in a given oncogene determine the specific phenotype and functional identity of leukemias. Meningioma 1 (MN1) is a transcriptional co-factor that is an independent prognostic marker for normal karyotype acute myeloid leukemia, with high expression linked to poor survival and resistance to treatment with the differentiation-inducing agent all-trans retinoic acid (ATRA). MN1 is also a potent and sufficient oncogene in murine leukemia, able to block differentiation and promote leukemic stem cell self-renewal through transformation of cells at the common myeloid progenitor level. Here, we have used these properties to dissect the functional aspects of different protein domains of the MN1 oncogene and their effect on leukemic phenotype. To elucidate the role(s) of each portion of MN1, we divided the gene into 7 regions of 200 amino acids and generated 16 mutants as distinct region deletions or cumulative deletions from the N- or C-termini. Mouse bone marrow was retrovirally transduced and assayed for: (1)

leukemogenicity and self-renewal activity, by CFU colony formation and in vivo transplantation; (2) block in lineage differentiation in myeloid and lymphoid differentiation by immunophenotypic analysis of transplanted mice, and at the erythro-megakaryocyte level by red blood cell (RBC) engraftment and CFU-Mk colony formation; and (3) ATRA resistance, as measured by in vitro response to drug treatment. The MN1 C-terminal regions were shown to be critical for the myeloid and lymphoid differentiation blocks characteristic of MN1 leukemia. C-terminal deletions showed increased Gr1 and CD11b expression in GFP+ peripheral blood and delayed disease onset in vivo, and a reversal of ATRA resistance in vitro (n=6, P<0.05). Gene expression profiling showed C-terminally deleted MN1 cells clustering with Gr1+CD11b+ cells, underscoring the importance of the C-terminus in blocking myeloid differentiation. Most strikingly, a larger C-terminal deletion lacking 606 amino acids from the C-terminus (MN1Δ5-7) led to a change in the lineage identity of the disease to T-cell acute lymphoblastic leukemia (n=3, P<0.05), indicating a major role for the C-terminus in determining leukemic identify. In contrast, the N-terminus of MN1 was found to be essential for the leukemogenicity of the molecule, as animals transplanted with MN1 mutants lacking the N-terminal 221 amino acids (MN1Δ1) failed to develop leukemia after 181 days (n=4). This loss of leukemogenic activity can be tied, in part, to the role of the N-terminus in proliferation and self-renewal, as deletion of multiple N-terminal domains resulted in decreasing in vivo engraftment over 16 weeks. Interestingly, the MN1 N-terminus was also found to be important for blocking erythro-megakaryocyte differentiation. This study demonstrates that the abilities of MN1 to promote self-renewal/proliferation, inhibit differentiation, and induce leukemia can be attributed to specific, and largely distinct regions. The marked differential effects of wild type MN1 compared to the mutant forms provides a powerful experimental model to probe the cellular and molecular determinants of leukemic transformation and resulting leukemic phenotype.

T-1105
MICRORNA142-3P PROMOTES TUMOR-INITIATING PROPERTIES IN ATYPICAL TERATOID/RHABDOID TUMOR

Lee, Yi Yen

Department of Neurosurgery, Neurological Institute, Taipei Veterans General Hospital, Taipei, Taiwan

Primary central nervous system (CNS) atypical teratoid/rhabdoid tumor (AT/RT) is an extremely malignant pediatric brain tumor in infancy and childhood. It has been reported that subpopulation of $CD133^+$ cells isolated from ATRT tumors present cancer stem-like and radioresistant property. However, the exact biomolecular mechanism of ATRT or AT/RT-$CD133^+$ is still unclear. In this study, we firstly showed that CD133-positive AT/RT cells ($CD133^+$) have pluripotent differentiation ability and the capability of malignant cells to be highly resistant to ionizing radiation (IR). Using microRNA array and quantitative RT-PCR, our data showed that expression of miR142-3p was lower in AT/RT-$CD133^+$ than in AT/RT-$CD133^-$. MiR142-3p overexpression significantly inhibited self-renewal and tumorigenicity of AT/RT-$CD133^+$. On the contrary, silencing of endogenous miR142-3p dramatically increased tumor-initiating and stem-like cell capacities in ATRT or AT/RT-$CD133^-$ cells, and further promoted the mesenchymal-transitional and radioresistant properties of ATRT cells. Most importantly, therapeutic delivery of miR142-3p in ATRT effectively reduced its lethality by blocking tumor growth, repressing invasiveness, increasing radiosensitivity, and prolonging survival time in orthotropic-transplanted NOD-SCID mice. These results demonstrate the prospect of developing novel miRNA-based strategies to block the stem-like and radioresistant property of malignant pediatric brain cancer stem cells.

T-1106
TARGETING TREATMENT-NAÏVE CML STEM/PROGENITOR CELLS FROM IMATINIB-NONRESPONDERS BY COMBINATION TREATMENTS OF NEW JAK2 (BMS-911543) AND ABL INHIBITORS IN VITRO AND IN VIVO

Lin, Hanyang[1], Chen, Min[1], Rothe, Katharina[1], Lorenzi, Matthew[2], Woolfson, Adrian[2], Jiang, Xiaoyan[1]

[1]*Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada,* [2]*Discovery Medicine Oncology, Bristol-Myers Squibb, Princeton, NJ, USA*

The hallmark of chronic myeloid leukemia (CML) is the presence of a BCR-ABL oncoprotein with constitutive tyrosine kinase activity that drives the pathogenesis of the disease. Imatinib Mesylate (IM) and other tyrosine kinase inhibitor (TKI) therapies have had a major impact on the treatment of chronic phase CML, but relapses and persistence of leukemic stem cells remain problematic. Our group has recently identified an AHI-1-BCR-ABL-JAK2 protein complex that contributes to the transforming activity of BCR-ABL and IM-resistance in CML stem/progenitor cells. We therefore hypothesized that dual suppression of BCR-ABL and JAK2 activities might be more effective in eliminating CML stem/progenitor cells. Several JAK2 inhibitors are currently in various stages of clinical trials, but their off-target effects on normal hematopoietic cells remain a concern. We have now examined the biological effects of an orally bioavailable, highly selective JAK2 inhibitor, BMS-911543 (BMS), in combination with TKIs, including IM, dasatinib (DA) and nilotinib, both on IM-resistant CML cell lines and primary CD34+ treatment-naïve IM-nonresponder cells. Western blot analyses showed that combination treatment was more effective at reducing pSTAT5 levels in IM-resistant K562 cells than single agents. In colony-forming cell (CFC) assays, combination treatment resulted in a greater reduction in colonies produced from these cells compared to single agents (2-3 fold, p<0.05). Similarly, in CD34+ CML cells, intracellular staining analyses showed that combined exposure to BMS-911543 and TKIs produced a deeper suppression of pSTAT5 and pCRKL activities than single agents (n=4, 40-45% vs. 15-20%, p<0.05). Apoptosis assays also revealed that combination treatments increased Annexin V+ cells compared to single agents (n=3, 30-40% vs. 10-20%, p<0.05). Furthermore, combination treatments resulted in greater inhibition of colony growth compared to single agents (n=7, 74-86% vs. 40-50%, p<0.001). Long-term culture-initiating cell assays also showed that more primitive cells were significantly eliminated by combination treatments (n=3, 2-3 fold, p<0.05). Importantly, our CFC data indicated that BMS-911543 is less toxic to normal bone marrow (BM) CD34+ cells than CML cells (n=7, 2-3 fold, p<0.001). For in vivo studies, we injected primitive CML cells intravenously into NSG mice and treated mice with inhibitors by oral gavage for two weeks. Mice undergoing combination treatment showed significantly reduced weight loss and engraftment levels in peripheral blood, BM and spleen compared to mice treated with single agents (0.21% vs. 6.14%, 1.17% vs. 26.3%, and 2.46% vs. 51.5%, respectively, p<0.05). Immunohistochemical staining revealed that combination treatments resulted in less infiltration of leukemic cells into mouse spleens and livers than single agents. Quantitative RT-PCR analyses further demonstrated a significant reduction in BCR-ABL transcript levels in spleen, liver, and BM of mice treated with combinations (15-20 fold, p<0.05). Most importantly, combination treatments significantly prolonged survival of leukemic mice compared to mice treated with single agents (median survival of IM + BMS vs. IM: 70 days vs. 60 days, P<0.05; DA + BMS vs. DA: 96.5 days vs. 81 days, p<0.001). Taken together, this study suggests that targeting both BCR-ABL and JAK2 activities in CML stem/progenitor cells may improve outcomes in patients, especially those destined to develop TKI resistance.

T-1107

ERK1/2 PATHWAY IS REQUIRED FOR DIFFERENTIATION OF AML TRIGGERED BY ANTI-CD44 MONOCLONAL ANTIBODIES

Madhoun, Nour, Gadhoum, Samah, Merzaban, Jasmeen
King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by accumulation of immature hematopoietic cells due to a blockage in myeloid differentiation at various stages. Since the current chemotherapy rarely eradicates the leukemic clones entirely, differentiation induction therapy has evolved. Previous experiments have shown that ligation of CD44, a cell surface glycoprotein strongly expressed on all AML cells, with anti-CD44 monoclonal antibodies (mAbs) could reverse the differentiation blockage of leukemic blasts and inhibit their proliferation in most AML subtypes, rendering CD44 a promising target for AML therapy. However, the underlying molecular mechanisms of the induction of differentiation by anti-CD44 mAbs have not been fully elucidated. Here, we investigated the signaling pathways triggered during anti-CD44 mAb (A3D8)-induced granulocytic differentiation of HL60, a human leukemic cell line commonly used as a model of human AML type2/3. Extracellular signal regulated kinase (ERK1/2) pathway plays a major role in the regulation of differentiation and proliferation for myeloid cells and has been reported to be aberrantly activated in AML. In our studies, we observed an increase of ERK1/2 phosphorylation by mitogen-activated ERK kinase (MEK) following A3D8 treatment. The use of U0126 (a MEK inhibitor) significantly abrogated the anti-CD44 induced granulocytic differentiation of HL60 cells, suggesting that ERK1/2 is critical for the induction of differentiation via CD44 in AML-M2/3. Our work provides the first evidence for the importance of the ERK pathway in granulocytic differentiation via CD44 and adds a new argument to the use of CD44 in differentiation therapy of AML.

T-1108

SELF-RENEWAL OF A DE NOVO POPULATION OF C-KIT+ CELLS CONFERS A SUSTAINABLE GROWTH OF NEUROBLASTOMA

Lau, Sin-Ting[1], Hansford, Loen M.[2], Kaplan, David R.[3], **Ngan, Elly**[1]
[1]Department of Surgery, University of Hong Kong, Hong Kong, [2]University of Toronto, Vancouver, ON, Canada, [3]University of Toronto, Toronto, ON, Canada

High cellular heterogeneity within neuroblastomas (NB) may account for the non-uniform response to treatment. c-KIT+ cells are frequently detected in NB, but how they influence NB behavior still remains elusive. Here, we used NB tumor initiating cells to reconstitute NB development and demonstrated that c-KIT+ cells are de novo generated and dynamically maintained within the tumors to sustain tumor progression. c-KIT+ NB cells express higher levels of neural crest and stem cell markers (SLUG, SOX2, NANOG) and are endowed with high clonogenic capacity, differentiation plasticity and are refractory to drugs. With serial transplantation assays, we found that c-KIT expression is not required for tumor formation, but c-KIT+ cells are more aggressive and can induce tumors 9-fold more efficiently than c-KIT-/low cells. Intriguingly, c-KIT+ cells exhibited a long-term in vivo self-renewal capacity to sustain the formation of secondary and tertiary tumors in mice. In addition, we showed that Prokineticin signaling and MAPK pathways are crucial for the maintenance of c-KIT+ cells in tumor to promote NB progression. Our results highlight the importance of this de novo population of NB cells in sustainable growth of NB and reveal specific signalling pathways that may provide targets leading to more effective NB therapies.

T-1109

PHARMACOLOGICAL TARGETING OF COOPERATION BETWEEN THE WNT/BETA-CATENIN AND PI3K/AKT STEM CELL SELF-RENEWAL PATHWAYS SELECTIVELY ELIMINATES LEUKEMIC STEM CELLS

Perry, John M.[1], He, XI (CiCi)[1], Roy, Anuradha[2], Tao, Fang[1], Ding, Sheng[3], Li, Linheng[1]
[1]Stowers Institute for Medical Research, Kansas City, MO, USA, [2]University of Kansas, Lawrence, KS, USA, [3]Gladstone Institutes, San Francisco, CA, USA

The existence of a subpopulation of cancer cells with exclusive or highly enriched tumorigenic capacity has stimulated the search for drugs that can target these cancer stem cells (CSCs), but these efforts have met only limited success. Similar to normal stem cells, CSCs have the capacity for self-renewal. However, while normal stem cells rely on multiple genetic pathway networks, CSCs are heavily dependent on mutated oncogenic pathways for their self-renewal ability. This offers an opportunity for targeting the aberrant self-renewal of CSCs and thus eliminating the 'root' of cancer while potentially sparing normal stem cells. Previous studies in our lab and others have shown that cooperative interaction between the Wnt/β-catenin and PI3K/Akt pathways is especially relevant to both self-renewal and tumorigenesis. Specifically, while neither pathway is sufficient for long-term self-renewal, simultaneous activation of both pathways using pharmacological agents allows for expansion of normal hematopoietic stem cells (HSCs) in culture. However, when both pathways are permanently activated by genetic mutation in HSCs, leukemic stem cells (LSCs) develop. We found that, while conventional chemotherapeutic treatment eliminates most leukemic cells, the LSC population actually expands in response to chemotherapy, resulting in relapse. Since simultaneous pharmacological activation of the Wnt/β-cat and PI3K/Akt pathways expands normal stem cells, we hypothesized that pharmacological inhibition of them, or their cooperative interaction, might prevent LSC self-renewal. Furthermore, LSC 'addiction' to this mechanism of self-renewal may allow for targeting of LSC self-renewal while sparing normal stem cells, thus facilitating hematopoietic recovery. Because the Wnt/β-cat and PI3K/Akt pathways broadly regulate normal cells, inhibition of either was found to be toxic. However, our previous studies have shown that these pathways interact. Specifically, Akt activates β-catenin by phosphorylation at serine residue 552. Thus, we designed a high-throughput screen to search for compounds that could inhibit pS522-β-cat while having minimal effects on total β-catenin. We found that combining chemotherapeutic treatment with targeted inhibition of pS522-β-cat not only prevented chemotherapy-induced LSC expansion but effectively eliminated the phenotypic LSC population. Transplantation experiments revealed that bone marrow from mice treated with chemotherapy alone resulted in rapid leukemia development in transplant recipients (median survival 44.5 days); however, when combined with pS522-β-cat inhibitor treatment, survival was significantly enhanced (median survival 94 days). Furthermore, combined chemotherapy with pS522-β-cat inhibitor treatment significantly improves survival of primary leukemic mice relative to vehicle or chemotherapy alone (median survival 57 days vs. 35 and 48 days, respectively). Indeed, the targeted elimination of LSCs allowed for partial recovery of normal HSCs, which are apparently less 'addicted' to the pS522-β-cat-mediated self-renewal mechanism than LSCs. Thus, our studies show that the aberrant self-renewal capacity of LSCs can be targeted by inhibiting the cooperative interaction between the Wnt/β-cat and PI3K/Akt pathways. Considering the common role of these pathways in multiple human cancers, future application of

these findings to the clinic may reduce the incidence of relapse.

T-1110
MESENCHYMAL STEM CELL-MEDIATED ENHANCEMENT OF GLIOBLASTOMA CELL PROLIFERATION

Rodini, Carolina Oliveira, Silva, Patrícia Benites Gonçalves, Okamoto, Oswaldo Keith
University of São Paulo, São Paulo, Brazil

Mesenchymal stem cells (MSC) display tropism to tumors and may exert paracrine effects influencing their development, although through mechanisms still poorly known. Here, we investigated whether MSC-secreted TGFbeta1, a multifunctional cytokine with roles in immunomodulation as well as, proliferation, migration and epithelial-mesenchymal transition of tumor cells, would contribute to development of glioblastoma multiforme (GBM). Secreted TGFbeta1 protein levels in culture supernatants of MSC isolated from different biological sources were found to be higher, as determined by Elisa, in MSC of human umbilical cord compared with those of bone marrow and adipose tissue. Human U87MG glioblastoma cells were then cultivated in the presence or absence of umbilical cord MSC concentrated supernatants, and tumor cell proliferation was analyzed after 72 hours. Under such conditions, a significant increase in the amount of viable tumor cells was found after treatment with MSC supernatant. This effect on tumor cells was reduced in the presence of SB-431542, a specific inhibitor of the TGFbeta1 pathway. To further investigate this phenomenon, umbilical cord MSC with a stable TGFB1 gene expression knockdown were generated by retroviral transduction. TGFB1 knockdown was confirmed at the transcript and protein levels by real time PCR and Elisa assay, respectively. Culture supernatants of transduced cells with higher than 80% knockdown in TGFbeta1 protein levels were then used in the tumor cell proliferation assays. In agreement with the previous findings, the enhancement of tumor cell growth by TGFbeta1-containing MSC supernatants was also abolished when tumor cells were cultivated with supernatants of MSC with stable TGFB1 knockdown. These results suggest that TGFbeta1 secreted by MSC may be an important component of their paracrine effects on GBM cells, thereby contributing to tumor cell proliferation. These findings highlight a pro-tumorigenic effect of MSC and a possible mechanism of their contribution within the tumor niche in GBM, the most frequent and aggressive malignant tumor of the central nervous system. Financial Support: CNPq-INCT, FAPESP, FAPESP-CEPID.

T-1111
THE CORE AUTOPHAGY PROTEIN ATG4B IS CRITICAL TO THE SURVIVAL OF LEUKEMIC STEM/PROGENITOR CELLS AND PREDICTS CLINICAL OUTCOMES OF CML PATIENTS TREATED WITH IMATINIB THERAPY

Rothe, Katharina[1], Lin, Hanyang[1], Lin, Kevin[1], Leung, Amy[2], Hui Mi, Wang[1], Malekesmaeili, Mehrnoush[1], Brinkman, Ryan[1], Forrest, Donna[3], Gorski, Sharon[2], Jiang, Xiaoyan[1]
[1]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada, [2]Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada, [3]Leukemia/BMT program of BC, BC Cancer Agency, Vancouver, BC, Canada

Although autophagy is a well-studied catabolic process that takes place at basal levels in the majority of mammalian cells, its roles in the regulation of hematopoiesis and pathogenesis of leukemia have not been fully explored yet. Previous studies have shown that imatinib mesylate (IM) induces autophagy in CML and that this process is critical to the survival of leukemic stem/progenitor cells upon IM therapy. However, it is still not known if autophagy differs between CML patients and healthy individuals and if pre-treatment CML cells harbor unique autophagy characteristics that could be predictive of patients' clinical outcomes. We demonstrate for the first time that several key autophagy genes, and their protein products, are differentially expressed in CD34+ subpopulations obtained at diagnosis from chronic phase CML patients who were retrospectively classified, after initiation of IM therapy, as IM-responders (n=14) and IM-nonresponders (n=14), as well as normal, healthy individuals (n=10). The cysteine protease ATG4B, a crucial enzyme of the autophagic process, was found to be the most highly expressed gene and protein in CD34+ hematopoietic stem/progenitor cells, with 110-fold higher gene expression than BECLIN-1. Interestingly, the transcript and protein levels of ATG4 family members, ATG5 and BECLIN-1 were significantly increased in CD34+ CML cells compared to CD34+ normal bone marrow cells ($p<0.05$). Importantly, ATG4B expression was significantly increased in CD34+ CML cells from subsequent IM-nonresponders vs. IM-responders ($p=0.014$); this finding was further confirmed using a logistic regression model analysis ($p<0.05$). In addition, transcript levels of several ATG genes, including ATG4B, were also higher in the stem-cell enriched CD34+CD38- population from IM-nonresponders compared to the same cells from IM-responders. Exposure to IM in vitro consistently further elevated the transcript and protein levels of ATG4B, and induced autophagic flux in CD34+ CML cells. Strikingly, lentiviral-mediated ATG4B knockdown using three different ATG4B short hairpin RNA (shRNA) sequences in K562 and CD34+ CML cells reduced viability (25-60%; $p<0.05$) and proliferation (30-40%; $p<0.05$), increased apoptosis (10-20%), and decreased the formation of colonies (CFC, 30-70%; $p<0.05$) in comparison to a non-targeting control. Suppression of ATG4B also impaired the growth of long-term culture-initiating cell (LTC-IC)-derived CFCs (20-50% reduction) generated from CD34+ CML cells, as compared to the non-targeting control. The observed phenotypic changes were further enhanced upon IM-treatment. Moreover, deregulated expression of ATG4B in CD34+ CML cells inversely correlated with transcript levels of miR-34a, a miRNA predicted to target ATG4B. Interestingly, overexpression of miR-34a could reduce ATG4B expression in CML cells to levels comparable to normal and significantly reduce CFC formation upon IM treatment compared to a scrambled control ($p<0.05$). In addition, luciferase assays further confirmed specific targeting of ATG4B by miR-34a. In conclusion, this study identifies ATG4B as a potential biomarker in treatment-naïve CML stem/progenitor cells that may be useful for predicting therapeutic response and indicates that ATG4B may be a potential drug target in CML stem cells.

T-1112
TRACING THE ORIGINS AND EVOLUTION OF CLONAL HETEROGENEITY IN A SHH-SUBTYPE MEDULLOBLASTOMA MOUSE MODEL

Selvadurai, Hayden[1], Vanner, Robert[2], Lee, Lilian[1], Dirks, Peter B.[1]
[1]Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada, [2]Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada

Many human cancers are functionally and genetically heterogeneous. This heterogeneity is central to the limitations of many standard therapies that target cancers as a homogenous population, rather than distinct populations that may possess different genetic or epigenetic aberrations driving tumour growth. The brain tumour medulloblastoma, a largely pediatric malignancy of the cerebellum, is one such cancer that displays a considerable degree of cellular heterogeneity. There is also evidence that these tumours may contain genetically distinct populations, suggestive of multiple independent clones present within the tumour as a whole. Given the confounding

effect of clonal heterogeneity on cancer therapy, it is thus important to understand the extent to which medulloblastoma is comprised of distinct clones, and how they arise and evolve over time. We have sought to answer these questions with a series of genetic lineage tracing experiments in a mouse model of medullobalstoma. Mice harbouring a Ptc1 loss of function allele reproducibly develop medullobastoma bearing a resemblance to the human Sonic hedgehog-driven disease subtype. In order to examine clonal heterogeneity in this model, we utilised the multi-coloured Confetti reporter allele in combination with a ubiquitously expressed, inducible Cre-recombinase allele, allowing stochastic marking of cell lineages with one of four fluorescent colours. We have first tested whether clonal heterogeneity can be ascribed to a polyclonal tumour origin by inducing widespread confetti labeling shortly after birth prior to any detectable cellular abnormalities. Analysis of pre-neoplastic lesions in early adult mice revealed striking multi-coloured heterogeneity within each individual mass. To test whether this clonal diversity persists throughout tumour development we have analysed end-point tumours with the same labeling strategy and found that tumours maintain multiple coloured cell populations, present as either cellular admixtures or large regions of homogenous labeling. These data suggest that the tumours in this model may represent multiple, distinct genetic lesions co-existing in a single mass - a finding with important implications for disease therapy. We are presently testing this hypothesis further by employing a late embryonic labeling strategy and are continuing our analysis to investigate the evolution of clonal heterogeneity from the cancer stem cell population.

T-1113
CYCLIN D1 IN REGULATION OF LIVER CANCER STEM CELLS

Wang, Xiao Qi, Xia, Wei, Tsao, George, Poon, Ronnie
University of Hong Kong Department of Surgery, Hong Kong, China

Cancer stem cells (CSCs) are capable of self-renewal, multipotency, in vivo tumorigenicity, and driving metastasis, leading to recurrence of tumor. Thus, novel therapies capable of eliminating CSCs are needed. Cyclin D1 is deregulated in many types of cancers. Beyond being a cell cycle regulator and oncogene, cyclin D1 also promotes stem cell (SC) self-renewal and induced pluripotent stem cells (iPSCs) reprogramming efficiency. Yet, the role of cyclin D1 in regulation of CSC is not well defined. In this study, we investigated potential role of cyclin D1 in regulation of liver cancer stem cell properties. In cyclin D1 overexpressed two lines of liver cancer cells, anchorage-independent spherical colony (spheres) formation was enriched. From dissected single spherical cells, the capacity of the secondary and the third sphere formation was significantly higher in cyclin D1-expressing liver cancer cells compared to parental cells, suggesting an enhanced self-renewal capacity. Cyclin D1 expression conferred liver cancer cells more CSC and SC properties, shown by the increased CD90+ and EpCAM+ liver CSC populations and the enhanced expression of SC-associated genes Nanog, Oct4 and Nodal. At longer culturing time, cyclin D1-expressing spheres maintained a good spherical morphology, whereas parental spheres became more differentiated. Interestingly, Smad2/3, a TGF-β signaling effector, was highly phosphorylated in cyclin D1-expressing spherical cancer cells compared to spherical cells without cyclin D1. Application of TGF-β/Smad inhibitor (SB431542) significantly reduced the CSC sphere formation in cyclin D1-expressing cells but had no effects on parental cell sphere formation. In addition, Erk inhibitor (UO126) showed no inhibiting effects, a further proof of the cyclin D1-mediated activation of Smad2/3. Our preliminary data also indicated the effects of SB431542 in induction of CSC sphere differentiation and reduction of tumorigenicity. Thus, TGF-β/Smad inhibitor might have therapeutic potential for targeting liver CSCs. Liver cells are known being highly resistance to chemotherapeutic drugs. We further investigated the sphere formation capacity of cyclin D1-expressing cells under the treatment of chemotherapeutic agents in combination with Smad inhibitor and found that there was an sensitised effect in low dose and synergistic effect in high dose of SB431542. In summary, our results suggest that cyclin D1 might promote liver CSC proliferation and self-renewal via activation of TGF-β/Smad signaling pathway, with underlining mechanism is under investigation.

T-1114
GLIOMA CANCER STEM CELLS SECRETE BMP ANTAGONISTS TO PROMOTE TUMOR HIERARCHY MAINTENANCE

Yan, Kenneth, Yan, Diana, Lee, Christine, Wu, Qiulian, Rahim, Nasiha, Tritschler, Isabel, Hjelmeland, Anita, Rich, Jeremy N.
Cleveland Clinic, Cleveland, OH, USA

Glioblastomas are the most prevalent and lethal primary brain tumor and are comprised of hierarchies with self-renewing cancer stem cells (CSCs) at the apex. Like neural stem cells (NSCs), CSCs reside in functional niches that provide essential cues to maintain the cellular hierarchy. Bone morphogenetic proteins (BMPs) instruct NSCs to adopt an astrocyte fate and are proposed as anti-CSC therapies to induce differentiation. However, paradoxically, tumors express high levels of BMPs, suggesting that BMPs are elements of the CSC niche. Here we demonstrate that BMP antagonists are specifically secreted by CSCs within their niches as protection from endogenous BMPs. These antagonists co-localize with CSCs but not differentiated cancer cell populations. Further, these antagonists block pro-differentiation effects of BMPs and their overexpression in non-CSCs decreases endogenous BMP signaling to promote stem-like features. Consequently, these cells display increased growth and tumor formation abilities. Targeting these BMP antagonists in CSCs results in impaired growth and self-renewal. This study establishes CSC-derived BMP antagonists as driving forces in maintaining glioblastoma tumor proliferation and glioblastoma hierarchies through the modulation of endogenous pro-differentiation signals.

EMBRYONIC STEM CELL CLINICAL APPLICATION

T-1115
RECENT ADVANCES IN STEM CELL TRANSLATIONAL RESEARCH AND THE FUTURE OF MEDICAL GENETICS IN SAUDI ARABIA

Al-Aqeel, Aida I.A.
Pediatrics, Prince Sultan Military Medical City, Riyadh, Saudi Arabia

Although Medical genetics has taken the lead in developing genetic technology in clinical practice, this may become increasingly diffficult as genetics goes “main stream” and is incorporated in different specialities. It has to take the lead in translational research and in development of treatement in single gene disorder, One of these advances in translational genomic research 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. In Saudi Arabia like other countries in the middle East first cousin marriages account for almost 60-70% of all marriages, leading to uniquely common disorders. A review of our patients files documented more than 150 varieties of neurodegenerative disease

among 2,000 children; 27 of which constitute more than half of these files. Some autosomal recessive disorders are common eg. sickle cell anaemia and thalassaemia. Others are unique eg. Al-Aqeel-Sewairi syndrome. For the last ten years pre- implantation genetics diagnosis and newborn screening for genetics metabolic disorders are the most important preventive programs in Saudi Arabia. The Stem Cell Therapy Program was also established at King Faisal Specialist hospital and research centre with launching of ten projects. If the regulatory policies in each country are put forward for such research, the major remaining barriers to realizing the medical benefits of translational genomics might be 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: personalized translational medicine should not be limited to diagnostic and prognostic approaches, and must include personalized therapeutic strategies. Therefore we will explore the challenges as well as the progress and recent advances in the implementation of personalized genomics in the clinical setting, including diagnostic and preventive strategies and new therapeutic strategies like stem cell therapy and recent advances in personalized therapeutics strategies at the genomics level. In addition we will explore the need of developing international approaches to address the ethical concerns across the continuum of such research, from bench to bedside and to publication with the attention to global equity and benefit sharing.

T-1116
CLEAN UP OF THE HESC LINES TOWARDS CLINICAL APPLICATIONS

Baek, Jin Ah[1], Seol, Hye Won[1], Jung, Juwon[1], Yoon, Bo Ae[1], Oh, Sun Kyung[1], Moon, Shin Yong Yong[1], Choi, Young Min[2]
[1]IRMP, MRC, Seoul National University, Seoul, Republic of Korea, [2]Seoul National University, College of Medicine, Department of OB/Gyn, Seoul, Republic of Korea

Human embryonic stem cells (hESCs) promise to be used in cell therapeutic applications. For this reason, hESC culture system has been changing from conventional culture condition to xeno-free culture condition without the risk of transmitting non-human pathogens and non-human immunogenic molecules. Neu5Gc (N-Glycolylneuraminic acid), which is a widely expressed in mammalian cell and found on the cell surface, could be a useful indicator of xenogenic contaminations in hESC culture because human cells cannot produce genetically. We have set up the humanized culture condition using CELLstart™, human foreskin fibroblast and xeno-free medium containing knockOut™ SR XenoFree instead of animal-derived materials. SNUhES4 and H1, previously established in conventional culture condition, were maintained using xeno-free culture condition and performed the detections of Neu5Gc. For the early passages, hESCs in xeno-free culture condition were showed lower attachment and higher spontaneous differentiation rates. However, hESCs showed the same morphology and characters as that of conventional culture condition and were negative for Neu5Gc in humanized culture condition within 5 passages. This study indicated that clean-up of the previously established hESC lines using this humanized culture condition would take one step closer to clinical-grade hESCs. Efficient production of clinical-grade hESC lines could be more easily attainable for clinical application with this method. This research was supported by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (2012M3A9C6049722).

T-1117
IDENTIFICATION OF AN ANTI-INFLAMMATORY, NEURO-PROTECTIVE COMPOUND USING A HUMAN IN VITRO STEM CELL-BASED MODEL OF NEURO-INFLAMMATION.

Boyer, Leah[1], Tse, Chris[1], Kim, Yongsung[1], Crotti, Andrea[2], Paz, Jose[1], Benner, Chris[1], Montminy, Marc[1], Glass, Chris[2], Gage, Fred H.[1]
[1]Salk Institute for Biological Studies, La Jolla, CA, USA, [2]UC San Diego, La Jolla, CA, USA

Neurodegeneration has historically been considered a strictly neuronal disease; however evidence from clinical and animal models suggest a role for inflammation in its progression. It remains controversial whether glial activation, and the resulting inflammatory cascade, is a result or a cause of neuronal death. We have established cultures of primary human astrocytes and microglia to investigate the glial-derived inflammatory response to extracellular insults, and found that the glial derived pro-inflammatory response can be serially propagated between astrocytes and microglia following a single inflammatory insult. We have also used hESC-derived neural cultures to establish the neurotoxic response to a glial derived inflammatory cascade, allowing us to study the inflammatory contribution to the pathological development of neurodegeneration. By using this model as a platform for drug discovery we have identified a compound that is both anti-inflammatory and neuro-protective, partially through targeting both NFkB and CREB pathways. These results have been extended to murine models of inflammation and neurodegeneration. This human in vitro stem cell-based model represents an innovative strategy for investigating non-cell autonomous aspects of neurodegeneration, towards identifying novel approaches to therapeutic intervention.

T-1119
CLINICAL GRADE CULTURE MEDIUM FOR EXPANSION, LARGE-SCALE CULTURE AND GENOME ENGINEERING APPLICATIONS OF HUMAN PLURIPOTENT STEM CELLS

Ellerstrom, Catharina, Jenndahl, Lachmi, Malmberg, Helene, Nilsson, Tina, Aspegren, Anders, Karlsson, Camilla
Cellectis AB, Gothenburg, Sweden

Lack of robust methods for clinical expansion of human pluripotent stem (hPS) cells still hampers their clinical use. Here we report the development of a culture system for expansion of hPS cells that is totally free from human- or animal-derived components, chemically defined, containing only raw materials of clinical quality with traceable production processes. Cells expanded in regular T-flasks in monolayer in this culture system maintain a high expression of stem cell markers and lack expression of differentiation markers up to 30 serial passages, display long-term self-renewal potential, retained stem cells characteristics and differentiation potential. The culture system further supports the expansion of an array of hPS cell lines using a very robust and straightforward single-cell based culture protocol. Generation of clinically relevant quantities of hPS cells, ranging from 108 or beyond, is essential for their clinical use. In addition to the regular T-flask expansion described above, the culture medium also support large-scale non-adherent expansion of hPS cells in suspension culture. Genome engineering is a process that presents an additional challenge for hPS cells and robust and pampering culture conditions is required in order to succeed. Here we show data on how this xeno-free, defined and feeder-free culture system support hPS cells also during the genome engineering process. This system can thus facilitate the use of hPS cells for research as well as in large-scale clinical applications.

T-1120

GENERATION OF CAR-CONTAINING NK AND T CELLS FROM HUMAN EMBRYONIC STEM CELLS

Nguyen, Bao[1], Scripture-Adams, Deirdre[1], Lowe, Emily[1], Zhong, Nianxin[1], Chen, Hongying[1], Pannell, Katie[1], Kamata, Masakazu[1], Chen, Irvin[1], Zack, Jerome A.[2], **Galic, Zoran**[1]

[1]University Of California, Los Angeles, Los Angeles, CA, USA, [2]David Geffen School of Medicine at University Of California, Los Angeles, Los Angeles, CA, USA

Cell-based approaches utilizing hematopoietic stem cells (HSC) and T cells with naturally occurring or experimentally introduced genetic modifications that confer either HIV resistance or enhanced anti-HIV properties have emerged recently as a viable approach to treating HIV infection. However, a number of technical issues, such as a lack of protocols to maintain and expand HSC in vitro, inefficient gene transfer technologies, and the induction of T cell exhaustion due to extensive in vitro expansion prior to adoptive transfer have impeded the progress in this area. Human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC), which can be used to derive any type of immune cells including T and NK cells, may be a superior source of genetically modified cells compared with primary T cells or HSC. hESC and iPSC can be maintained in culture in an undifferentiated state indefinitely, and as such they can be extensively genetically manipulated, characterized for potential genotoxic events at the nucleotide level, and expanded to clinically relevant quantities. Given that these cells can be clonally expanded from a single cell, virtually all of the progeny of the genetically engineered hESC and iPSC will carry the same modification at same genomic position. Furthermore, as these cells can be effectively cryopreserved and differentiated into the desired lineage at different time points, the same cell populations can be used for preclinical studies and patient treatment. Importantly, in the case of lymphoid lineages, due to the extended length of their telomere ends, hESC/iPSC-derived T/NK cells are less likely than their normal counterparts to undergo cellular senescence and immunological exhaustion upon expansion. We recently generated hESC lines that express an anti-HIV CAR, which is a fusion molecule consisting of human CD4 with the signaling domain of the CD3 complex zeta-chain. CD4 binding to HIV-1 gp120 envelope on the surface of infected cells will trigger NK/T-cell recognition of infected cells and activation of their effector functions through zeta-chain signaling. Importantly, this vector also carries two anti HIV shRNA sequences that confer protection from HIV-1 infection to the transduced cells. Here we present the phenotypic and in vitro functional characterization of the hESC-derived, anti-HIV CAR-expressing T and NK cells.

T-1121

WHICH STEM CELLS ARE OPTIMAL FOR HUMAN REGENERATIVE CELL THERAPY

Hovatta, Outi[1], Rodin, Sergey[2], Antonsson, Liselotte[3], Tryggvason, Karl[2]

[1]Clinical Science, Intervention and technology, Karolinska Intitutet, Stockholm, Sweden, [2]Biochemistry and Biophysics, Karolinska Intitutet, Stockholm, Sweden, [3]CLINTEC, Karolinska Institutet, Stockholm, Sweden

Prospects for using stem cell derived adult/differentiated cells for cell therapy purposes are improving as many differentiation protocols are showing great promise. Many human SC types have been suggested as candidates: hES cells, iPS cells, mesenchymal stem cells, and very recently the "acid-bath cells". There are strict safety requirements for cells to be used for human cell therapy purposes. These include genetic stability, GMP quality, cultured in chemically defined human environments, ethical approval in the country in question and demonstrated functionality. hES cells: Fullfil principally all requirements. We have demonstrated that pluripotent hES cells can be derived clonally from genetically normal early human embryos without destroying the embryo under xeno-free and chemically defined conditions, which minimizes ethical conflicts. The hES cells are normal not genetically modified cells that can be expanded clonally under GMP conditions using cell suspension passaging and they can be derived and cultured for long periods while maintaining genetic stability. We have used the natural extracellular matrix secreted by human embryonic stem cells, laminin 521 combined with E-cadherin, all manufactured in human cell cultures, as the feeder-free matrix, and a chemically defined xeno-free culture medium. iPS cells: Pluripotent, but they are genetically modified with extra transcription factors which raises concerns about genetic stability and reproducibility. There are also concerns about possible tumorigenicity of such cells. Mesenchymal stem cells: Are not widely regarded as pluripotent but may be applicable for for certain purposes such as adipose and connective tissue and possibly bone and cartilage. STAP cells: Pluripotent stem cells generated via stimulus-triggered acquisition of pluripotency using exposure to low pH. A very recently described method to reprogram differentiated cells to stem cell like cells. Thus far it has been only used in a mouse system. There is no documentation about control of the reprogramming procedure or genetic stability, or if the method works for human cells. There are still significant concerns about the STAP method . Is is a too premature SC type to consider for human cell therapy. Conclusions: High quality hES cell lines are now easily available which questions the use of other SC types. Why even think of using reprogrammed adult type cells as the reprogramming procedure can generate mutations and insecurity factors? However, iPS cells provide very interesting model systems for other purposes such as drug testing in genetic disease.

GERMLINE CELLS

T-2001

HUMAN ENDOMETRIAL STEM CELLS ARE USEFUL FOR RESTORING OVARIAN FUNCTION IN A MOUSE MODEL OF PRIMARY OVARIAN FAILURE

Lai, Dongmei[1], Xiang, Charlie[2]

[1]The International Peace Maternity and Child Health Hospital, School of medicine, Shanghai Jiaotong University, Shanghai, China, Shanghai, China, [2]State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou China, Hangzhou, China

Cancer patients who receive chemotherapy or radiation often suffer reproductive damage, especially women younger than 40 years-of-age and this damage is frequently associated with premature ovarian failure (POF) and infertility. Recent clinical interest has emerged for the application of human endometrial stem cells (EnSCs) derived from menstrual blood to treat intractable diseases or traumatic tissue damage. To this end, specifically to investigate restoration of injured ovaries, we prepared a mouse model with damaged ovaries using busulfan and cyclophosphamide (B/C) treatment. The human EnSCs were derived from menstrual blood of a 40 year-old Chinese woman and was morphologically similar to fibroblast-like cells. The characteristics of mesenchymal stem cells were evidenced by phenotype assayed by flow cytometry and the ability to differentiate into adipocytes, chondroblasts and, osteoblasts in vitro. We then investigated the restorative effects of transplanted EnSCs on ovarian function. EnSCs were capable of improving total body weight and cyclicity. Importantly, human EnSC transplantation re-established

fertility in sterilized mice. In addition, the migration and localization of GFP-labeled EnSCs was investigated by live imaging and immunofluorescent methods. GFP-labeled cells were undetectable 48 h after cell transplantation, but were detected in and localized to the ovarian stroma where they transdifferentiated to granulose cells two months after transplantation. Recently germline stem cells (GSCs) have successfully been isolated from ovaries of neonatal and adult mice as well as from human ovaries, which challenged the viewpoint that the bank of ovarian oocytes is not renewed in postnatal female mammals. To identify and confirm whether EnSCs affect oogenesis in sterilized mice, GSCs were immunohistochemically quantified in mouse ovaries from different treatment groups. 5′-bromodeoxyuridine (BrdU) and mouse vasa homologue (MVH) protein double-positive cells were immunohistochemically detected in mouse ovaries, and the results showed that EnSC transplantation reduced depletion of the germline stem cells (GSCs) pool caused by chemotherapy. Thus, EnSCs, as autologous stem cells, may have a role in restoring damaged ovarian function and could be useful for regenerative medicine.

T-2002
DYNAMIC CHANGES OF METHYLATION PATTERNS IN IMPRINTING GENES DURING NEURONAL DIFFERENTIATION OF GERMLINE-DERIVED PLURIPOTENT STEM CELLS

Lee, Hye Jeong, Choi, Na Young, Ryu, Jae-Sung, Ko, Kinarm
Department of Stem Cell Biology, School of Medicine, Konkuk University, Seoul, Republic of Korea

Germline stem cells (GSCs), often called spermatogonial stem cells, are precursor cells in the testis to give rise to sperms. In our previous study, we reported that under defined culture conditions GSCs, unipotent stem cells, can self-induce pluripotency to be converted into germline-derived pluripotent stem cells (gPS cells). DNA methylation status of imprinting genes (*H19* and *Igf2r*) in GSCs showed an androgenetic pattern, which were still maintained even after conversion of GSCs into gPS cells. The androgenetic imprinting pattern in gPS cells is a particularly interesting result, as it suggests that gPS cells can represent a unique model system to study the role of paternal genomes in normal development and the contribution of imprinting in disease development. Therefore, studies on *in vitro* differentiation of gPS cells can provide insights into the paternal-specific contribution to specialized organ development. In this study, we used *in vitro* neuronal differentiation model to determine whether androgenones affects neuronal differentiation capacity of gPS cells. We differentiated gPS cells into neural stem cells (NSCs) and further differentiated the NSCs into to neuron and astrocytes, and compared them with embryonic stem cells (ESCs) and fetal brain derived NSCs. Analysis of molecular and cellular characterization revealed that gPS cells can be differentiated into neural lineages, which suggests that androgenetic imprinting status did not affect neuronal differentiation of gPS cells in *in vitro*. Additionally we investigated using bisulfite sequencing and pyrosequencing whether paternal imprinting genes (*H19* and *Ube3a*) and maternal imprinting genes (*Snrpn*, *Peg1/Mest*, *Ndn*, and *Impact*) are maintained or altered into biparental state when gPS cells are differentiated into neuronal cell types. We found that DNA methylation status of *H19*, *Snrpn*, *Peg1/Mest*, and *Impact* was androgenetic in gPS cells same as in GSCs, which was maintained even after differentiation of gPS cells into neuronal cells whereas fully unmethylated pattern of *Ndn* in GSCs was altered into partially methylated somatic patterns in gPS cells, which did not reverted into the androgenetic status in gPS cell-derived NSCs. Realtime PCR analysis showed that mRNA expression of H19, Peg1/Mest, Ube3a, Igf2r, and Igf2 in gPS cell-derived NSCs were similar to the levels of biparental NSCs, such as ESC-derived NSCs and fetal brain-derived NSCs. Notably, expression level of Impact and Snrpn were higher than those of biparental NSCs, which implies the correlations between expression levels of paternal imprinting genes and DNA methylation. Our study demonstrated dynamic changes of methylation patterns and expressions in imprinting genes during induction of pluripotency in GSCs and differentiation of gPS cells, suggesting that gPS cells can be useful model system to investigate imprinting-associated neurodevelopmental disorder or neuropsychiatric disease.

T-2003
UTF-1, A PLURIPOTENCY STEM CELL MARKER, IS EXPRESSED IN PORCINE SPERMATOGONIAL STEM CELLS

Lee, Wonyoung[1], Lee, Hoontaek[2], Song, Hyuk[1]
[1]Department of Food Bioscience, College of Biomedical and Health Science, Konkuk University, Chungju-si, Republic of Korea, [2]Department of Animal Biotechnology, Konkuk University, Seoul, Republic of Korea

Spermatogenesis is a continuous process starting with spermatogonial stem cells (SSCs), which has an ability to balance self-renewal and unipotency to generate haploid mature spermatozoa. Although the some of the markers of SSCs were investigated in rodent, species and each SSC developmental stage specific markers of SSC have not been identified. The objective of this study was to identify the expression of undifferentiated embryonic cell transcription factor1 (UTF1) as a marker for SSC in porcine testis and to characterize the UTF1 expressed testicular cells. In 5 day old porcine testis tissues, UTF1 was expressed in PGP9.5 positive spermatogonial stem cells, and 91.14% of PGP9.5 expressed cells showed UTF1 positive. However in 180 day old porcine testis tissues, expression of UTF1 was not identified or very weak in PGP9.5 positive spermatogonial stem cells. In vitro culture of porcine spermatogonial germ cells (pSGCs) from 5 day old testis revealed that expression of PGP9.5, UTF1, and NANOG was identified in the pSGC colonies. Relative levels of mRNA expressions of PGP9.5, NANOG and UTF1 among the pSGC colonies, testis tissues and muscles showed that the expressions of PGP9.5, NANOG and UTF1 were significantly high in the pSGC colonies. Consequently, UTF1 is expressed in PGP9.5 positive porcine spermatogonial germ cells in 5 day testis, and its expression was maintained in cultured pSGC colonies suggested that UTF1 could be a putative marker for early stage of porcine spermatogonia in neonatal testis. This finding will facilitate the study of spermatogenesis and application of germ cell research.

T-2004
ANALYSIS OF SHORT-TERM ADULT QUAIL COTURNIX COTURNIX TESTICULAR CELL CULTURE IN PREPARATION FOR XENOTRANSFER TO HOST CHICKEN GALLUS GALLUS EMBRYOS

Mannie, Chelsea[1], Roe, Mandi[1], Durrant, Barbara[2], Jensen, Thomas[3]
[1]California State University San Marcos, San Marcos, CA, USA, [2]San Diego Zoo Institute for Conservation Research, Escondido, CA, USA, [3]San Diego Zoo Institute. for Conservation Research, Escondido, CA, USA

As advanced reproductive technologies become routine in domestic animals, their application to the field of endangered species conservation is inevitable. For avian conservation, techniques such as in vitro fertilization and cloning are not viable methods for routine germplasm rescue. One technique that does show potential as a way to recover valuable germplasm is xenotransfer of the germline stem cells (spermatogonia Ad) from deceased donor gonads to chicken (Gallus gallus) host embryos. For this method to be practical, donor cells must be cultured for 2.5-3 days until chicken host embryos reach

developmental stages 14-17, when xenotransfer is possible. This project describes changes observed in quail (Coturnix coturnix) testes cells following 3-day culture in modified DMEM/F12. We used RT-qPCR to determine relative changes in expression of the germline specific genes Csf, C-Kit, Plzf, cVh and Scp3. The percent change in presumed germline stem cells was quantified by flow cytometry using anti- cVH, SSEA-1, and SSEA-3 antibodies. While relative Csf mRNA expression remained level, the C-Kit, Plzf, cVh and Scp3 mRNA expression decreased significantly following culture. The percent of cells expressing the cVH (1.92%, 3.47%, 0.72%, 0.60%) or SSEA-3 (1.29%, 2.99%, 1.32%, 1.12%) antigens both increased from fresh to day 1, but decreased by day 2 and day 3. There were no significant changes in the percent SSEA-1 positive cells (93.7%, 94.9%, 95.8%, 95.2%). Cell division as detected by EdU incorporation of suspended cells was minimal at <0.2%, while overall cell counts decreased from 2.00x106 cells/mL to 1.61x106 cells/mL. Previous research has demonstrated it is imperative for germline and Sertoli cells to be in contact during spermatogenesis. Suspension culture likely causes a loss of paracrine signaling between germline and Sertoli cells, resulting in mRNA expression changes of the C-Kit, Plzf, cVh and Scp3 genes. Csf expression may rely on either internal cell signaling or the growth factors provided in culture. Theoretically, each avian spermatogonia Ad stem cell produces 32 spermatids, yielding a total of 64 cells per spermatogonia Ad. Therefore, the theoretical minimum percent of total germline cell population of spermatogonia A is 1.6%, and the minimum percent of spermatogonia A and B is 12%. We propose the increase of cVH and SSEA-3 positive cells during culture is due to de-differentiation of spermatogonia B to spermatogonia A by day 1, followed by loss of these germline stem cells. The SSEA-1 antigen appears to be detected on all germline cells. These results indicate this culture system is not a practical method to sustain adult germline stem cells from deceased adult birds for the three days until host embryos reach stages 14-17. However, the data do suggest that 24-hour culture of donor cells in these conditions is sufficient or even beneficial prior to transfer.

T-2005
SURFACE MARKER DOLICHOS BIFLORUS AGGLUTININ (DBA) IS NOT SUITABLE FOR ENRICHING BOVINE TYPE A SPERMATOGONIA BY CELL SORTING TECHNIQUES

McFarlane, James[1], Xie, Jianshan[2], Fan, Ruiwen[2], Dong, Changsheng[2], Herrid, Muren[1]
[1]Centre for Bioactive Discovery in Health and Ageing, University of New England, Armidale, Australia, [2]College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu, China

The identification and purification of type A spermatogonia represent the first two steps required to enhance the success of germ cell transplantation or establishment of long term culture in vitro. However, the identification and enrichment of spermatogonial stem cells (SSCs) has been difficult due to the lack of specific markers and the small proportion of these cells in the testis [1]. A plant lectin - Dolichos Biflorus Agglutinin (DBA), which bind to terminal N-acetylgalactosamine residues, specifically binds to gonocytes or type A spermatogonia in livestock species, including cattle [2]. Therefore, in the present study, we used enriched type A spermatogonia to determine the suitability of DBA lectin in labeling type A spermatogonia for magnetic-activated cell sorting (MACS) or fluorescent-activated cell sorting (FACS). Firstly, enriched type A spermatogonia population were cultured with different concentrations of DBA-FITC (0, 100, 200 and 300 μg/ml) at 34°C. Duplicate samples from each culture were stained using a Live/Dead kit and cell viability of the whole population and DBA-FITC positive cells were assessed by FACS at 3, 24 and 48 hours after culture. DBA-FITC had a concentration dependent effect on cell viability with more dead cells in the DBA-FITC positive population than the controls. The higher the concentration the lower the viability over the time. In a separate experiment, 10×106 cells from single cell suspensions were incubated with or without 200 μg/ml DBA-FITC in 1 ml MACS buffer on ice for 15 min with gentle agitation. The cells were isolated on the columns according to the manufacture's recommendation (Milteny Biotech, Bergisch Gladbach, Germany). Using triplicate samples from each culture, cell viabilities of DBA-FITC positive and negative fractions were assessed by FACS at 3, 24 and 48 hours after cultures. MACS isolation resulted in a significant increase in DBA positive cells from 13.9% of input cells to 49.1% in the positively selected fractions. The cell viabilities in both the positive or negative fraction was unaffected at 3 hours, the viability of the positive fractions were significantly lower than that of the controls at 24 (40%) and 48 (13%) hours of culture compared to the controls (79% and 58%). In summary, DBA lectin specifically binds to bovine type A spermatogonia and can be used to enrich these cells but unfortunately exhibits a highly toxic effect. Therefore, surface marker DBA is not suitable for enriching bovine type A spermatogonia for cell culture in vitro by cell sorting techniques, such as MACS and FACS.

T-2006
PROPAGATION OF SPERMATOGONIA STEM CELLS OF AZOOSPERMIC PATIENT USING HUMAN EMBRYONIC STEM CELL MEDIUM

Md Isa, Muhammad Lokman Sal, Abdul Wahab, Azantee Yazmie
Basic Medical Sciences, International Islamic University Malaysia, Kuantan, Pahang, Malaysia

Azoospermia is found in about 1% of all men and 10% to 15% of infertile men. Obstructive azoospermia is considered one of the most favourable prognostic conditions for male infertility since spermatogenesis is not disrupted, unlike in non-obstructive azoospermia. Spermatogonial stem cells (SSCs) are the basis of male reproduction and spermatogenesis. This study is carried out to detect and tried to propagate the presence of SSCs in azoospermic patient by using immunofluorescent staining of CD29 (integrin beta-1) and CD49f (integrin alpha-6) cell surface specific protein markers to ensure either the patient is completely absence or harvest small number of cells. But characterization and identification of human SSCs in are very limited and poorly understood. Therefore VASA expression is also attempted to be detected using conventional PCR method. The isolated azoospermic spermatogonial stem cell is proliferated in human embryonic stem cell (HESC) medium over 21 days. The idea of using HESC is by understanding that this medium is able to maintain the undifferentiated stage of embryonic stem cells. The results showed significantly increase the number of SSCs after 21 day of culture with relative of VASA expression detected. This approach method should be streamed down in order to establish standard procedure of propagating SSCs in azoospermic patient and soon potentially to differentiate these cells in producing mature spermatozoa for in vitro fertilization. Eventually the findings may give new hope for infertile couple for having an offspring.

T-2007
IDENTIFICATION AND CHARACTERIZATION OF OCT4-EGFP EXPRESSING CELLS IN TRANSGENIC PIG TESTIS

Nowak-Imialek, Monika[1], Lachmann, Nico[2], Herrmann, Doris[1], Jacob, Franziska[1], Niemann, Heiner[1]
[1]Biotechnology, Friedrich-Loeffler-Institut, Mariensee, Neustadt, Germany, [2]Hannover Medical School, Hannover, Germany

We have produced the first germ line transgenic pigs carrying an 18

kb genomic sequence of the murine Oct4 gene fused to the enhanced green fluorescent protein (EGFP) cDNA (OG2 construct) in order to allow the identification of pluripotent cells by monitoring Oct4 expression by GFP fluorescence (Nowak-Imialek et al., 2011, Stem Cells and Development). Expression of the GFP reporter construct was confined to the inner cell mass and trophectoderm of blastocysts, germ line cells, and testicular germ cells. In the adult Oct4-EGFP reporter mouse early Type A Spermatogonia are the only adult stem cells that express Oct4 gene. Spermatogonial stem cells (SSCs) are unipotent adult stem cells that have the potential for both self-renewal and the production of functional spermatozoa. In pig, very limited information about SSCs is available and the isolation of the true SSCs from the complex cell population of the testis is still unknown. We used Oct4-EGFP transgenic pigs as model to isolate and characterize Oct4-EGFP expressing cells in the adult porcine testes. Fluorescence microscopy of testicular tissue isolated from transgenic piglets revealed minimum numbers of GFP positive cells, whereas testicular tissue isolated from adult transgenic boars contained a high amount of GFP fluorescent cells. Northern blot analysis confirmed stronger GFP expression in the testis of adult transgenic pigs than in the testis from transgenic piglets. Time course and the signal intensity of GFP expression in Oct4-EGFP testis paralleled mRNA expression of the endogenous Oct4 gene. Here, we used adult Oct4-EGFP transgenic pigs as model for fluorescence-activated cell sorting (FACS) based isolation of GFP expressing cells from testis. To obtain a single-cell suspension, the testes were enzymatically dissociated using two digestion steps. Thereafter, FACS based on GFP expression was successfully used to purify specific testicular cell populations. Two cell populations, i.e. GFP+ (14%) and GFP- (45%) could be isolated. Subsequently, qPCR analyses were performed on GFP+, GFP- and on unsorted cell populations using marker genes specific for pluripotency and on undifferentiated germ cells (OCT4, PGP9.5, UTF1, FGFR3, GFRα1, SALL4, THY-1), meiosis (BOLL), spermatids (PRM2) and somatic cells (VIM, LHCGR). All of the genes, including OCT4, PGP9.5, UTF1, FGFR3, GFRα1, SALL4 and THY-1 were strongly expressed in the GFP positive population. Vimentin, which is mainly expressed in Sertoli cells and LHCGR, which is mainly expressed in Leydig cells, were expressed in unsorted and GFP- cell populations and at very low level in GFP+ cells. Moreover, higher expression of the PRM2 marker was detected also in GFP+ cell population indicating that these cells contain also differentiating spermatids. To explore the characteristics of the Oct4-EGFP expressing cells in detail, localization in the porcine testis sections and analysis of co-expression with germ cell markers using immunohistochemistry is currently underway.

T-2008
GAP JUNCTION-MEDIATED SIGNALLING REGULATES PROLIFERATION AND DIFFERENTIATION OF SOMATIC CYST STEM CELLS IN THE DROSOPHILA TESTIS

Smendziuk, Chris, Islam, Fayeza, Messenberg, Anat, Tanentzapf, Guy
University of British Columbia, Vancouver, BC, Canada

Gametogenesis is a conserved process in animals that requires intricate signalling between germ cells, which will give rise to sperm or eggs, and somatic cells, which support germline development. A key feature of gametogenesis is the involvement of two types of specialized stem cells that give rise to soma and to the germline. Previous work in *Drosophila* has illustrated that soma-germline interactions control stem cell behaviour. Failure to achieve proper regulation of germline-soma communication within the stem cell niche or during spermatogenesis can result in infertility or the formation of tumours. Flies containing mutations in the gene *zero population growth/innexin4* (*zpg*) are sterile and possess tiny gonads. *zpg* has been shown to code for an innexin, a gap junction protein. Previous studies indicate that Zpg functions in the germline to regulate germ cell function but the precise role of Zpg has not yet been elucidated. The objective of this study is to characterize the role of gap junctions in regulating stem cell proliferation and differentiation during spermatogenesis. We are investigating the function of innexin in the fly testis, using mutational analysis and RNAi-mediated knockdown in somatic cells. Additionally, we are using a structure-function approach to investigate the role of key domains/residues of Zpg for mediating soma-germline interactions, Our preliminary data support the idea that Zpg mediates communication from the germline to the soma. We have uncovered previously uncharacterized defects in the soma of *zpg* mutants, including overproliferation and delayed differentiation of somatic cyst stem cells. In addition, we have disrupted the function of each fly innexin in early somatic cells and identified Innexin2 (Inx2) as a candidate for mediating gap junction signalling in the soma. Furthermore, we have identified defects in somatic stem cell proliferation upon disruption of Inx2. Our work demonstrates a role for Zpg in the germline for regulating somatic cell behaviour and provides evidence that Zpg works with Inx2 in the soma to mediate soma-germline interactions during spermatogenesis. Altogether, our study provides mechanistic insight into the regulation of somatic stem cell proliferation and differentiation in the fly testis, via signals from the germline.

TOTIPOTENT / EARLY EMBRYO CELLS

T-2009
COMPARATIVE PCR ARRAY ANALYSIS OF RAT PLURIPOTENT AND ADULT STEM CELLS

Rajanahalli, Pavan[1], He, Hong[1], Smith, J. Robert[1], Binas, Bert[2], Verfaillie, Catherine[3], Weiss, Mark[1]
[1]Anatomy and Physiology, Kansas State University, Manhattan, KS, USA, [2]Division of Molecular and Life Science, College of Science and Technology, Hanyang University, Seoul, Republic of Korea, [3]Department of Development and Regeneration, Stem Cell Biology and Embryology, KU Leuven, Leuven, Belgium

Recent developments in the isolation of new cell lines during early development in rats have demanded their characterization, function and identifying potential expression markers in relation to established pluripotent stem cell lines. By isolating and revealing the characteristics of these cell lines in detail, they can essentially be used as an alternate model system for drug development studies and therapeutic applications. We have previously shown that genuine rat embryonic stem cell (rESCs) lines can be screened and distinguished from other cell lines including trophoblast stem (TS) cells, differentiated ES cells and extraembryonic endoderm (Xen) cells using a customized PCR microarray. By applying unbiased sorting via principle component analysis (PCA), and clustering analysis algorithms with more than 10 fold differences in gene expression using Qlucore bioinformatics software, we found that rat multipotent adult progenitor cells (MAPCs) and rat hypoblast stem cells (HypoSCs) provided by Drs. Catherine Verfaillie and Bert Binas share a close similarity in gene expression compared to Xen cells, extraembryonic endoderm precursor (Xen-P) cells and epiblast stem cells (EpiSCs). Undifferentiated rESCs and rat induced pluripotent stem cells (riPSCs) are more similar to EpiSCs. Recent studies have shown high gene expression similarities between MAPCs, HypoSCs, Xen cells and Xen-P cells. Our array analysis found that a small set of markers are different between these cell lines. MAPCs were positive for Gata6 Gata4, Sox17, Sox7 and Foxa2 (which are positive markers for mouse MAPCs) and negative for Nanog

and Sox2. Certain lines of MAPCs do express Oct4, but the lines we received had lower expression of Oct4 that rESCs (<10 fold change). HypoSCs shared a similar expression pattern to MAPCs and can be distingushed from ESCs. Xen cells and Xen-P cells also express Gata4, Gata6 and Sox17. EpiSCs can be differentiated from rESCs by Cer-1, Afp and Gata6 expression. HypoSCs express Pitx2 and MAPCs express very low levels of Pitx2. We found that Tcl1a and Fgf2 are expressed in MAPCs (>10 fold) than HypoSCs. MAPCs and Xen cells can be differentiated by Klf4 expression detected only in MAPCs. Gdf3, Cdx2, Ascl1 and RGD1563046 (predicted to be similar to Cerberus-like) expressed by Xen-P cells can differentiate them from MAPCs. The array also detected increased expression for Sox2, Nanog, Fgf4, GDF3, Foxd3, Ecat1 and Kdr by EpiSCs compared to MAPCs. Identifying the right culture conditions can further delineate the differences in gene expression profiles between the closely related cell lines MAPCs, HypoSCs, Xen cells, Xen-P cells and EpiSCs. Our custom array has taken us a step further in the advancement of gene expression changes that occur between pluripotent stem cells and adult stem cells.

T-2010
MAINTENANCE OF PLURIPOTENCY CONTROLLED BY KLF5

Azami, Takuya[1], Matsumoto, Ken[2], Jeon, Hyojung[1], Takahashi, Satoru[1], Ema, Masatsugu[2]
[1]Department of Anatomy and Embryology, University of Tsukuba, Tsukuba, Japan, [2]Shiga University of Medical Science, Research Center for Animal Life Science, Otsu, Japan

Pluripotency is maintained by the core transcription factors, such as Oct4, Sox2, and Nanog. These transcription factors also have important functions in embryonic development and can reprogram somatic cells into iPS cells. Among the Yamanaka factors, Klf4 is dispensable for ES cells self-renewal and early embryo development. Klf4 together with Klf2 and Klf5 play redundant functions in ES cells. However, our previous study clearly indicated that Klf5 is indispensible for blastocyst development (Ema et al., 2008). Therefore, it is important to elucidate the molecular mechanism underlying these functions. To understand the molecular mechanism, we amplified the cDNA from WT and Klf5 KO embryos and performed the microarray analysis. Then we found that FGFR signaling pathway related genes was hyper-activated in Klf5 KO embryos. Pharmacological inhibition for this pathway by specific inhibitor resulted in significant rescue for Klf5 KO phenotypes. Interestingly, Nanog positive pluripotent cells were emerged in ICM of Klf5 KO embryos after pharmacological inhibiton of FGFR signaling pathway. We examined whether Klf5 KO ES cells are derived from rescued blastocyst. We obtained 60 ES cell lines from 92 embryos, and remarkably, 7 lines of Klf5 KO ES cell were derived from rescued embryos. These results clearly show that FGFR signaling pathway is hyper-activated in Klf5 KO embryos. Since our previous study revealed that Klf5 KO ES cells showed upregulation of Fgf5 expression, we examined whether FGFR signaling pathway is hyper-activated in Klf5 KO ES cells. Compared to WT ES cells, Klf5 KO ES cells exhibited elevated levels of the phosphorylation of ERK1/2. Moreover, when Klf5 KO ES cells were cultured in the presence of Mek inhibitor or FgfR inhibitor, defective proliferation was partially, but significantly rescued. Additionally, it is reported that Klf2 and Klf4 can reprogram EpiSCs into naïve ES Cells state in 2i/LIF condition. We also demonstrate that Klf5 also have a EpiSCs reprogramming potential even in CHIRON/LIF, without MEK inhibitor condition. These results suggest that Klf5 suppresses FGFR signaling pathway in the embryo, ES cells and EpiSCs reprogramming processes, and act as a safeguard for the maintenance of pluripotent stem cells.

T-2011
LINEAGE TRACING DEMONSTRATES THE EXISTENCE OF MULTI-FATED NEURAL CREST CELLS IN THE MOUSE EMBRYO

Baggiolini, Arianna[1], Varum, Sandra[1], Mateos, José María[2], Bettosini, Damiano[1], John, Nessy[1], Joyner, Alexandra L.[3], Ziegler, Urs[2], Goetz, Magdalena[4], Clevers, Hans C.[5], Furrer, Reinhard[6], Sommer, Lukas[1]
[1]Institute of Anatomy, University of Zürich, Zürich, Switzerland, [2]Center for Microscopy and Image Analysis, University of Zürich, Zürich, Switzerland, [3]Memorial Sloan-Kettering Cancer Center, New York, NY, USA, [4]Institute of Stem Cell Research, Helmholtz Zentrum Muenchen, Neuherberg/Munich, Germany, [5]Hubrecht Institute, Utrecht, Netherlands, [6]Institute of Mathematics, University of Zürich, Zürich, Switzerland

Neural crest (NC) is an embryonic cell population that arises between the neural plate border and the non-neuronal ectoderm. During vertebrate development NC cells undergo an epithelial to mesenchymal transition (EMT), migrate extensively throughout the embryo, and segregate into distinct cell fates. Whether mouse trunk NC cells are multi-fated or are already committed to specific cell fates at the pre-migratory stage is still highly controversial. We address this problem by performing in vivo fate mapping of NC cells both at the pre-migratory and migratory stages using the R26R-Confetti mouse model. We combine quantitative analysis with mathematical modeling to show that NC population consists of few fate-restricted cells and a majority of multi-fated cells both at the pre-migratory stage and, most intriguingly, also during migration. Our work is the first to use a transgenic mouse model to demonstrate the existence of multi-fated NC cells and suggests that NC displays the broadest developmental potential besides the inner cell mass.

T-2012
COMPARATIVE PROFILING OF RODENT AND PRIMATE PREIMPLANTATION EMBRYOS REVEALS DIFFERENCES IN SIGNALLING PATHWAY SUSCEPTIBILITY

Boroviak, Thorsten[1], Loos, Remco[2], Behr, Ruediger[3], Sasaki, Erika[4], Nichols, Jennifer[1], Smith, Austin G.[1], Bertone, Paul[2]
[1]Wellcome Trust - Medical Research Council Cambridge Stem Cell Institute, Cambridge, United Kingdom, [2]European Molecular Biology Laboratory, European Bioinformatics Institute, Cambridge, United Kingdom, [3]German Primate Centre, Goettingen, Germany, [4]Central Institute for Experimental Animals Animal Experimentation Center, Kanagawa, Japan

Current primate embryonic stem cells (ESCs), including human ESC, are postulated to represent a primed state of pluripotency, which is associated with biased differentiation capacity, compromised clonogenicity and lack of chimeric contribution. It is unclear if some of these differences are due to species-specific characteristics of the preimplantation embryo or result from suboptimal culture conditions. The establishment of a new type of primate ESC in an unrestricted, naïve pluripotent state will strongly depend on better knowledge of the signalling pathways regulating early primate development and pluripotency. To identify potential differences in transcription factor expression and pathway activities, we performed whole transcriptome analysis of individual mouse and marmoset monkey embryos at various preimplantation stages. Importantly, the use of a non-human primate model system allowed us access to unperturbed, in vivo conceived embryos. Furthermore, if the results from this study can be utilised to derive novel, potentially more naïve-like marmoset ESCs in the future, their ability to re-enter embryonic development could be tested by blastocyst injection. Individual morulae as well as individual early,

mid- and late inner cell masses of mouse and marmoset blastocysts were profiled using single cell preamplification techniques followed by next generation sequencing (RNA-seq). Increasing the starting amount from one cell to a small pool of cells (10-20 cells) allowed us to perform lineage specific transcriptome profiling from single embryos at a resolution comparable to conventional RNA-seq. At the early blastocyst stage core pluripotency factors such as Nanog, Pou5f1, Sox2 were expressed in both mouse and marmoset. The mouse naïve pluripotency markers Klf4, Klf5, Tbx3, Tfcp2l1, Dppa3 (Stella) and Pecam1 were found to be expressed in marmoset, with the exception of Klf2, Zfp42 (Rex1) and Gbx2, suggesting differences in the wider primate pluripotency transcription factor network. Primitive endoderm specification occurred later in the marmoset, but was correlating with the same set of transcription factors (GATA6, PDGFRA, SOX17, GATA4), known to be important in mouse. Segregation of these factors towards the primitive endoderm lineage at the late blastocyst stage was confirmed by immunofluorescence staining. Detailed pathway analysis showed an upregulation of receptors in marmoset for Erbb, Bmp, Activin-Nodal-Tgfb and Fgf signalling pathway, which may render primate ESC more susceptible to extracellular signalling activity, specification and exit from pluripotency. FGF4 and BMP4 were absent in the early ICM in primates, but upregulated at the late blastocyst stage, together with primitive endoderm markers. Finally, we found metabolic differences including an increase in lysine biosynthesis and differential expression of genes required for glycolysis. The ability of mouse, but not primate ESCs to metabolise threonine, is reflected by high expression of threonine dehydrogenase in mouse, but not marmoset ICMs. In conclusions our dataset provides an extensive resource to compare preimplantation development between mouse and a non-human primate. While the majority of pluripotency factors are expressed in both species, we uncover a substantial increase of signalling receptor expression in the marmoset for a wide range of signalling pathways.

EMBRYONIC STEM CELL DIFFERENTIATION

T-2014
SCL/TAL1 ENHANCES MEGAKARYOCYTIC SPECIFICATION OF HUMAN EMBRYONIC STEM CELLS BY ACTIVATING A MEGAKARYOCYTE-SPECIFIC TRANSCRIPTIONAL NETWORK

Toscano, Miguel G.[1], Navarro-Montero, Oscar[2], Ayllon, Veronica[2], Ramos-Mejia, Veronica[2], Bueno, Clara[3], **Laricchia-Robbio, Leopoldo**[4], Romero, Tamara[2], Cobo, Marien[2], Martin, Francisco[2], Menendez, Pablo[3], Real, Pedro J.[2]

[1]Amarna Therapeutics SL., Sevilla, Spain, [2]GENYO. Centre for Genomics and Oncological Research: Pfizer-University of Granada-Andalusian Regional Government, Granada, Spain, [3]Josep Carreras Leukemia Research Institute, Barcelona, Spain, [4]Andalusian Initiative for Advanced Therapies, Sevilla, Spain

Human embryonic stem cells (hESCs) are a unique in vitro model for studying human developmental biology and may represent a potential source for cell replacement strategies. Platelets can be generated from cord blood progenitors and hESCs; however, the molecular mechanisms and determinants controlling the in vitro megakaryocytic specification of hESCs remain elusive. We have recently shown that SCL overexpression accelerates the emergence of hemato-endothelial progenitors from hESCs and promotes their subsequent differentiation into blood cells with higher clonogenic potential. Given that SCL participates in megakaryocytic commitment, we hypothesized that it may potentiate megakaryocytic commitment in hESCs. Here, we show that ectopic SCL expression enhances the emergence of megakaryocytic precursors, mature megakaryocytes and platelets in vitro. SCL-overexpressing megakaryocytic cells and their derived platelets respond to different activating stimuli, similarly to their control counterparts. Gene expression profiling of megakaryocytic precursors showed that SCL-overexpression renders a megakaryopoietic molecular signature. The Connectivity Map analysis revealed that trichostatin A (TSA), which is ahistone deacetylase (HDAC) inhibitor, functionally mimicked the SCL overexpression-induced effects. Finally, we confirmed that TSA treatment of hESCs promotes the emergence of megakaryocytic precursors. We demonstrated that SCL gain-of-function and HDAC inhibitors are regulators of hESC-derived megakaryocytic cells

T-2015
IN VITRO TITRATION OF WNT/BETA-CATENIN SIGNALING ENABLES EFFICIENT FATE SPECIFICATION OF LATERAL PLATE MESODERM DERIVATIVES

Palpant, Nathan[1], Pabon, Lil[1], Roberts, Meredith[1], Hadland, Brandon[1], Jones, Daniel[1], Ruzzo, Walter[1], Bernstein, Irwin[1], Zheng, Ying[1], Murry, Charles[2]

[1]University of Washington, Seattle, WA, USA, [2]University of Washington - Center for Cardiovascular Biology, Seattle, WA, USA

During development, dosage of morphogens like Wnt/β-catenin, Activin/nodal and BMPs are critical for defining the patterning of lateral plate mesoderm and specifying down-stream derivatives including cardiomyocyte and endothelial fates. Based on these embryological data, we used Activin A and BMP4 titration to direct hESC-derived mesoderm as a basis for generating highly enriched definitive populations including cardiomyocytes, endothelial cells, and blood derivatives. During the gastrulation phase of differentiation (day 2), we found that Wnt/β-catenin signaling is low in cells initiated under 100 ng/mL Activin A and 5 ng/mL BMP4 (A100/B5) compared A50/B40 conditions. Similarly mesendoderm markers like Nodal and Goosecoid were higher in A100/B5 compared A50/B40 conditions whereas genes involved in posterior lateral plate mesoderm such as CDX1 were higher in A50/B40 compared to A100/B5. When cells were further differentiated under cardiogenic conditions, cardiomyocytes developed robustly from cardiac progenitor cells (CPCs) differentiated under conditions of A100/B5 (90% cTnT+) compared to A50/B40 (14% cTnT+). Using the same Activin A/BMP4 tritration in combination with conditions permissive to endothelial development, we found that endothelial cells (ECs) can be generated with equal efficiency (90% KDR+/CD34+) from A50/B40 as well as A100/B5 conditions. We matured ECs to determine whether they had the capacity to generate mature endothelial cells. We found that both populations of differentiated ECs formed greater than 90% CD31+ endothelial cells without sorting and expressed mature endothelial markers such as von Willebrand Factor. Interestingly, when placed in type I collagen in the absence of any supporting stromal cell, A100/B5-derived endothelial cells showed significant sprouting angiogenesis compared to A50/B40-derived endothelial cells. Since endothelium can have hemogenic potential in the developing embryo, we tested the hematopoietic competence of hESC-derived ECs using a methylcellulose CFU assay. We found that A50/B40-EC hematopoietic derivatives predominantly gave rise to macrophage and granulocyte/macrophage mixed colonies, and few primitive erythroid colonies. In contrast, the A100/B50-ECs generated cells gave rise to mostly primitive erythroid and macrophage colonies. These data showed that multiple types of functional hemogenic endothelium can be generated from hESCs. We tested that hypothesis that hemogenic endothelium could be re-directed into the cardiac lineage by manipulating Wnt/β-catenin signaling. Interestingly,

inhibiting Wnt/β-catenin signaling in A100/B50-ECs activated the cardiac gene program resulting in a fate switch to cardiomyocyte development with greater than 90% efficiency. The global transcriptional profile of A100/B50-ECs was indistinguishable from A100/B50-CPC derived cardiomyocytes by RNA-seq analysis. However, A50/B40-ECs failed to generate cardiomyocytes under the same conditions. On the basis gene expression, signaling activities, and derivation of lineage specific definitive cell types, we have concluded that subtle titrations of Activin A and BMP4 are sufficient to pattern an anterior to posterior polarization of hESCs that recapitulates normal events during primitive streak formation and gastrulation. Furthermore, we have revealed a novel mechanism by which to manipulate endothelial progenitor cell subtypes into the cardiac lineage.

T-2016
GASZ PROMOTES GERM CELL DERIVATION FROM EMBRYONIC STEM CELLS BY INTERACTING WITH DAZL

Wang, Yuan
East China Normal University, Shanghai, China

Primordial germ cells (PGCs) are the first germ-line population that forms from the proximal epiblast of the developing embryo. Despite their biological importance, the regulatory networks whereby PGCs arise, migrate, and differentiate into gametes during embryonic development remains elusive, largely due to the limited number of germ cells in the early embryo. To elucidate the molecular mechanisms that govern early germ cell development, we utilized an in vitro differentiation model of embryonic stem cells (ESCs) and screened a series of candidate genes with specific expression in the adult reproductive organs. We discovered that gain of function of Gasz, a gene previously reported to participate in meiosis of postnatal spermatocytes, led to the most robust upregulation of PGC formation from both human and murine ESCs. In contrast, Gasz deficiency resulted in pronounced reduction of germ cells during ESC differentiation and decreased expression of MVH and DAZL in genital ridges during early embryonic development. Further analyses demonstrated that GASZ interacted with DAZL, a key germ cell regulator, to synergistically promote germ cell derivation and modulate target RNA stability from differentiated ESCs. Thus, our data reveal a potential role of GASZ during embryonic germ cell development and provide a powerful in vitro system for dissecting the molecular pathways in early germ cell formation during embryogenesis.

T-2017
TRANSCRIPTION FACTOR CUX2 IN EMBRYONIC STEM CELLS

Abdelhady, Shaimaa, Ahmed, Najla, Kele Olovsson, Julianna, Andäng, Michael
Karolinska Institute, Stockholm, Sweden

Self-renewal implies the ability to proliferate continuously in an undifferentiated state while maintaining pluripotency. Embryonic stem cells (ESCs), derived from the inner cell mass of the blastocyst, proliferate rapidly without a mitogen controlled G1 restriction point and have capacity to self-renew indefinitely and suppress differentiation. Although proliferative capacity is a key component of self-renewal, little is known about the mechanisms that regulate stem cell proliferation. In 2008, Andäng et al. described a novel mechanism whereby proliferation is controlled in embryonic stem cells and fetal stem cells via an S-phase checkpoint pathway in response to ion channel activity. An auto-/paracrine ligand gated ion channel, the GABAA receptor, activates Cl- ion currents, causing membrane hyperpolarization and cell volume increase. The same mechanism has been studied and confirmed in adult neural stem cells (NSCs) in vivo. However, the regulation of expression of components of a GABA signaling circuit, i.e. GABAAR subunits, glutamic acid decarboxylase 1 (Gad1/Gad67) and Slc32a1 (the vesicular GABA transporter) in ESCs and NSCs has not been characterized. Interestingly the transcription factor cut-like homeobox 2 (Cux2) is expressed in ESCs. Cux2 has been shown to regulate cell cycle progression and development of neural progenitor cells. By binding both Neurod and p27(Kip1) promotors, Cux2 influences cell cycle progression as well as cell cycle exit and cell fate determination. Our preliminary results with siRNA knockdown of Cux2 in ESCs indicate that there is a correlation between expression of Cux2 and the β3-subunit of the GABA-A receptor. To identify potential target genes for Cux2 in ESCs as well as effects on the cell cycle, we are establishing ESC lines from homozygous Cux2 knockout mice.

T-2018
DERIVATION, DIFFERENTIATION AND CHARACTERIZATION OF HUMAN AND MOUSE EMBRYONIC STEM CELL DERIVED GABAERGIC INTERNEURON PROGENITORS

Anderson, Nickesha C.[1], Chen, Christopher Y.[1], Moakley, Daniel F.[1], Henderson, Katharine W.[1], Plocik, Alex[2], Graveley, Brenton[2], Naegele, Janice[1], Grabel, Laura[1]
[1]Biology, Wesleyan University, Middletown, CT, USA, [2]Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT, USA

The selective loss of GABAergic inhibitory interneurons is characteristic of numerous neurodegenerative diseases. Absence of these inhibitory subtypes creates an electrical imbalance in the hippocampal and cortical neural circuits. Our long term goal is to replenish these inhibitory interneuron subtypes using an embryonic stem cell (ESC) source. During embryonic development, these inhibitory interneuron progenitors arise from a transient ventral forebrain structure known as the medial ganglionic eminence (MGE) and are characterized by the expression of Nkx2.1. We have optimized an adherent monolayer protocol for the generation of Nkx2.1+ neural progenitors from both mouse and human ESC lines using sonic hedgehog treatment. The Nkx2.1+ enriched cell population expresses elevated levels of MGE markers, including Nkx2.1 and Nkx6.2, based upon qRT-PCR analysis. Transcriptome analysis using high throughput mRNA sequencing is underway to further characterize the Nkx2.1+ cell population. To test the differentiation potential of the Nkx2.1+ cells in vitro, we used co-culture with mouse cortical astrocytes and obtained an enriched population of interneurons in which 75% of the MAP2+ cells are also GABA+ after 8 weeks. Preliminary studies examining the fate of human ESC-derived Nkx2.1+ progenitors transplanted into the mouse hippocampus demonstrate the expression of neuronal markers 3 weeks post-transplant

T-2019
METABOLIC REGULATION OF HUMAN NEURAL STEM CELL DIFFERENTIATION THROUGH O-GLCNACYLATION

Andres, Lissette, Blong, Ian, Yamaguchi, Teppei, Thompson, Pamela, Bertozzi, Carolyn
University of California at Berkeley, Berkeley, CA, USA

Stem cells maintain a balance of self-renewal and differentiation programs through the activation and repression of specific developmental genes. Recent evidence suggests that this regulation is modulated, in part, via the post-translational modification known as *O*-GlcNAcylation. This modification involves the addition of a single monosaccharide (GlcNAc), onto serine and threonine residues of

nuclear and cytoplasmic proteins. *O*-GlcNAcylation has been shown to cross talk extensively with phosphorylation. Additionally, the enzymes responsible for the addition and removal of *O*-GlcNAc, *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA), respectively, have been shown to target key transcriptional and epigenetic regulators, and they are both abundantly expressed in the brain. Furthermore, perturbations to the regulation of *O*-GlcNAc are associated with the etiology of a number of neurodegenerative diseases. However, little is known about how perturbations in *O*-GlcNAcylation affect human embryonic stem cell (hESC) differentiation. Here, we used a chemical inhibitor of OGT to determine the effects of perturbing *O*-GlcNAcylation during hESC neural differentiation. By treating neural stem cells (NSCs) with an inhibitor of *O*-GlcNAcylation (Ac-5SGlcNAc), we observed a dramatic decrease in global levels of *O*-GlcNAc, as expected. Additionally, upon inhibition of OGT, we observed that NSCs gained a morphology reminiscent of mature neurons, and acquired the neuronal markers beta-III tubulin and MAP2. We performed RNA-seq analysis of NSCs treated with OGT inhibitor and found that genes involved in neuronal differentiation and axonal guidance signaling were significantly up-regulated in OGT-inhibited cells. Among the most down-regulated genes were PPP1R3C, a protein phosphatase, and FGF23, which is involved in regulating phosphate concentrations. The mechanism by which OGT inhibition induces neuronal differentiation was analyzed using the Ingenuity Pathway Analysis (IPA) software. Transforming growth factor, beta 1 (TGF-β 1) was identified as the most represented upstream regulator, in addition to being up-regulated in OGT-inhibited cells. These results, and an increase in tyrosine kinase gene expression, suggest that by inhibiting OGT and maintaining low levels of *O*-GlcNAc in cells, we are promoting the phosphorylation of molecules involved in the activation of TGF-β signaling, such as TGF-β receptor I and Smad2/3, and the transcription of neuronal genes. These findings illustrate a novel mechanism by which modulation of *O*-GlcNAcylation influences the differentiation state.

T-2020
HYPER-ADAPTABILITY AND DEFENSE MECHANISM OF EMBRYONIC STEM CELLS UPON ARSENITE EXPOSURE

Beeravolu, Naimisha Reddy[1], Perez-Cruet, Mick[2], Chaudhry, G. Rasul[1]
[1]Oakland University, Rochester, MI, USA, [2]Beaumont Health System, Royal Oak, MI, USA

Environmental arsenite exposure has known correlations with cancer as well as other severe afflictions including neurological and cardiovascular diseases, presenting an important public health issue. Arsenite exposure is mainly via food and drinking water contamination leading to specific and proportional adverse effects. Arsenite exposure has been investigated in various animal models and with a plethora of cell types, yet its toxicity and developmental effects using embryonic stem cells (ESCs) has not been investigated. In this study, ESCs and mouse embryonic fibroblasts (MEF) were exposed to varying concentrations of inorganic arsenite (iAs^{III}) to investigate the chronic effects. For the comparable concentrations, toxicity of arsenite was higher in MEF then in ESCs. The cell death was 10 fold higher in MEFs exposed to 100 μM of arsenite as compared to ESCs. The results showed a clear distinction in the population doubling time between the MEF and the ESCs, suggesting a special survival mechanism as well as innate resistance in ESCs. The defense mechanism of ESCs appeared to be mediated by BCL2 which significantly decreased the percentage of cells undergoing apoptosis. The expression of Jun was increased while levels of Fos deceased with increased in arsenite concentration indicating that the escape plan for the cell survival was stimulated by both Map kinase and JNK pathways. Also, it was observed that the arsenite exposure repressed pluripotency maintaining markers and forcing ESCs to undergo non-specific differentiation. Arsenite exposed cells differentiated into various lineages such as chondrogenic, osteogenic, myogenic as well as neural specific markers. These findings may have implications in the developmental effects of acute and chronic exposure of arsenite during the early fetus development and warrant careful further studies.

T-2022
PROTEOMIC PROFILING OF THE O-GLCNAC MODIFICATION IN HUMAN EMBRYONIC STEM CELLS UNDERGOING NEURAL DIFFERENTIATION

Andres, Lissette[1], **Blong, Ian**[1], Myers, Samuel[2], Rumachik, Neil[1], Pham, Nam[3], Kohler, Jennifer[3], Burlingame, Alma[2], Bertozzi, Carolyn[1]
[1]UC Berkeley, Berkeley, CA, USA, [2]UC San Francisco, San Francisco, CA, USA, [3]UT Southwestern, Dallas, TX, USA

The dynamic and reversible modification of intracellular proteins with O-linked β-N-acetylglucosamine (O-GlcNAc) has emerged as a ubiquitious and important regulator of diverse cellular processes. O-GlcNAcylation involves the attachment of a single GlcNAc monosaccharide onto serine or threonine residues of nucleocytoplasmic proteins. The significance of this modification is emphasized by the fact that it is required for cell viability and perturbations to the regulation of O-GlcNAc is involved in the development of several diseases. The modification has been found on proteins critical for human embryonic stem cell (hESC) proliferation (e.g., myc), differentiation and reprogramming (e.g., Sp1, Oct-4 and Sox-2), neuronal plasticity and brain development, and the enzymes responsible for the modification are expressed highest in the brain. Given the importance of O-GlcNAc in stem cell development, we expect that changes in O-GlcNAcylation status may mirror the vast transcriptional changes that accompany differentiation. To investigate the developmental changes of protein O-GlcNAcylation, we analyzed global O-GlcNAcylation in samples from different stages of neural differentiation by immunoblotting against O-GlcNAc. We found that O-GlcNAc levels oscillate dramatically during neural differentiation of hESCs. Additionally, we found that the levels of UDP-GlcNAc, the nucleotide sugar donor for O-GlcNAcylation, mirrored the pattern of oscillation of O-GlcNAc during differentiation, suggesting that overall protein O-GlcNAcylation may be regulated by UDP-GlcNAc levels. To assess the dynamics of O-GlcNAcylation during neural differentiation, we utilized mass spectrometry-based proteomics technologies. O-GlcNAcylated proteins were enriched by lectin weak affinity chromatography (LWAC), using wheat germ agglutinin to bind terminal GlcNAc residues. Following LWAC enrichment, we performed mass spectrometry (MS) analysis on four different stages of neural differentiation. Utilizing electron transfer-dissociation, we identified more than 200 O-GlcNAcylated peptides, as well as the sites of O-GlcNAc modification on these peptides. After mapping the peptides back to proteins, we found that several of the proteins identified were previously known to be modified by O-GlcNAc, thus validating our method. From the glycopeptides identified, the majority were involved in transcription, transcriptional regulation, transport, and mRNA processing and splicing, and was localized in the nucleus. These observations provide fundamental knowledge of stage-dependent protein modification by O-GlcNAc and will help to elucidate the roles of O-GlcNAc in brain development.

T-2023

MESP1 INDUCES CARDIOPHARYNGEAL MESODERM LEADING TO CARDIAC AND CRANIAL SKELETAL MYOGENIC FATES

Chan, Sunny Sun-Kin, Hagen, Hannah R., Arpke, Robert W., Kyba, Michael
University of Minnesota, Minneapolis, MN, USA

Mesp1 has been thought of as a cardiac master regulator, however we have recently demonstrated that during *in vitro* differentiation of ES cells, Mesp1 actually promotes multiple lineages, including blood and cardiac in the presence of serum, or skeletal muscle in the absence of serum. We show here that under serum-free skeletal muscle conditions, if serum is added back at a later stage, Mesp1-induced presumptive skeletal muscle progenitors adopt a cardiac fate. The dual cardiac/skeletal muscle potency of the cell population induced by Mesp1 under serum-free conditions raised the possibility that this culture system might be modeling cardiopharyngeal mesoderm, which in the early embryo contributes to both the skeletal muscle of the face, and the second heart field. We find that *Tbx1* and *Pitx2*, transcription factors that distinguish and are necessary for craniofacial myogenesis but not trunk / limb myogenesis, are upregulated, even after Mesp1 is withdrawn, confirming the craniofacial identity of Mesp1-induced skeletal myogenic progenitors. Due to the scarcity of cells in an early embryo, cardiopharyngeal mesoderm and its derivatives are poorly defined, thus an *in vitro* system presents a unique opportunity to dissect the molecular underpinnings of its fate choices. By screening factors that convert the default skeletal muscle program under serum-free conditions into cardiac, we show that BMP signaling promotes cardiac differentiation at the expense of skeletal myogenesis from Mesp1+ cardiopharyngeal progenitors, via a rapid upregulation of the second heart field-specific pro-cardiogenic factor *Isl1* and downregulation of the pro-skeletal myogenic factor *Myf5*. Furthermore, we find that Mesp1+ skeletal myogenic progenitors can be traced by the dynamic expression of PDGFR α and VCAM-1: these cells initially express PDGFR α subsequently acquire VCAM-1 to become double-positive and later on differentiate into a VCAM-1+ single-positive population. To further validate the myogenic potential of Mesp1+ cells *in vivo*, we transplanted ES-derived Mesp1+ VCAM-1+ cells into injured adult muscle, where they readily contributed to new muscle fibers. We are currently evaluating potential satellite cell contribution, and examining the role of Mesp1 in cranial skeletal myogenesis *in vivo*. These findings support the notion that Mesp1 induces a cardiopharyngeal mesoderm population with dual cardiac and cranial skeletal myogenic potentials, and provide a model system in which to dissect the molecular regulation of this developmentally important population.

T-2024

DISCOVERY OF DEVELOPMENTAL PROCESSES THAT REGULATE FUNCTIONAL MATURATION OF HUMAN EMBRYONIC STEM CELL-DERIVED NEURONS

Chander, Praveen, Weick, Jason
University of New Mexico, Albuquerque, NM, USA

Differentiation of human embryonic stem cells (hESCs) to neuroectoderm recapitulates many of the early events during in vivo development, at both morphological and molecular levels. Furthermore, in vitro differentiated hESC-derived neurons (hPSNs) possess the functional hallmarks of neurons that develop within an intact central nervous system. Here we sought to use hPSNs to better understand the molecular events underlying later stages of development; specifically the transition from proliferating neuroepithelial cells to post-mitotic neurons, and subsequent functional maturation. We built on previous microarray analyses performed from days 0-17 to examine two time points that are defined by distinct cellular behavior: day 30; when neurons are post-mitotic but do not possess functional connectivity, and day 50; following the development of action potentials and synaptic activity. As expected, numerous transcripts whose protein products are known to regulate the functional properties of neurons, were found highly upregulated at this time, including neurotransmitter receptors and metabolic enzymes, ion channels, transport and synaptic proteins. In addition, we discovered a number of uncharacterized proteins, including NSG2. Based on sequence homology, NSG2 belongs to a family of single transmembrane domain-containing proteins including Calcyon and NEEP21. Both calcyon and NEEP21 are endosomal proteins shown to regulate AMPA receptor (AMPAR) trafficking during synaptic plasticity. Based on a single report showing golgi localization and its expression at day 30 of hPSN development, we hypothesized that NSG2 was involved in trafficking newly-synthesized AMPARs to nascent synaptic sites. In addition to golgi localization, overexpression of NSG2-mCherry showed clear punctate localization within developing neurites. Time-lapse imaging revealed dynamic antero- and retrograde transport (~1μm/sec), consistent with active transport. Further, endogenous NSG2 was found co-localized with numerous intracellular compartments including endosomes and golgi apparatus. Co-immunoprecipitation experiments showed that NSG2 bound to both the overexpressed and endogenous forms of the GluA2 subunit of AMPARs. Knock down of NSG2 mRNA using morpholinos in zebrafish revealed deficits in mechanosensory transduction, indicating an impairment of functional neurotransmission. Together, these data support the notion that NSG2 is a critical mediator of AMPAR trafficking in developing neurons and that hPSNs can be used to discovery new developmental processes even at later stages of neuronal development which apply to in vivo processes.

T-2025

SPECIFICATION OF MIGRATING INTERNEURONS FROM HUMAN PLURIPOTENT STEM CELLS BY DORSOVENTRAL AND ROSTROCAUDAL MODULATION

Chung, Sangmi[1], Kim, Tae-Gon[1], Cho, Jun-Hyeong[1], Vasudevan, Anju[1], Bolshakov, Vadim[1], Kim, Kwang-Soo[2]
[1]Mclean Hospital/Harvard Medical Sch, Belmont, MA, USA, [2]Harvard Medical School McLean Hospital, Belmont, MA, USA

GABAergic interneurons regulate cortical neural networks by providing inhibitory inputs, and their malfunction, resulting in failure to intricately regulate neural circuit balance, is implicated in brain diseases such as Schizophrenia, Autism and Epilepsy. During early development, GABAergic interneuron progenitors arise from the ventral telencephalic area such as Medial Ganglionic Eminence (MGE) and caudal ganglionic eminence (CGE) by the actions of secreted signaling molecules from nearby organizers, and migrate to their target sites where they form local synaptic connections. In this study, using combinatorial and temporal modulation of developmentally relevant dorsoventral and rostrocaudal signaling pathways (SHH, Wnt and FGF8), we efficiently generated MGE cells from multiple human pluripotent stem cells. Human MGE cells spontaneously differentiated into Lhx6-expressing GABAergic interneurons and showed robust migration. These human MGE-derived neurons generated GABA, fired action potential and displayed robust GABAergic postsynaptic activity. Transplantation into rodent brains results in well-contained neural grafts enriched with GABAergic interneurons that extensively migrate in the host. Thus, we propose that signaling modulation recapitulating normal developmental patterns efficiently generates human GABAergic interneurons. This strategy represents a novel tool in regenerative medicine, developmental studies, disease modeling,

bioassay, and drug screening.

T-2026
ANALYSIS OF THE SOX17-DEPENDENT PROTEOME IN ESC-DERIVED HEMOGENIC ENDOTHELIUM REVEALS A STAGE SPECIFIC REQUIREMENT FOR INDIVIDUAL STAT PROTEINS DURING EMBRYONIC HEMATOPOIESIS

Clarke, Raedun[1], Robitaille, Aaron[2], Moon, Randall T.[2], Keller, Gordon M.[1]
[1]*McEwen Centre for Regenerative Medicine, Toronto, ON, Canada,* [2]*Department of Pharmacology, Institute for Stem Cell and Regenerative Medicine, Seattle, WA, USA*

Understanding the molecular mechanisms that underlie embryonic hematopoietic development will facilitate the directed differentiation of hematopoietic stem cells (HSCs) from embryonic and induced pluripotent stem cells (ESCs; iPSCs). During embryonic hematopoiesis emerging HSCs are first detected in the major arterial vessels, the most well characterized site being the intra-embryonic region comprising the developing aorta, gonad and mesonephros (AGM). Within the AGM, HSCs arise from a specialized subpopulation of endothelium, hemogenic endothelium (HE), through a process known as the endothelial to hematopoietic transition (EHT). We have recently demonstrated that the transcription factor Sox17 is required for the generation of HSCs in the AGM in vivo and the development of functional definitive HE in mouse ESC differentiation cultures in vitro. To better understand how Sox17 regulates this stage of embryonic development, we deciphered the Sox17-dependant proteome in HE generated from mESCs using a novel form of label-free quantitative proteomics. Analysis of protein expression in HE derived from wildtype, Sox17-overexpressing and Sox17-null mESCs successfully quantified 36000 unique peptides corresponding to 4200 proteins, 412 of which were specifically regulated by Sox17 expression. Of the this set of Sox17 regulated proteins, Stat1 was among those that showed the greatest difference between the wild type and the Sox17-null HE. Based on these findings, we next evaluated the expression pattern and functional requirement of individual Stat proteins during specific stages of embryonic hematopoiesis in both mouse and human ESC differentiation cultures. The outcome of these analyses suggests a stage specific role for these proteins in this process with Stat1 involved in the generation of HE and Stat3 at the level of EHT. Collectively, these findings provide new insights into the regulation of embryonic hematopoiesis by Sox17 and suggest that two critical steps in this process, the generation of HE and its hematopoietic specification through the EHT, are regulated by distinct signaling pathways.

T-2027
META ANALYSIS OF MICROARRAY DATA OF BMP4 TREATED HUMAN EMBRYONIC STEM CELLS SHOWS LITTLE EVIDENCE FOR TROPHOBLAST CELL TYPES

Cox, Brian[1], Leavey, Katherine[2], Nosi, Ursula[2]
[1]*University of Toronto, Toronto, ON, Canada,* [2]*Physiology, University of Toronto, Toronto, ON, Canada*

Trophoblast cells are an essential cell type of human reproduction, involved in embryo uterine implantation and the formation of the pregnancy unique organ, the placenta. The essential nature of trophoblast in human reproduction and in several prevalent pathologies, has fuelled intense interest in developing trophoblast stem cells orthologous to those available from the mouse, to create in vitro research tools. The need for human specific cells is driven by the high degree of species differences in the placenta organ that the trophoblast are the primary driver. There was great excitement when the first report appeared on the generation of human trophoblast cells from BMP4 treatment of human embryonic stem cells. However, there is some controversy as other groups propose that these cells are a form of extra embryonic mesoderm. Multiple groups have deposited microarray data on in vitro derived human trophoblast cells using different culture conditions yet a systematic analysis of these data sets has not been done. This current study was motivated to first resolve if the cell types produced are truly trophoblast via integrative genome wide analysis and second if there may be a transitory trophoblast progenitor or stem cell population during the in vitro differentiation process. While it is very difficult to definitively show the absence of a cell type, genome wide meta analysis indicates that the vast majority of gene expression during BMP4 mediated differentiation is linked to cardiac cell types and not primary trophoblast. We find string concordance of gene expression between in vitro cardiac and BMP4 mediated trophoblast derivation from human embryonic stem cells. Commonly used trophoblast markers, namely CDX2, EOMES, GCM1, HAND1, CGB (beta-hCG) and HLA-G are not sufficient to distinguish early cardiac progenitors and trophoblast. Specific markers of cardiac cell fates are observed strictly in both in BMP4 derived trophoblast and cardiac cells and not in placental derived trophoblast. Many genes specific to placental trophoblast are not observed in the in vitro trophoblast. We present sets of markers that should enable the distinction of human trophoblast and cardiac cell types. Functionally, syncytialization is a feature common to trophoblast and cardiac cells and our analysis of microarray data supports other published observations that the genes myoferlin and dysferlin are expressed in both tissues during this process. We also find evidence of expression of enzymes involved in estrogen and progesterone production two other hallmarks of trophoblast. It is interesting that trophoblast appears to have co-opted a great number of genes from cardiac lineage development, but this may have confounded efforts to develop methods to direct human embryonic stem cells in to trophoblast lineages. The markers that we provide will hopefully lead to better validation of cell differentiation protocols and the eventual production of trophoblast from in vitro sources.

T-2028
INHIBITION OF MASTER TRANSCRIPTION FACTORS IN PLURIPOTENT CELLS INDUCES THE EARLY STAGE DIFFERENTIATION

De, Debojyoti
Sungkyunkwan University, Suwon, Republic of Korea

The potential for pluripotent cells to differentiate into diverse specialized cell types has given much hope to the field of regenerative medicine. Nevertheless, the low efficiency of cell commitment has been a major bottleneck in this field. Here we provide a strategy to enhance the efficiency of early differentiation of pluripotent cells. We hypothesized that the initial phase of differentiation can be enhanced if the transcriptional activity of master regulators of stemness is suppressed, blocking the formation of functional transcriptomes. However, an obstacle is the lack of an efficient strategy to block protein-protein interactions. In this work we take advantage of the biochemical property of Skp, a bacterial molecular chaperone that binds directly to Sox2. The small angle x-ray scattering analysis reveals that the transactivation domain of Sox2 wraps into a cleft on the Skp trimer. Upon the transduction of Skp into pluripotent cells, the transcriptional activity of Sox2 was inhibited and the expression of Sox2 and Oct4 was reduced, which resulted in the expression of early differentiation markers and appearance of early neuronal and cardiac progenitors. These results suggest that the initial stage of differentiation can be accelerated by inhibiting master transcription factors of stemness. This strategy can possibly be applied to increase the efficiency of stem

cell differentiation into various cell types and also provides a clue to understanding the mechanism of early differentiation.

T-2029
KLF4 NUCLEAR EXPORT INITIATES GENE EXPRESSION CHANGES DURING EMBRYONIC STEM CELL DIFFERENTIATION

Dhaliwal, Navroop Kaur, Mitchell, Jennifer
Cell and Systems Biology, University of Toronto, Toronto, ON, Canada

In embryonic stem (ES) cell, pluripotency is regulated by an interconnected network of transcription factors including OCT4, SOX2, KLF4 and NANOG which bind in combination to numerous loci throughout the genome. KLF4 (Kruppel-like factor 4), maintains pluripotency by supporting transcription of the pluripotency transcription factors in response to LIF (Leukemia inhibitory factor) signaling. Although differentiation of ES cells to specific cell types occurs over several days in culture, significant changes in chromatin architecture occur even within the first 24 hours of differentiation. These changes include an increase in condensed chromatin observable by electron spectroscopic imaging, a decrease in heterochromatin protein mobility and a decrease in global transcriptional output. Despite these observations, most studies of gene expression during differentiation focus on changes that occur after 24 hours. Here we focused on investigating changes in gene expression that occur within the first 24 hours of differentiation and identifying their underlying mechanisms. By monitoring gene expression using real-time PCR we observed that down regulation of *Klf4* primary transcript occurs within 6 hours of LIF/2i withdrawal; *Klf4* is the first of the pluripotency transcription factors to decrease. We observe *Nanog* primary transcript decrease only after 12 hours, following *Klf4* down regulation. This led us to investigate changes in the sub-cellular localization of pluripotency transcription factors using immunofluorescence and cellular fractionation followed by western blot; we observed that KLF4 protein becomes more cytoplasmic after 6 hours of differentiation while NANOG levels gradually decrease over the first 24 hours. During this time SOX2 and OCT4 protein show no change in levels or sub-cellular localization. Because KLF4 nuclear exit occurs simultaneously with the decrease in *Klf4* primary transcript we hypothesized that *Klf4* transcription is dependent on nuclear KLF4. We explored this possibility by inhibiting KLF4 nuclear export using the nuclear export inhibitor Leptomycin B. Indeed, *Klf4* and *Nanog* expression was maintained at 12 hours when KLF4 nuclear export was inhibited. These findings suggest that KLF4 nuclear export initiates the cascade of gene expression changes that occur within the first 24 hours of LIF/2i withdrawal as ES cells exit pluripotency and commit to a differentiated state.

T-2030
HUMAN DEFINITIVE HEMOGENIC ENDOTHELIUM REPRESENTS A DISTINCT LINEAGE FROM ARTERIAL VASCULAR ENDOTHELIUM

Ditadi, Andrea[1], Sturgeon, Christopher[1], Awong, Geneve[1], Kennedy, Marion J.[1], Ng, Elizabeth S.[2], Stanley, Ed[2], Elefanty, Andrew George[2], Keller, Gordon M.[3]
[1]*McEwen Centre for Regenerative Medicine, Toronto, ON, Canada,* [2]*Murdoch Childrens Research Institute, Parkville VIC, Australia,* [3]*McEwen Centre for Regenerative Medicine Ontario Cancer Inst, Toronto, ON, Canada*

The successful derivation of hematopoietic stem cells (HSCs) from human pluripotent stem cells (hPSCs) will be dependent on our ability to accurately recapitulate critical aspects of embryonic hematopoiesis in the differentiation cultures. In the embryo, HSCs arise from a progenitor population known as hemogenic endothelium (HE), through a Notch dependent process that involves an endothelial-to-hematopoietic transition (EHT). Given that HE expresses endothelial markers and is integrally associated with the major arteries in the embryo, it is assumed that this progenitor population derives from the arterial vascular endothelium (VE). However, despite this close association, a clear lineage relationship between HE and arterial VE has not been established. To address this question, we analyzed the hPSC-derived CD34+ population that we have previously shown to contain both HE and VE for expression of the vascular markers CD73 and CD184. Based on their expression pattern, we could resolve three distinct fractions within the CD34+ population: CD73-CD184-, CD73medCD184+ and CD73hiCD184-. Of these, only the CD73-CD184- fraction displayed the capacity to undergo EHT and generate CD34+CD45+RUNX1C+ definitive hematopoietic cells, as measured by T lymphoid potential. The emergence of this population was abrogated by the addition of a chemical inhibitor of the Notch pathway, indicating that hematopoietic specification of these progenitors is dependent on Notch signaling. qPT-PCR analyses indicated that the CD73medCD184+ and CD73hiCD184- fractions that do not display hematopoietic potential are enriched for cells with arterial and venous VE potential respectively. When transplanted into immunocompromised mice, these cells generated vessels that maintained their arterial and venous endothelial identity and integrated into the host vasculature, confirming that they do represent the hPSC-derived arterial and venous VE progenitors. Together, these findings provide new insights into human hematopoietic development and strongly support the interpretation that HE represents a lineage distinct from VE.

T-2031
GENERATION, CHARACTERIZATION AND IN VIVO SAFETY EVALUATION OF EXPANDABLE GMP GRADE HUMAN PLURIPOTENT EMBRYONIC STEM CELL-DERIVED NEURAL STEM CELLS.

Dolezalova, Dasa[1], Hruska-Plochan, Marian[1], Goldstein, Lawrence S.B.[2], Marsala, Martin[1]
[1]*Anesthesiology, University of California, San Diego, La Jolla, CA, USA,* [2]*University of California San Diego, La Jolla, CA, USA*

Background: Regeneration and replacement of neurons and glia that undergo cell death due to progressive neuro-degenerative disorders and/or CNS injury are the main goals of all stem cell-based therapies. However, well-defined and clinically-relevant methods for generation of neural stem cells (NSCs) from human embryonic stem cells (hESCs) as well as their safety in vivo remain to be defined. The aim of the present study was to develop an in vitro technique for isolation of NSCs from pluripotent hES cell lines. The potency and safety of generated cell lines were probed by their differentiation potential both in vitro and in vivo and by spinal grafting into immunodeficient rats. Methods: Three independent cell lines of pluripotent hESCs (H9, UCSF4, UCSF4.3) were expanded and induced to form neural rosettes. Morphologically distinct population of proliferating NSCs was harvested from the periphery of neural rosettes and further expanded in the presence of FGF2 as a solo mitogen. NSCs were expanded at least up to 40 passages while being periodically characterized by flow cytometry for pluripotency and NSC-specific markers (Nanog, Sox2, Sox1, Pax6, Nestin, GFAP, CD24, CD44, CD184, CD271). To evaluate safety, NSCs were grafted spinally into immunodeficient rats and the presence and phenotype of grafted cells analyzed from 2 weeks up to 6 months using human-specific antibodies. Results: Newly generated cell lines of NSCs showed homogenous morphology upon extensive propagation (>40 passages) in vitro. Established lines displayed consistent expression of markers

typical for NSCs (>75% of Nestin, Sox1, Sox2 and Pax6 expression) and no residual expression of pluripotency transcription factor Nanog. A stable karyotype as well as the ability to differentiate into glial cells and functional neurons was seen in vitro. After transplantation in vivo, cells integrate into host tissue and show morphology and markers typical for mature neurons and/or astrocytes such as DCX, NeuN and/or GFAP. Grafted cells showed moderate KI67 immunoreactivity which was quantitatively similar to endogenous pool of proliferating Ki67+ precursors. Importantly no tumor formation or the appearance of any aberrant cell type formation (rosettes or malignant cell clusters) was noted. Conclusion: This isolation protocol can be effectively used to generate high number of transplantable NSCs from pluripotent hESCs which demonstrate a favorable safety profile and can potentially be used in future human clinical trials.

T-2032
ELONGATING AND ELONGATED SPERMATIDS MANUFACTURED IN VITRO FROM NON-HUMAN PRIMATE PLURIPOTENT STEM CELLS

Simerly, Calvin[1], Chan, Anthony[2], Schatten, Gerald P.[1], **Easley, Charles Allen**[3]

[1]OB/GYN and Reproductive Sciences, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA, [2]Department of Human Genetics, Emory University School of Medicine/Yerkes National Primate Center, Atlanta, GA, USA, [3]Cell Biology, Emory University School of Medicine, Atlanta, GA, USA

We have recently shown that human embryonic (hESCs) and induced pluripotent stem cells (hiPSCs) can differentiate into advanced spermatogenic cells including round spermatids by in vitro culture. Here, we confirm and extend these findings showing a non-human primate, rhesus embryonic (nhpESCs), somatic cell nuclear transfer-derived embryonic (nhpNT-ESCs), and induced pluripotent stem cells (nhpiPSCs) can be differentiated into spermatogonial stem cell (SSC) - like cells that express PLZF, a marker for stem and progenitor spermatogonia, and VASA, a known germ cell marker expressed by several germ cell types. Differentiating nhp pluripotent stem cells in SSC culture conditions also produces haploid spermatids. Most of these in vitro haploid cells resemble round spermatids as assessed by protamine 1, acrosin, Golgin3, Giantin and VAMP1 immunostaining as well as microtubule and centrosome marker analyses. However, round spermatids do not fertilize human or non-human primate oocytes. We have observed a small population of haploid spermatids derived in vitro that structurally resemble elongated spermatids, a spermatid population capable of fertilizing primate oocytes by intracytoplasmic sperm(atid) injection (ICSI). We are currently assessing whether these in vitro "elongating" haploid spermatids are capable of fertilizing rhesus oocytes. If successful, this model will provide the first evidence that functional gametes can be manufactured in vitro from non-human primate pluripotent stem cells and may provide insight into potentially novel methods for diagnosing and even treating human male infertility.

T-2033
MAKING STEM CELLS A THERAPEUTIC TOOL FOR RESTORATION OF ARTICULAR CARTILAGE AND SYNOVIAL JOINT FUNCTION

Wu, Ling[1], Bluguermann, Carolina[2], Van Handel, Ben[1], **Evseenko, Denis**[1]

[1]University of California LA, Los Angeles, CA, USA, [2]FLENI, Buenos Aires, Argentina

Articular cartilage injury and the lack of cartilage regeneration often lead to osteoarthritis, characterized by the degradation of joints, including articular cartilage and subchondral bone. Arthritis affects an estimated 50 million people in the US at an annual cost of $100 billion, making identifying treatments for this disease a priority. None of the current cell-based repair strategies including in vitro expanded chondrocytes or mesenchymal stroma cells (MSCs) generate long lasting hyaline articular cartilage. Limited survival, hypertrophy and degeneration of implanted cells, as well as the excessive collagen type I (COL I) deposition and formation of mechanically inferior fibrotic tissue in the site of injury, are the standard outcomes of current techniques. Human pluripotent stem cells (hPSC) have been successfully used by several groups to generate cartilage-like tissue. However, none of the previously published studies have reported the generation of a purified population of PSC-derived chondrocytes, but rather the generation of undefined cells with unknown ontogeny capable of depositing chondrogenic matrix. We have recently described a population of putative cartilage-committed cells and factors that control survival, proliferation and differentiation of these cells. Detailed analysis of the transcriptional and epigenetic signatures have been also carried out and revealed close similarity of hPSC-derived cartilage progenitors and primary human chondrocytes; these data offer a novel strategy for articular cartilage restoration. Our group is currently investigating this novel hPSC-derived cell subset in pre-clinical (pig) model of joint injury. Altogether, these innovations are expected to significantly improve existing therapeutic approaches used to treat articular cartilage injury.

T-2034
EARLY NEURAL CREST FORMATION IN CHICK AND HUMAN MODELS

Garcia-Castro, Martin[1], Uribe-Querol, Eileen[2], Leung, Alan[1], Murdoch, Barbara[3], Marquez, Jonathan[1]

[1]Molecular Cellular and Developmental Biology, Yale University, New Haven, CT, USA, [2]División de Estudios de Posgrado e Investigación, Facultad de Odontología, Universidad Nacional Autónoma de Mexico, Mexico city,, Mexico, [3]Department of Biology, Eastern Connecticut State University, Vancouver, CT, USA

The neural crest is a unique population of cells in vertebrate embryos that arises early in development, migrate extensively and generate a bewildering array of numerous and diverse derivatives. Aberrant development and homeostasis of NC cells results in developmental malformations (cleft lip/palate), rare syndromes (Waardenburg) and aggressive cancers (melanoma). Despite their relevance, our understanding of their formation in amniotes, and particularly in humans, is very limited. Here we present chick and human evidence suggesting that NC arise via an unanticipated early origin that does not require the previously accepted tissue interactions of neural and non-neural ectoderm or mesodermal contribution. We have assessed early events in NC development in amniotes using avian embryos, and found that prior to primitive streak formation, NC cells are specified in the lateral edges of the blastula epiblast. Fate map experiments identify the same territory as NC-contributing. Surprisingly, we identify a significant and robust bias for the lateral epiblast to acquire NC marker expression in the absence of contact mediated interactions. To address human NC development we developed a fast surrogate model based in human ESC that provides unprecedented efficiency. hESC can be directed to drop stem cell markers and adopt dual Pax7-Sox10 expressing NC (~90-95%) character in just 5 days. These hNC-cells co-express many other NC markers and can generate expected NC-derivatives including muscle, melanocytes, peripheral neurons and glia. We have addressed the ontogeny of NC in this system through immunofluorescence, Q-RT-PCR, and next generation RNA sequencing analysis. Our results are consistent with our chick observations and suggest that neither neural, non-neural ectoderm or

mesodermal contributions are at play.

T-2035
CALCINEURIN SIGNALING CONTROLS THE CONVERSION OF AN ANALOGUE BMP SIGNAL TO A BINARY CELL FATE DECISION DURING NEURAL INDUCTION

Graef, Isabella[1], Wernig, Marius[2], Davila, Jonathan[3], Cho, Ahryon[4]
[1]Stanford University, Stanford, CA, USA, [2]Stanford University, Palo Alto, CA, USA, [3]Stanford University, Palo Alto, CA, USA, [4]Pathology, Stanford University, Stanford, CA, USA

Morphogenesis is controlled by complex signaling networks, which have to work in concert with one another. However, it has been challenging to establish the molecular mechanisms by which cells integrate these signaling pathways. Genetic and biochemical analyses reveal that the Ca2+-activated phosphatase, calcineurin, regulates neural induction by connecting FGF and Ca2+-signaling to the BMP-pathway. Specifically, calcineurin is required to adjust the strength and transcriptional output of BMP signaling. We demonstrate that calcineurin directly dephosphorylates BMP-regulated Smad1/5 proteins and that reduced calcineurin signaling leads to increased Smad1/5-activated transcription. As a result, FGF-activated calcineurin signaling has the potential to convert a continuous BMP gradient into a threshold function, thereby enabling stable, binary cell fate decisions.

T-2036
PLASMALEMMAL SYNTAXINS REGULATE CELL ADHESION PROPERTIES IN EC CELLS, LEADING TO THEIR CYTODIFFERENTIATION

Hagiwara, Natsumi, Hirai, Yohei
Kwansei Gakuin University, Sanda, Japan

The proteins in the syntaxin family are known to mediate fusion of cytoplasmic vesicles to the target membrane, yet subpopulations of certain plasmalemmal syntaxins, including epimorphin (syntaxin2) and syntaxin4, translocate across the cell membrane in response to external stimuli so as to function as signaling molecules. Here, we show that extracellularly supplied syntaxin impacted cell behaviors and regulated the differentiation direction in teratocarcinoma (EC) cells. We found that undifferentiated F9 cells extruded a small subpopulation of extracellular syntaxin4 at the lateral cell membrane, while this polarized expression pattern was abolished by the differentiation induction with all-trans retinoic acid. The forced apolar expression of extracellular syntaxin4, but not syntaxin2, in F9 cells resulted in the dramatic enhancement of cell-substrate interaction and suppression of cell-cell adhesion, with an alternation in the expression profile of vinculin, a regulator of both cell-substrate and cell-cell interactions. F9 cells expressing extracellular syntaxin4 up-regulated several differentiation markers for endodermal lineages, together with another family member syntaxin3 in these cells. The exogenous over-expression of cytoplasmic syntaxin3 elicited cell responses similar to the stimulation with extracellular syntaxin4, indicating that syntaxsin4's effects were attributed, at least in part, to the up-regulation of syntaxin3. We also found that the addition of a circular peptide composed of the predicted functional core in syntaxin4 (AIEPQK; a.a. 103~108) facilitated the substrate adhesion property in F9 cells, while a syntaxin4-mutant lacking the functional core (Δsyntaxin4) lost even cell surface-binding ability. Pull down experiments using recombinant syntaxin4 indicated the existence of a putative receptor of extracellular syntaxin4: full length syntaxin4, but not Δsyntaxin4, bound a protein of around 65 kDa in F9 cells. In contrast to the clear effects on F9 cells, another EC cell line P19 did not clearly respond to the extracellular syntaxin4. On the other hand, an artificial expression of extracellular syntaxin2 conferred the substrate-adherent property upon P19 cells, along with the expression induction of several mesodermal markers including that of cardiomyocyte. When P19-CL6 cells, P19-derivative cells, that have been introduced with GFP reporter gene connected with myosin promoter were treated with extracellular syntaxin2, green fluorescence became detectable within a few days even without any differentiation stimuli. Taken these results together, plasmalemmal syntaxins emerged as potential initiators and/or regulators of differentiation of anaplastic EC cells.

T-2037
A LINEAGE-SPECIFIC REQUIREMENT FOR ERK ACTIVITY DURING MOUSE EMBRYONIC STEM CELL DIFFERENTIATION

Hamilton, William B.
DanStem, University Of Copenhagen, Copenhagen, Denmark

Fgf signaling via Erk activation has been associated with both neural induction and the generation of a primed state for the differentiation of embryonic stem cells (ESCs) to all somatic lineages. As a means to understand the role of Erk in both ESC self-renewal and lineage specification we explore the requirements for this pathway in various in vitro differentiation settings. A combination of pharmacological inhibition of Erk signaling and genetic loss of function experiments reveal a role for Erk signaling in suppressing endodermal differentiation, but not neural specification, as a result of sustained support for the expression of pluripotency transcription factors. While Nanog responds directly to Erk inhibition, we find that the response of ESCs to Erk signaling is predominantly Nanog independent. Taken together, our results suggest that Erk signaling suppresses pluripotent gene expression to enable endodermal differentiation.

T-2038
IDENTIFYING NOVEL MODULATORS OF BLOOD VESSEL FORMATION USING EMBRYONIC STEM CELL DIFFERENTIATION ASSAYS

Hammoud, Lamis, Rossant, Janet
The Hospital for Sick Children Research Institute, Toronto, ON, Canada

Many events that occur during embryonic vascular development are recapitulated during adult neoangiogenesis, which is critical to tumour growth and metastasis. While the latest antiangiogenic drugs, such as Avastin® (bevacizumab), have been shown to prolong life expectancy in cancer patients, they have serious side effects. Furthermore, relapses often occur, necessitating the need for novel therapeutic targets. The aim of this study was to develop a robust in vitro embryonic vascular differentiation assay suitable for small molecule screens to identify novel modulators of angiogenesis. Mouse embryonic stem (ES) cells containing a vascular reporter (Flk1-EGFP) were aggregated to form embryoid bodies (EBs) using the hanging drop method. EBs were then grown in a collagen type I gel in the presence of medium supplemented with 10% fetal bovine serum (FBS) and containing one or more of four previously established angiogenic growth factors (VEGF, bFGF, IL-6 and EPO). Flk1 positive vascular sprouts were quantified following 6-8 days of culture. VEGF-treated EBs generated reproducible Flk1 positive vascular sprouts regardless of the presence or absence of other factors. Using the Cellomics ArrayScan platform, we developed an algorithm, based on the Neuronal Profiling software, to quantify total expression of Flk1 as well as the number of fluorescent sprouts. We used this algorithm to demonstrate that this assay can discriminate between positive and negative responses to signaling pathway inhibitors. Upon treatment with a gamma secretase inhibitor (L685458), excessive

angiogenic sprouting was observed, as expected for a Notch pathway inhibitor. Conversely, treatment with a Flk1 inhibitor (SU5416) blocked angiogenic sprouting. We have used our vascular differentiation assay to screen a kinome small molecule inhibitor library provided by the Medicinal Chemistry Platform of the Ontario Institute for Cancer Research (OICR). Promising candidates showing quantitative deviation from control cultures have been observed and validated through dose response curves. We have identified many kinases with already well-established roles in angiogenesis such as Flk1, Tie2, Alk, FGFR, IGFR, MEK1/2, and ERK1/2. In addition, we have identified kinases that have not been previously implicated in angiogenesis. Novel hits identified using this screen, belong to the ribosomal and cell cycle checkpoint kinase families. Currently, we are exploring the identity of the isoforms involved. We have also assessed the validity of these molecules using human umbilical vein endothelial cell (HUVEC) tube formation assays, and are now testing their effectiveness in preclinical tumour models in mice.

T-2039

MODULATING INNATE IMMUNITY IMPROVES HEPATITIS C VIRUS INFECTION OF STEM CELL DERIVED HEPATOCYTES

Zhou, Xaioling[1], Sun, Pingnan[1], Angus, Allan[2], Lucendo-Villarin, Baltasar[1], Szkolnicka, Dagmara[1], Farnworth, Sarah[1], Cameron, Kate[1], Patel, Arvind[2], **Hay, David**[1]

[1]University of Edinburgh, Edinburgh, United Kingdom, [2]University of Glasgow, Glasgow, United Kingdom

Hepatitis C virus (HCV) infects an estimated 2-3% of the world population, and is a major cause of liver disease and cancer. It is estimated that more than 350,000 people die of HCV-related liver disease each year. Modelling virus lifecycle is therefore key to the development of a better understanding of virus and host interactions and the disease process. Current cell models which support HCV propagation possess some major drawbacks, including a defect in retinoic acid-inducible gene (RIG)-I which results in diminished IFN production and immune response. A detailed understanding of virus life cycle and pathogenesis therefore requires the establishment of biologically relevant model systems which more closely mimic human physiology. For this reason, primary human hepatocytes (PHH) have been employed, however, scarcity, inconsistency and rapid dedifferentiation in culture impede their widespread deployment. Human embryonic stem cell-derived hepatocytes (hESC-Heps) offer an alternative novel model for virus-host study and are the focus of this study. We employed hESCs to deliver homogeneous hepatocyte populations as described (Szkolnicka et al 2014). Viral infection of hepatocytes was carried out in a controlled environment as previously described (Iro et al,2009). hESC-Heps were investigated for their ability to support hepatitis C virus (HCV) infection and replication. hESC-Heps were capable of supporting full viral life cycle, including the release of new infectious particles. While supportive, hESC-Hep viral infection levels were not as great as that observed in Huh7 cells. We reasoned that the innate immune response may limit HCV replication in hESC-Heps, leading to low infection efficiency. Indeed, upon further investigation, we found that the hESC-Heps displayed a strong innate immune response triggered by HCV infection. Specific inhibition of the Jak-Stat signalling pathway led to a significant decrease in innate immunity and a corresponding increase in HCV replication in hESC-Heps. Notably, innate immunity was not evident in Huh7 cells and we did not observe alterations in viral infection in response to modulating Jak-Stat signalling.. In conclusion, we have successfully established a novel in vitro model system which facilitates the in-depth study of HCV life cycle and pathogenesis. We believe our model offers great potential as a platform technology to study innate immunity and for the development of novel anti-virals.

T-2040

RUVB LIKE PROTEIN 2 (RUVBL2) AFFECTS EARLY NEUROECTODERM SPECIFICATION IN MOUSE EMBRYONIC STEM CELLS

Hong, Soomin[1], Jo, Junghyun[1], Kim, Hyung Joon[2], Lee, Jeoung Eun[3], Lee, Sung-Geum[3], Baek, Ahmi[2], Lee, Dong Ryul[1]

[1]Department of Biomedical Science, College of Life Science, CHA University, Seoul, Republic of Korea, [2]Fertility Center of CHA Gangnam Medical Center, CHA University, Seoul, Republic of Korea, [3]CHA Stem Cell Institute, CHA University, Seoul, Republic of Korea

Introduction: Since embryonic stem cells (ESCs) are able to differentiate into various cell types in vitro, ESCs have been considered as a great resource for studying early embryonic development. During the early embryonic development, the stepwise cell fate transition is occurred by activation and/or inactivation of lineage-specification factors, and cells are converted into specific types of cells such as three germ layers. In developmental biology, one of the well-studied processes during the gastrulation is neuroectoderm specification. However, it is still important to figure out which factors are responsible for conversion of pluripotent stem cells into neuroectoderm in mammals. RuvB like protein 2 (*Ruvbl2*) is a member of the AAA+ family (ATPase associated with diverse cellular activities) of DNA helicase and implicated in diverse cellular processes, such as cell growth, DNA repair, chromatin remodeling and regulation of gene expressions. Although there is a report that *Ruvbl2* expression is increased during the neural stem cell differentiation in human ESCs (Barthéléry et al. 2009), the role of *Ruvbl2* in ESCs is still not clear and has not been studied much yet. In this study, we established *Rubl2* over-expressing mouse ESCs (mESCs) and examined their capacity of differentiation especially into neuroectoderm lineage. Our results suggest that *Ruvbl2* seems to promote the early neuroectoderm differentiation in mESCs. Materials and Methods: *RuvBl2* over-expressing mESCs were established by transfection of lentiviral vectors, and we used a VENUS (YFP) reporter system to assess gene transfer and expression. In order to measure extent of over-expression of *Ruvbl2*, we conducted RT-PCR, real-time RT-PCR and Western blot analysis. Expression changes in neuroectoderm lineage-specific markers (*Pax6, Nestin, Tubb3*) were examined by RT-PCR and real-time RT PCR in *Ruvbl2* over-exprssing mESCs. Also, mESCs were induced into embryoid bodies (EBs) by withdrawal of LIF and then RA was added into the differentiation medium after the first 4 days and left for the last 4 days for examination of differentiation capacity especially in neuroectoderm. After differentiation, gene expressions of mEBs were examined with several lineage-specific markers (*Pax6, Nestin, Tubb3*). Results: Assessment of *Ruvbl2* gene integration into mESCs genome was validated by YFP expression. Also, higher gene expression of *Ruvbl2* in *Ruvbl2* over-expression mESCs was shown by RT-PCR and real-time RT-PCR analysis. In those cells, increased RUVBL2 protein was confirmed by Western blot analysis. In *Ruvbl2* over-expressing mESCs, expression level of neuroectoderm specific molecular marker *Pax6, Nestin, Tubb3* was increased. In addition, *Ruvbl2* over-expressing mEBs showed increased expression level of neuroectoderm markers (*Pax6, Nestin, Tubb3*). Conclusion: Our results showed that *Ruvbl2* up-regulate the transcription levels of neuroectoderm markers (*Pax6, Nestin, Tubb3)*. Also, *Ruvbl2* directs the differentiation of mESCs into neuroectoderm lineage. Thus, this study provides evidence that *Ruvbl2* is a potent gene for promoting neuroectoderm differentiation in mESCs and contributes to find out certain genes which are responsible for neuroectoderm determinant in animals.

T-2041

EPHRINB2 REGULATES HUMAN PLURIPOTENT STEM CELL FATE

Hoo, Nicholas W.[1], Palomares, Karina[2], Lee, Benhur[3], Pyle, April[4]
[1]Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, Westwood, CA, USA, [2]University of California, San Diego, La Jolla, CA, USA, [3]Department of Microbiology, Immunology and Molecular Genetics, Icahn School of Medicine at Mount Sinai, New York City, NY, USA, [4]University Of California, Los Angeles, Broad Stem Cell Center, Los Angeles, CA, USA

EphrinB2, a member of the eph/ephrin receptor network shown to regulate numerous developmental processes, is also considered a key molecular marker for "stemness". Gaining attention for its role in permitting cell entry of the Nipah Paramyxovirus Virus (NiV), we have developed a number of tools to study EphrinB2's impact on stem cell fate. Studies thus far suggest that EphrinB2 does not mark for a phenotypically stable population of hESC, but may instead exist heterogeneously for the maintenance of pluripotency. Microarray analysis suggests that EphrinB2 may influence a movement of cells towards a mesoderm lineage bias and may also have an impact on the expression of a secondary cognate receptor, EphA4. qPCR analysis is being performed to confirm these results. Additionally, to validate the role of EphrinB2 in modulating stem cell fate, we are evaluating the effects on mRNA expression using soluble tetrameric and monomeric forms of sNiV-G protein on stem cell culture and embryoid bodies.

T-2042

COMBINED SMALL MOLECULE INHIBITION ACCELERATES GENERATION OF FUNCTIONAL CORTICAL NEURONS FROM HUMAN PLURIPOTENT STEM CELLS

Qi, Yuchen[1], Zhang, Xinjun[1], Studer, Lorenz[2]
[1]Memorial Sloan Kettering Cancer Center, New York, NY, USA, [2]Sloan-Kettering Institute for Cancer Research, New York, NY, USA

Significant progress has been made to convert human pluripotent stem cells into functional neurons by modulating signaling pathways directing the development and differentiation. The human cerebral cortex is one of the most complex structures in brain with high-order functions including perception, cognition and language. Extensive loss of cortical neurons has been observed in many neurodegenerative diseases, and great efforts have been made to direct differentiation of human pluripotent cells into functional cortical neurons as cell replacement therapy. However, most of the in vitro differentiation follows the slow, stepwise process, mimicking the protracted timing of human development. Recently, we developed a protocol based on small molecule inhibition of key signaling pathways directing forebrain neural fate and cortical neuron specification, which yield up to 60% Tuj1+ postmitotic neurons within 13 days of induction, and showed abundant expression of cortical layer markers of Tbr1 and Reelin, with up-regulated expression of other cortical layer marker. These neurons showed accelerated functional electrophysiological properties of excitatory neurons around 25 days of induction, and incorporate into host brain and synaptic networks after grafting into neonatal mouse. These data suggested the usage of small molecule inhibition as a general strategy for accelerating developing timing in vitro.

T-2043

SINGLE CELL TRACKING AND MOLECULAR ANALYSIS REVEALS DISTINCT MESENDODERM PROGENITORS

Yang, Dapeng[1], Burtscher, Ingo[1], Schwarzfischer, Michael[2], Irmler, Martin[3], Marr, Carsten[2], Lickert, Heiko[1]
[1]Institute of Stem Cell Research, Institute of Diabetes and Regeneration Research, munich, Germany, [2]Institute of Computational Biology, munich, Germany, [3]Institute of Experimental Genetics, munich, Germany

In the mouse embryo 6.5 days after fertilization, gastrulation starts and pluripotent epiblast cells undergo epithelial-mesenchymal transition to ingress into the posterior primitive streak region to form mesoderm and definitive endoderm (DE). Our previous results indicated that endoderm and mesoderm is specified in the epiblast, where T positive cells mark mesoderm progenitors and Foxa2-positive cells mark the DE progenitors (Burtscher and Lickert, 2009). However, it is still not clear when and how precursor cells are specified and differentiate. In this study, we have developed an *in vitro* ES cell differentiation system that enables us to track the endoderm and mesoderm differentiation process in detail by a dual knock-in reporter cell line (T-GFP x Foxa2 tag-RFP cell line). The linage tracking analysis indicates that majority of the endoderm and mesoderm cells were formed directly from ES cells, but not undergoing mesendoderm stage. These results are consistent with the idea that DE cells might directly delaminate into the visceral endoderm from the epiblast without migrating through primitive streak (Tam and Beddington, 1992). Furthermore, this *in vitro* ES cell differentiation system enables us to identify and characterize mesendoderm ($Foxa2^+T^+$ cells), endoderm ($Foxa2^+$ cells), mesoderm (T^+ cells). In combination with the epithelial surface marker CD24 it allows us to follow their linage progression through differentiation. Profiling data from those sorted subsets reveals those subsets display similar characteristics of developmental stages of gastrulation. However, those endoderm and mesoderm progenitors show distinct properties with mesendoderm cells. Taken all together, this in vitro differentiation model nicely recapitulates gastrulation *in vivo* and revealed novel insight into the segregation of lineages during germ layer formation.

T-2044

USING A FEZF2: EYFP HESC REPORTER LINE TO MODEL HUMAN CORTICAL NEURON DEVELOPMENT IN A DISH

Feltham, Casey[1], Smith, Anders[1], Hinckley, Sandra[1], Zheng, Binhai[2]
[1]Neurosciences, University of California, San Diego, La Jolla, CA, USA, [2]University of California San Diego, La Jolla, CA, USA

The corticospinal tract (CST) is involved in controlled motor movement. Damage to the human CST results in loss of voluntary motor function and control. The central nervous system has a limited ability for regeneration and recovery. Therefore, injury to the CST results in permanent damage. In order to better understand the CST, we sought to model the development of corticospinal motor neurons (CSMN) using human embryonic stem cells (hESCs). Fezf2, an essential transcription factor for CSMN development, was selected as a marker to trace the development of hESCs into Fezf2+ neural precursors and neurons. Fezf2 is highly expressed in layer V subcerebral projection neurons, including CSMN, and in Fezf2 knockout mice no CST develops. Previously our lab developed a Fezf2:EYFP hESC knockin reporter line via homologous recombination, in which the endogenous Fezf2 promoter controls EYFP expression. Fezf2:EYFP hESCs were differentiated into neurons. A time course was generated during neural differentiation and Fezf2:EYFP positive and negative cells were characterized with multiple markers to assess their molecular identity. The data suggests multiple roles for Fezf2 during human cortical

neuron development.

EMBRYONIC STEM CELL PLURIPOTENCY

T-2046
IDENTIFICATION OF GAIN OF CHROMOSOME 20Q IN HUMAN EMBRYONIC STEM CELL LINES DERIVED AT HUMANIZED CULTURE CONDITION

Seol, Hye Won[1], Baek, Jin Ah[1], Jung, Juwon[1], Yoon, Bo Ae[1], Kim, Hee Sun[2], Oh, Sun Kyung[2], Ku, Seung Yup[2], Kim, Seok Hyun[2], Moon, Shin Yong Yong[1], Choi, Young Min[2]
[1]Institute of Reproductive Medicine and Population, Medical Research Center, Seoul National University, Seoul, Republic of Korea, [2]Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, Republic of Korea

Recently, several reports have described the gain of 20q11.21 by high-resolution molecular cytogenetic methods for the screening of genetic changes in human embryonic stem cells (hESCs). 20q11.21 amplification is a recurrent mutation that has been repeatedly reported in human pluripotent stem cells (hPSCs) as well as human cancer, suggesting that this mutation confers a selective advantage to these cells. We established 9 hESC lines at humanized culture conditions (human foreskin feeder layer + DMEM/F12 with 15% KSR™ xenofree + defined, humanized substrate CELLstart™) and these cell lines were maintained using mechanical transfer with retaining pluripotent stem cell characteristics. There were diploid in all hESC lines except one cell line when cell lines were derived. But, after in vitro culture, 5 out of 9 hESC lines (55.5%) acquired subline with gain of chromosome 20q and this aberration was confirmed by G-banding, FISH and array CGH. These genetic changes showed regardless of early or late passage in spite of mechanical transfer method. In array CGH analysis, we observed a little different gene expression pattern at each of 5 cell lines with gain of 20q and specially one cell line was simultaneously analyzed gene expression profiling of gain of 20q and trisomy 20. We have only experienced chromosome 20 aberration of hESCs at humanized culture condition excluding conventional culture condition (STO feeder + DMEM/F12 with 20% KSR + gelatin). We suggest that occurrence of this specific chromosome aberration may be related to the nature of cells to be lived to make energy for cell by itself to overcome unbalance between self renewal, differentiation and apoptosis come from environmental factor such as cell growth media, substrate and oxygen level, etc. Besides, regular screening by FISH and array CGH in hESCs is essential to ensure the quality of hESC humanized culture in future therapeutic applications. This research was supported by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MEST) (2012M3A9C6049722).

T-2047
A NOVEL PROTEIN PARTICIPATE IN REGULATING TELOMERE LENGTH SPECIALLY IN EMBRYONIC STEM CELLS

Huang, Junjiu[1], Feng, Xuyang[1], Luo, Zhenhua[1], Yu, Jianping[1], Jiang, Shuai[1], Shi, Guang[1], Songyang, Zhou[2]
[1]Sun Yat-sen University, Guangzhou, China, [2]Baylor College of Medicine, Houston, TX, USA

Telomere length and integrity is important for genomic stability and affects cell aging. Telomere length has been link to the pluripotency of embryonic stem cells (ESCs). Telomere shortening may reduce pluripotency of ESCs and induced pluripotent cells (iPSCs). Telomerase is considered to be a crucial factor in maintaining telomere length. In recent years, more and more evidences indicate that telomeres elongation in ESCs was not only depended on the telomerase pathway but also regulated by another mechanism called Alternative Lengthening of Telomeres (ALT). Herein, we found a novel protein that co-localized with telomeres in pluripotent stem cells such as ESCs and iPSCs specially. This protein would be released from telomeres when induced ESCs differentiation in vitro. And it would rebind to telomeres during reprogramming somatic cells to iPSCs. In addition, its telomere-location was neither relating to the telomere length nor the status of DNA methylation. Knocking down this protein didn't affect telomerase activity but would repress the ALT activity by decreasing ALT-associated promyelocytic leukaemia (PML) nuclear bodies (APBs),the presence of telomere C-circles and telomere sister-chromatid exchange (T-SCE) result in telomere shortening significantly in ESCs. This study implicates that this novel protein play an important role in ALT activity participating in maintaining telomere length in ESCs.
Embryonic Stem Cell Pluripotency

T-2048
ATP INDUCES INTRACELLULAR CALCIUM RELEASE THROUGH IP_3 RECEPTORS IN HUMAN EMBRYONIC STEM CELLS

Huang, Jijun, Zhang, Min, Liang, He, Zhang, Peng, Yang, Huang-tian
SIBS, Shanghai, China

Human embryonic stem cells (hESCs), which are derived from the inner cell mass of blastocyst, have the ability to propagate indefinitely in culture while still remain the capacity to differentiate into all cell types of three germ layers. Thus they are not only a potentially inexhaustible resource for the repair and restoration of organ functions but also a unique model for the study of human early development process. So far, much progress has been achieved on the understanding of regulatory mechanism of self-renewal and differentiation in hESCs, including the key transcription factors, signaling pathways and epigenetic regulators. However, little is known about the physiological properties and regulations of hESCs, including Ca^{2+} signaling systems, while such knowledge would enrich our understanding of the regulation of pluripotency and self-renewal. Extracellular adenosine triphosphate (ATP) is an important signaling molecule in many embryonic cell types that increases the intracellular Ca^{2+} concentration, which can regulates cell proliferation, migration, and differentiation. In the present study, we aimed to (i) characterize Ca^{2+} signaling in hESCs under resting or ATP stimulation; and (ii) elucidate the regulatory mechanism of Ca^{2+} signaling in hESCs. We therefore determined the expression profiles of genes coding for major Ca^{2+} handling proteins in hESCs and then investigated the function of these proteins by confocal microscope and flow-cytometry, combined with pharmacological approaches and small RNA interference technique. We found that extracellular ATP induced a Ca^{2+} transient in a concentration dependent manner in hESCs, while such response was completely abolished by pre-treatment of P2 receptor antagonist suramin or pyridoxal-phosphate-6-azophenyl-2',4'-disulfonate (PPADS). In addition, the Ca^{2+} transient was not affected by withdrawal of extracellular Ca^{2+} but was abolished by IP3 receptor (IP_3R) inhibitors. Meanwhile, IP_3R knockdown significantly reduced the percentage of the responding cells to ATP. Using an extracellular solution with high Ca^{2+} concentration, store operated Ca^{2+} entry (SOCE) was observed when intracellular Ca^{2+} stores were depleted by endoplasmic reticulum Ca^{2+} ATPase blockers. These results suggest that intracellular Ca^{2+} store accounts for the ATP-induced Ca^{2+} release through IP_3R in hESCs, and hESCs already have the molecular basis responding to extracellular stimuli by mobilizing a robust Ca^{2+}

signaling cascade.

T-2049
SP5 MODULATES STEM CELL SELF RENEWAL AND DIFFERENTIATION DOWNSTREAM OF WNT SIGNALING

Huggins, Ian J.[1], Gaasterland, Terry[2], Willert, Karl[1]
[1]Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, USA, [2]Scripps Genome Center, La Jolla, CA, USA

Tight control of stem cell self renewal and differentiation is crucial for proper development and tissue homeostasis. Wnt proteins regulate these processes in multiple stem cell types throughout embryogenesis and in adult tissues such as the brain, intestines, and skin. To determine the transcriptional effects of Wnt signaling on human embryonic stem cells (hESCs), we performed whole transcriptome sequencing on these cells following Wnt3a treatment. The gene encoding the transcriptional repressor SP5 was among the most highly upregulated, a finding that was confirmed by quantitative RT-PCR in hESCs as well as in hESC-derived neural progenitor cells. SP5 antagonizes the action of SP1, a ubiquitously expressed transcription factor which occupies the promoter regions of thousands of genes. We hypothesize that SP5 mediates the effects of Wnt signaling in stem cells primarily through repression of SP1-regulated genes. In support of this hypothesis, treatment of hESCs with IWP-2, an inhibitor of PORCN that blocks Wnt protein secretion, leads to decreased SP5, POU5F1, and NANOG expression. Knockdown of SP5 by shRNA recapitulates this effect. Overexpression of SP5 together with GFP in hESCs leads to an increase in the percentage of GFP-positive cells at each passage versus overexpression of GFP only, indicative of a proliferative or survival advantage conferred by SP5. Finally, chromatin immunoprecipitation followed by sequencing reveals that Wnt3a stimulation of hESCs drives a genome wide decrease in SP1 binding with a concurrent increase in SP5 binding at many loci. Together these data suggest an important role for SP5 in Wnt-mediated regulation of stem cell self renewal and differentiation.

T-2050
NRF2, A REGULATOR OF THE PROTEASOME, CONTROLS SELF-RENEWAL AND PLURIPOTENCY IN HUMAN EMBRYONIC STEM CELLS

Jang, Jiwon
Neuroscience Research Institute, University of California Santa Barbara, Santa Barbara, CA, USA

Nuclear factor, erythroid 2-like 2 (Nrf2) is a master transcription factor for cellular defense against endogenous and exogenous stresses by regulating expression of many antioxidant and detoxification genes. Here, we show that Nrf2 acts as a key pluripotency gene and a regulator of proteasome activity in human embryonic stem cells (hESCs). Nrf2 expression is highly enriched in hESCs and dramatically decreases upon differentiation. Nrf2 inhibition impairs both the self-renewal ability of hESCs and reestablishment of pluripotency during cellular reprogramming. Nrf2 activation can delay differentiation. During early hESC differentiation, Nrf2 closely co-localizes with OCT4 and NANOG. As an underlying mechanism, our data show that Nrf2 regulates proteasome activity in hESCs partially through proteasome maturation protein (POMP), a proteasome chaperone, which in turn controls the proliferation of self-renewing hESCs, three germ layer differentiation and cellular reprogramming. Even modest proteasome inhibition skews the balance of early differentiation toward mesendoderm at the expense of an ectodermal fate by decreasing the protein level of cyclin D1 and delaying the degradation of OCT4 and NANOG proteins. Taken together, our findings suggest a new potential link between environmental stress and stemness with Nrf2 and the proteasome coordinately positioned as key mediators.

T-2051
FOXD3 COUNTERACTS NFAT-INDUCED DIFFERENTIATION TO MAINTAIN SELF-RENEWAL OF EMBRYONIC STEM CELLS

Jin, Ying[1], Zhang, Shiyue[2], Zhu, Lili[3]
[1]Institute of Health Sciences, Shanghai, China, [2]Shanghai Institutes for Biological Sciences, CAS, Shanghai, China, [3]Institute of Genetic Medicion, Newcastle Upon Tyne, United Kingdom

Pluripotency-associated transcription factor Foxd3 is required for maintaining pluripotent cells. However, the molecular mechanisms underlying its function are largely unknown. Here, we report that Foxd3 maintains the ESC identity through counteracting differentiation induction of Calcienurin-NFAT signaling. Mechanistically, Foxd3 interacts with NFAT proteins and represses NFATc's transcriptional activities via recruiting co-repressor Tle4, a member of the Tle suppressor family highly expressed in undifferentiated ESCs. Further global transcriptome analysis shows that Foxd3 and NFATc3 co-regulate a set of differentiation-associated genes in ESCs. Collectively, our study establishes a direct molecular and functional link between a pluripotency-associated factor and an important ESC differentiation-inducing pathway.

T-2052
PLURIPOTENT STEM CELL VERIFICATION: 3 WAYS TO UTILIZE A SINGLE WELL OF A 24-WELL PLATE

Johnson, Wade, Wegner, Greta, Anderson, Marnelle, Tousey, Susan, Aho, Joy L.
R & D Systems, Minneapolis, MN, USA

Pluripotent stem cell verification is a crucial process both for newly established cell lines as well as during routine culture. Here, we describe three methods for easy stem cell verification using the small sample size of a 24-well plate: 1) GloLIVE™, live-cell imaging antibodies allow for quick verification of pluripotent marker expression in live cells within 30 minutes, 2) Membrane-based multiplex antibody array analysis verifies the expression of 15 different pluripotent and differentiated cell markers from the lysate of a single well of a 24-well plate, in less time than it takes to perform a single Western blot, and 3) Directed tri-lineage differentiation functionally verifies cell potency within 5 days. Together these tools assess cell quality with ease and reliability, leading to consistency between experiments while saving time, money, and reagents.

T-2053
FEEDER-FREE CULTURE MODEL SUPPORTING THE PROPAGATION OF HUMAN PLURIPOTENT STEM CELLS ON GELATIN SUBSTRATUM BY CXCR2

Jung, Ji Hye[1], Yong, Park[2], Byung Soo, Kim[2]
[1]Biomedical and Science, Institution of Stem Cell Research, Korea University, Seoul, Republic of Korea, [2]Internal Medicine, Institution of Stem Cell Research, Korea University, Seoul, Republic of Korea

The purpose of this study is to establish the efficient humanized feeder-free culture system of human pluripotent stem cells(hPSCs) and explore the possible different mechanism of undifferentiated propagation of hPSCs in feeder-free culture condition. Currently, feeder-free propagation of human pluripotent stem cells (hPSCs) requires Matrigel or laminin and exogenous supplementation of bFGF which is known to be a master factor for sustaining pluripotency. We

previously reported that cells derived from human placenta chorion support ex vivo propagation of hPSCs without bFGF supplementation and showed the role of bFGFs synthesized in placenta feeder cells. Based on these studies, we investigated the role of human placenta-derived cell conditoned media (hPCCM) in maintaining pluripotency of hPSCs in vitro. Current study revealed that hPCCM support the pluripotency of hPSCs on gelatin substratum without exogenous bFGF supplementation and that CXCR2 and related ligands are a key factor in maintaining the pluripotency independently of bFGF. This culture system makes contamination by animal protein in hPSCs be avoidable and dramatically decrease the overall costs for ex vivo maintaining hPSCs. Therefore, we expect that safety and cost issues of cell therapeutics based on hPSCs might be resolved by using this system.

T-2054
WEEKEND-FREE PROTOCOL TO CULTURE HUMAN PLURIPOTENT STEM CELLS USING MTESR™1 OR TESR™-E8™

Kardel, Melanie Dawn, Kwok, Raymond, Norberg, Jessica, Olson, Melanie, Hadley, Erik, Antonchuk, Jennifer, Thomas, Terry E., Eaves, Allen C., Louis, Sharon A.
STEMCELL Technologies Inc., Vancouver, BC, Canada

Human pluripotent stem cells (hPSCs) have enormous potential for use in basic research, disease modeling, drug screening, and regenerative medicine. However, culturing hPSCs is labor-intensive, requiring daily medium changes and vigilant monitoring of colony morphology to determine the ideal passaging day. These protocols routinely require researchers to work on the weekend, which is problematic for many labs with regards to scheduling experiments or staff. A "weekend-free" (WF) protocol would require a 7-day passaging interval and permit undifferentiated cells to be maintained when left for two days without a medium change. By controlling the aggregate size and density such that cells were routinely passaged at 7-day intervals, we were able to eliminate the need to feed cultures for two days after seeding, and developed a WF protocol for use with mTeSR™1 and TeSR™-E8™ media. In the WF protocol, we used optimized conditions for a 7-day passaging interval in which small aggregates of about 80 - 150 µm in diameter were seeded at a low density of 35 - 50 aggregates/cm^2. Cultures were passaged on Fridays using these conditions; however media changes were performed either according to standard recommendations for TeSR™ culture (control) or daily after the first 2 days (WF). The protocols were tested with human embryonic stem cells (hESCs; H1, H9) or human induced pluripotent stem cells (hiPSCs; WLS-1C, WLS-4D1) maintained in mTeSR™1 or TeSR™-E8™ on Matrigel®, in duplicate, for 10 passages (p). Cultures were assessed at each passage for ideal colony morphology (dense centers with homogeneous and tightly packed cells) and expansion, and analyzed after 10p for expression of undifferentiated cell markers OCT4 and TRA-1-60. High expansion was observed over 10p using the WF protocol (mean fold-expansion per passage ± SEM: mTeSR™1: H1 63±10, WLS-1C 37±4, WLS-4D1 27±3; TeSR™-E8™: H9 63±6, WLS-1C 23±3, WLS-4D1 30±5; n=1 in duplicate). Morphology was consistently excellent in all cultures grown using the WF protocol, with cells expressing OCT4 (mTeSR™1 98.2±0.7%, TeSR™-E8™ 98.8±0.1%; mean ± SEM, n=3) and TRA-1-60 (mTeSR™1 98.8±0.6%, TeSR™-E8™ 97±2%; n=3) after 10p. These results were equivalent to those obtained with the control protocol. We demonstrated that cells maintained > 10p using the WF protocol retained a normal karyotype and the ability to differentiate into the three germ layers. Finally, the WF protocol was used in a completely defined culture system with H1 cells maintained on Vitronectin XF™ (developed and manufactured by Primorigen Biosciences) with either mTeSR™1 or TeSR™-E8™ (n=1 in duplicate for each medium). After 6p WF, H1 cells exhibited excellent morphology and expressed OCT4 (mTeSR™1 94.2%, TeSR™-E8™ 96.9%). High fold-expansion of these cultures was also achieved over 5p using the WF protocol (mean fold-expansion per passage ± SEM: mTeSR™1: 50±4, TeSR™-E8™: 27±6). These results were also equivalent to those obtained with the control TeSR™ protocol. In summary, by controlling hPSC aggregate size and seeding density from mTeSR™1 or TeSR™-E8™ cultures, we established a 7-day passaging interval, and showed that no medium change was required for the first 2 days after cell seeding. In practical terms, hPSCs cultured in TeSR™ media could be passaged on a Friday, and then left unmanipulated for Saturday and Sunday, thus providing a convenient weekend-free protocol for maintaining hPSCs.

T-2055
DERIVATION AND CHARACTERIZATION OF PRIMED EMBRYONIC STEM CELLS FROM PORCINE EMBRYOS OF DIFFERENT ORIGINS

Kim, Eunhye[1], Lee, Chang-Kyu[2], Hyun, Sang-Hwan[1]
[1]College of Veterinary Medicine, Chungbuk National University, Cheongju, Republic of Korea, [2]Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea

Embryonic stem cells (ESCs) derived from the inner cell mass of the developing embryo represent the ability to self-renew indefinitely and the capacity to differentiate into ectoderm, endoderm, and mesoderm either *in vitro* or *in vivo*. However, these properties have been demonstrated only for mouse ESCs whereas other species including pigs, cattle, rats, sheep, goat, primates and humans have been showed limited capacities until now. Pigs are significant as a disease model in translational research, although several putative ES cell lines have been reported from embryos, authentic ES cell lines don't established. In this study, total 7 primed ESC lines were derived from porcine embryos of various origins, including *in vitro* fertilized (IVF), parthenogenetic activation (PA) and nuclear transfer (iPS-NT) from a donor cell with induced pluripotent stem cells (iPSC). To characterize these cell lines we performed several approaches, including morphological observation, intensive alkaline phosphatase activity, the strongly pluripotency-associated genes such as *POU5F1* (formerly known as *OCT4*), Sox2 and *NANOG*, immunocytochemistry analysis, *in vitro* differentiation potential and embryoid body formation. Furthermore, we investigated morphological changes of colonies during the early process of the derivation of primed ESCs. During the culture of ESCs, we classified two colony types (normal and transformed colony) and three subpopulations of ES cells composing transformed colony with intrinsic morphological characteristics: petaloid rapidly self-renewing cells, small spindle-shaped cells and large flattened cells. It is thought that the distribution ratio of these ES cell types represents the current quality of transformed colony and helps to estimate the quality in the future. Establishment of porcine naïve ESCs, especially iPS-NT ESCs, will make possible sophisticated genetic manipulation to create ideal animal models for pre-clinical research and studies of human diseases. This study will help to approach the goal for establishing naive pluripotent stem cells in pigs.

T-2056

A VERSATILE AND ROBUST XENO- AND SERUM-FREE CULTIVATION SYSTEM FOR HUMAN PLURIPOTENT STEM CELLS

Kurtz, Annett[1], Bretz, Andrea[1], Finkbeiner, Judith[1], Juengerkes, Frank[1], Oleszynski, Christiane[1], Chatrousse, Laure[2], Kropp, Christina[3], Olmer, Ruth[3], Zweigerdt, Robert[3], Rockel, Thomas D.[1], Bosio, Andreas[1], Girard, Mathilde[2], **Knoebel, Sebastian**[1]

[1]Miltenyi Biotec GmbH, Bergisch Gladbach, Germany, [2]I-STEM, EVRY, France, [3]REBIRTH - Center for Regenerative Medicine, Hannover, Germany

Pluripotent stem cells (PSC) have traditionally been cultured on mouse embryonic feeder (MEF) cells which contribute to maintenance of pluripotency and deposit extracellular matrix components conferring cell attachment. Though constituting a relatively robust cultivation environment when monitored rigorously, mEF based systems are prone to lot-to-lot variances. Furthermore, the xenogeneic nature of mEF cells and commonly used media components is not compliant with current efforts to establish clinically compatible protocols for maintenance and differentiation of PSC. Different compositions have been devised in order to maintain pluripotency in feeder-independent conditions. However, most media require extensive adaption periods when cells are transferred from feeder-dependent to feeder-free culture conditions. We have optimized a xeno- and serum free media formulation that a) allows rapid adaption to feeder-free conditions, i.e. culture on Matrigel or Vitronectin, b) enables robust and efficient expansion of bona fide PSC as single cells as well as cell-clusters for more than 10 passages while maintaining pluripotency as evidenced by marker expression, in vitro differentiation potential, teratoma formation and karyotyping, c) allows rapid culture initiation after cryopreservation and genetic manipulation, and e) supports episomal reprogramming of human fibroblasts. Furthermore, preliminary observations suggest that the medium supports formation of aggregates from single cell inoculated hPSCs and their expansion in suspension culture. The formulation will allow a rapid translation into a clinical grade medium designed following the recommendations of USP <1043> on ancillary materials and will be suitable for clinical grade expansion of PSC.

T-2057

TEC KINASE MODULATES AUTOCRINE/PARACRINE FGF2 SIGNALING IN HUMAN PLURIPOTENT STEM CELLS

Konecna, Zaneta[1], Obadalova, Tereza[1], Kunova, Michaela[2], Krejci, Pavel[1], Dvorak, Petr[2]

[1]Masaryk University, Faculty of Medicine; FNUSA ICRC, Brno, Czech Republic, [2]Masaryk University, Faculty of Medicine, Brno, Czech Republic

Human pluripotent stem (hPS) cells are known for their unique ability to be propagated in undifferentiated state and for their large potential in clinical applications. The undifferentiated state of hPS cells is significantly supported by fibroblast growth factor 2 (FGF2) which is commonly supplemented into their culture media. Four isoforms of FGF2 are also produced by hPS cells themselves while only low molecular mass (LMM) isoform of FGF2 can be secreted into the extracellular space. It is believed that FGF2 secretion is followed by its autocrine/paracrine signaling, maintaining pluripotency of hPS cells. However, the mechanism by which LMM FGF2 is secreted remains largely unknown. Recently, TEC (tyrosine kinase expressed in hepatocellular carcinoma) kinase was described to be necessary for LMM FGF2 secretion in HeLa cells via direct interaction and phosphorylation of FGF2 by TEC. We therefore wondered whether TEC possessed this function also in hPS cells, and whether this could be used to precisely describe the autocrine/paracrine loop of FGF2 action. In our study, we found TEC kinase to be expressed in hPS cells and to interact with FGF2. TEC was preferentially localized in the cytoplasm where it often clustered in punctuated structures of yet unknown origin. Our data suggest that these structures are likely not connected with endocytosis, apoptosis, cell cycle or priming of hPS cells to differentiation. We found TEC to be expressed at high levels in hPS cells, rapidly diminishing during neural differentiation. In contrast, TEC levels remained stable during 15-day embryoid body differentiation, with only a minor decrease during first five days of differentiation. These data suggest that TEC kinase has an important role in undifferentiated hPS cells, but also in differentiation towards endoderm and/or mesoderm. To study the role of TEC in LMM FGF2 secretion, we generated hPS clones with downregulated TEC expression (TEC-KD). These clones possessed impaired proliferation and decreased colony forming efficiency. Importantly, the amounts of secreted LMM FGF2 were significantly lower in TEC-KD compared to hPS clones expressing scrambled shRNA. Taken together, we showed the importance of TEC for hPS phenotype. The main function of TEC probably lies in the modulation of FGF2 autocrine/paracrine loop. We believe that this could be used to manipulate hPS cells for more efficient differentiation.

T-2058

FGF19/BETA-KLOTHO REGULATE PHYSIOLOGY AND DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

Kunova, Michaela[1], Pavel, Krejci[2], Lukas, Trantirek[3], Dvorak, Petr[1]

[1]Masaryk University, Faculty of Medicine, Brno, Czech Republic, [2]Masaryk University, Faculty of Medicine; FNUSA-ICRC, Brno, Czech Republic, [3]CEITEC, Brno, Czech Republic

To propagate human pluripotent stem cells (hPSC), both embryonic (hESC) and induced pluripotent (hiPSC) in vitro, they must be supplied with several cytokines, with the most potent being fibroblast growth factor 2 (FGF2). Because of the instability of FGF2 and intense feeding requirements of hPSC, the search for alternate ligands emerged. Therefore, we focused on other members of the FGF family in order to identify novel players that trigger the pluripotency program in hPSC. We analyzed the expression of entire FGF family (FGF1-10, 16-23) in seven well-characterized hESC lines and two hiPSC lines. RT-PCR identified four FGF genes expressed in hPSC, i.e. FGF1, FGF2, FGF4 and FGF19. We have further focused on FGF19, as its role in hPSC had never been described. In addition to mRNA, we also found FGF19 proteins to be expressed in hPSC by immunoblotting and immunocytochemistry. The high-affinity FGF receptors (FGFR) for FGF19 - FGFR1IIIc and FGFR4 - are expressed in hPSC. In vivo, FGF19 also requires the co-receptor beta-Klotho. Indeed, we found beta-Klotho to be expressed in hPSC with an interesting pattern of beta-Klotho-high cells being present in multilayered centers of hPSC colonies. A typical feature of hPSC is the heterogeneity among cell population, representing issues with differentiation efficiencies and in drug screening. Typically, a centre-to-edge gradient among hPSC colonies is described. We found recFGF19 to induce phosphorylation of ERK1/2 predominantly in centers of colonies, thus in regions that were beta-Klotho-high. In addition to ERK1/2, immunoblotting identified FRS2α and AKT to be phosphorylated upon FGF19 treatment in hPSC. Thus, the FGFR signaling similar to that of FGF2-treated cells is induced upon FGF19 treatment in hPSC. Altogether, we showed the concomitant expression of FGF19 and its co-receptor beta-Klotho in hPSC, and the ability of exogenous FGF19 to activate FGFR signaling therein. One area of FGF19 function may lie in maintenance of hPSC undifferentiated phenotype in a fashion similar to the FGF2. Alternatively, FGF19 may have more metabolic actions in hPSC,

possibly regulating glucose uptake in beta-Klotho-high cells growing in the middle of the colonies, which suffer from limited glucose and oxygen supply. In support of this speculation, we found upregulation of β-Klotho expression in hESC growing in hypoxic environment, and increased glucose metabolism in hESC exposed to FGF19. In vivo, beta-Klotho is predominantly expressed in endodermal tissues. Indeed, we found beta-Klotho to become significantly more expressed during endodermal differentiation induced by Activin A and/or BMP4. Therefore, the FGF19/beta-Klotho signaling may represent a novel pathway to be targeted, as the endodermal derivatives are generally difficult to obtain from hPSC. Taken together, our data open an attractive possibility for yet another FGF ligand, FGF19, being involved in regulation of hPSC physiology and differentiation. Beta-Klotho-high hPSC may represent a cell population poised to a distinct cell fate, making them an interesting target for differentiation into a desired cell type.

T-2059
INVESTIGATING THE ROLE OF FOXM1 IN THE MAINTENANCE OF HUMAN EMBRYONIC STEM CELL PLURIPOTENCY

Kwok, Chun Ting Davis[1], Leung, Man Hong[1], Lee, Yin Lau[2], Yao, Kwok-Ming[3]
[1]The University of Hong Kong, Hong Kong, [2]The University of Hong Kong, Hong Kong, China, [3]University of Hong Kong, Hong Kong

Human embryonic stem cells (hESCs) are capable of infinite proliferation and hold the potential to differentiate into various cell lineages for cell and tissue transplantation. Many factors have been identified to regulate the self renewal and pluripotency of hESCs, yet the complete regulatory mechanism has not be fully elucidated1. The proliferation-associated Forhkead box transcription factor FOXM1 has been reported to be involved in the maintenance of pluripotency in mouse embryonal carcinoma cells. In this study, we showed that FOXM1 is expressed in the hESC line VAL3, with expression found in both the nucleus and the cytoplasm. Using bivariate flow cytometry analysis, FOXM1 was shown to display cell cycle-dependent changes in expression in asynchronus VAL3 cells, with peak levels observed at the G2/M phase. Using chromatin immunoprecipitation assay, FOXM1 was demonstrated to bind the promoters of OCT4 and NANOG, as well as the promoters of cell cycle genes. However, depleting FOXM1 in undifferentiated VAL3 cells using siRNAs did not result in significant down-regulation of OCT4 and NANOG. As VAL3 differentiated, either as embryoid bodies or after retinoic acid induction, FOXM1 expression was found to decrease gradually and reach low levels at late differentiation stages. Taken together, our findings suggest that FOXM1 is not likely to be an upstream regulator of the core pluripotent network in hESCs. Future work will focus on the role of FOXM1 in mediating the self renewal and proliferation of hESCs.

T-2060
FIP1 REGULATES MRNA ALTERNATIVE POLYADENYLATION TO PROMOTE STEM CELL SELF-RENEWAL

Lackford, Brad[1], Hu, Guang[2]
[1]NIEHS, RTP, NC, USA, [2]NIEHS/NIH, RTP, NC, USA

Alternate polyadenylation (APA) of mRNA transcripts has shown to play a critical role in post-transcriptional gene control and has been shown to be tightly regulated during development and in human diseases. However, biological consequences of APA and the regulatory mechanisms thereof have yet to be fully elucidated. Here, we show that Fip1, an mRNA 3' processing factor belonging to the CPSF complex, is indispensible for the maintenance of mESCs self-renewal and pluripotency as well as somatic cell reprogramming. Unlike the bulk of essential mESC genes that regulate self-renewal and pluripotency through transcriptional regulation, Fip1 exerts it control, in apart, by maintaining an ES cell specific APA program. The maintenance of this profile allows for optimal expression of a subset of critical self-renewal factors. This profile is absent in somatic cells such as MEFs and is restored to an ESC-like state during four factor reprogramming along with a concomitant increase Fip1 levels. Mechanistically, Fip1 acts through the recognition of a suboptimal poly-adenylation site (PAS) to produce mRNAs with a shorter 3' UTR and that this recognition is dependent upon the level of Fip1 expression. Taken together, our data, for the first time, assigns as specific biological significance to APA as a novel model for the post-transcriptional regulation of self-renewal and pluripotency in mESCs. Furthermore, we identify a specific regulator of APA in mESCs, Fip1, and provide new mechanistic insights into APA regulation and 3' mRNA processing.

T-2061
TGIF1 MAINTAINS THE IDENTITY OF MOUSE EMBRYONIC STEM (ES) CELLS BY DIRECT REPRESSION OF THE CORE PLURIPOTENCY FACTORS

Lee, Bum-Kyu, Shen, Wenwen, Rhee, Catherine, Cook, Kendra, Kim, Jonghwan
Department of Molecular Biosciences, Institute for Cellular and Molecular Biology, The University of Texas at Austin, Austin, TX, USA

TGFβ/Activin/Nodal signaling has been implicated in the maintenance of pluripotency in human ES cells and mouse epiblast stem cells via the positive regulation of Nanog. However, prior studies suggest TGFβ/Activin/Nodal signaling is not a critical pathway in the maintenance of mouse ES cells. A homeodomain transcription factor, Tgif1 (TG-interacting factor 1), is known to play an important role in TGFβ signaling as a terminal co-repressor of the pathway in somatic cells. Recent genome-scale target mappings of the core pluripotency factors, including Oct4, Sox2, and Nanog, in mouse ES cells have revealed the regulatory region of Tgif1 is occupied by multiple core pluripotency factors. More recent study of interactions among gene regulatory elements using ChIA-PET show a preferential regulation of Tgif1 by mouse ES cell specific enhancers, collectively suggesting Tgif1 may play important roles in mouse ES cells, possibly in a TGFβ/Activin/Nodal signaling pathway independent manner. Here, we report Tgif1 is a novel member of the previously known mouse ES cell core regulatory network, and precisely controls the activity of core pluripotency factors. Both knockdown (KD) and overexpression (OE) of Tgif1 abrogate typical colony morphology of mouse ES cells along with the loss of alkaline phosphatase (AP) activity. KD of Tgif1 also dramatically hampers the generation of induced pluripotent stem (iPS) cells, indicating that Tgif1 is required for not only the maintenance of ES cells, but also the acquisition of induced pluripotency. To further elucidate the regulatory mechanisms of Tgif1 in self-renewing ES cells, we mapped the chromosomal targets of Tgif1, revealing that Tgif1 directly regulates many ES cell core pluripotency factors. With further analyses of global gene expression profiles upon OE and KD of Tgif1, we found Tgif1 negatively regulates the core pluripotency-related gene expression program in mouse ES cells. From an additional protein complex pull-down assay, we confirmed Tgif1 is a novel integral member of the core pluripotency protein interaction network. Taken together, we identified a novel negative regulator of mouse ES cell core regulatory network. As an indispensable factor for the maintenance of mouse ES cells, Tgif1 functions as a molecular rheostat for controlling the precise level of ES cell core factors.

T-2062

FEEDER CELL DENSITY AFFECTS HUMAN PLURIPOTENT STEM CELL GROWTH AND SELF-RENEWAL

Lee, Sunray, Park, Hyun-Sook, Lee, Jin Yup, Park, Jang-Mi, Hwang, Yu-Sik
CEFO Research Center, Seoul, Republic of Korea

The initial derivation of human embryonic stem (hES) cells and induced pluripotent stem (iPS) cells is generally performed on mouse embryonic fibroblast (MEF) feeder layers. However, it has not been well reported how MEF feeder cells affect the growth of pluripotent stem cells. In this study, we investigated the effect of MEF feeder cell density on the self-renewal of pluripotent stem cell using three different hES cell lines: UC06 (HSF6), WA09 (H9) and Miz-hES4 (Miz4) and one iPS cell line (MC09-v3). The results showed that each pluripotent stem cell line has its own optimal range of MEF feeder cell densities in which the self-renewal and growth of stem cells are maximally supported. A less than optimal MEF feeder cell density could not fully support self-renewal, as indicated by positive alkaline phosphatase (AP)-staining, and by the expression of Oct4 and Tra1-60. On the other hand, an MEF feeder cell density that was too high hindered the attachment and growth of pluripotent stem cell colonies, and prevented cell expansion. Additionally, the degree of apoptosis, as measured by the TUNEL assay, revealed that stem cell survival was fully supported on the optimal range of MEF feeder cell densities. The factors from the optimal range of feeder cell density appeared the optimal concentration of extracellular matrix (ECM) rather than cytokines. These results suggested that the density of MEF feeder cells is critical when reconstructing an optimal culture environment for pluripotent stem cells via composition of ECM. This study was supported by a grant (N01090009) form Global R and D Project of the Korea Institute for Advancement of Technology, Ministry of Knowledge Economy and a grant of the Korea Healthcare technology R and D Project, Ministry for Health, Welfare and Family Affairs, Republic of Korea. (A121962).

T-2063

INVESTIGATING THE PROTECTIVE ROLE OF FOXM1 AGAINST OXIDATIVE STRESS AND DNA DAMAGE IN HUMAN EMBRYONIC STEM CELLS

Leung, Man Hong[1], Kwok, Chun Ting Davis[1], Lee, Yin Lau[2], Yao, Kwok-Ming[3]
[1]Biochemistry, The University of Hong Kong, Hong Kong, [2]The University of Hong Kong, Hong Kong, China, [3]University of Hong Kong, Hong Kong

Human embryonic stem cells (hESCs) hold immense potential for tissue engineering and regenerative medicine. For hESCs to function properly, it is hypothesized that they posses stringent mechanisms to maintain genome integrity. The Forkhead box transcription factor FOXM1 is ubiquitously expressed in embryonic tissues that are highly proliferative and regenerative. Recent studies have shown that FOXM1 is critically required for protection against cellular senescence and as a mediator of the DNA damage response. In human primary fibroblasts, FOXM1 regulates the expression of antioxidant enzymes, including MnSOD and catalase, which defend against reactive oxygen species. DNA repair genes like BRIP1 and RAD51 have recently been shown to be the transcriptional targets of FOXM1. We hypothesized that FOXM1 is a critical regulator for maintaining genome stability in hESCs. In this study, FOXM1 was found to be expressed at high levels in the hESC cell line VAL-3. Knockdown of FOXM1 in VAL-3 cells using specific siRNAs reduced proliferation but did not affect pluripotency. Moreover, FOXM1-depleted VAL-3 cells became sensitized to oxidative stress. Interestingly, comparison of mRNA and protein levels suggested that the FOXM1 protein was stabilized upon hydrogen peroxide treatment. These findings suggest that FOXM1 may play a role in protecting hESCs against oxidative stress. Further studies will be conducted to explore whether FOXM1 is also required for protecting against other genotoxic stresses and the downstream transcriptional targets involved.

T-2064

REPROGRAMMING AND CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELLS ON A SUBSTRATE WITH IMMOBILIZED CELL ADHESION PEPTIDES

Lin, Jia-yi, Li, Hsing-Fen, Higuchi, Akon
National Central Univeristy, Jhongli City, Taiwan

Human induced pluripotent stem cells (hiPSCs) have significant potential in therapeutic applications for many diseases because they have the specific ability to differentiate into all types of human somatic cells. However, the tentative clinical potential of hiPSCs is restricted by the use of mouse embryonic fibroblasts (MEFs) as a feeder layer. The feeder-free cultures using synthetic biomaterials having nanosegments as stem cell culture materials offer more reproducible culture conditions and lower the cost of production without introducing xenogenic contaminants. These improvements will increase the potential clinical applications of differentiated hiPSCs. Here we report that hiPSCs can be successively generated without usage of a feeder layer of MEFs during generation of hiPSCs by transfection of retrovirus containing pluripotent genes into human adipose-derived stem cells (hADSCs) where hiPSCs were cultured on polyvinylalcohol-co-itaconic acid (PVA-IA) grafted with several nanosegments (KGGPQVTRGDVFTMP [cell-binding domain derived from vitronectin, oligoVN], KGGNGEPRGDTYRAY [cell-binding domain from bone sialoprotein, oligoBSP], and GKKQRFRHRNRKG [heparin-binding domain, oligoHBD]). The elasticity of PVA-IA dishes grafted with nanosegments was regulated from 10.3 kPa to 30.4kPa by control of crosslinking time of PVA-IA. At day 4 after transfection, hADSCs transfected with pluripotent genes (Oct4, Sox2, Klf4, and c-Myc) were shifted to be cultured on MEFs as control experiments and on PVA-IA dishes grafted with nanosegments. hiPSC colonies were clearly observed for the cells cultured on MEFs at day 14 after transfection, while hiPSC colonies were clearly detected on dishes grafted with oligoVN and oligoBSP having elasticity from 10.3kPa to 30.4kPa after the passage of the cells. The number of colonies generated on MEFs was 120±28 per dishes, while that generated on VN-dishes was 20-80 per dishes when 105 hADSCs were seeded on the dishes. It was found that the efficiency of hiPSC generation on the VN-dish at feeder-free conditions was less than that on MEFs. However, the hiPSC colony showed alkali phosphatase activity much clearly, and immunohistochemistry suggested that the hiPSCs were generated on PVA-IA dishes grafted with oligoVN and oligoBSP expressing pluripotent protein of SSEA-4 at feeder-free conditions. hiPSCs prepared on MEFs as well as PVA-IA dishes having nanosegments generated teratoma and embryonic bodies containing different cell types of the three germ layers, which suggest hiPSCs cultured on PVA-IA dishes having nanosegments keep their pluripotency and can differentiate into the cells of three germ layers. There are several reports for the culture of hiPSCs on feeder-free conditions. However, hiPSCs were generated and cultured on MEFs at first before the culture of hiPSCs on feeder-free conditions in most of cases. This study reports that hiPSCs have been generated on synthetic dishes (PVA-IA dishes grafted with nanosegments) at feeder-free culture. It was found that the optimal elasticity (25.3kPa to 30.4kPa) and specific nanosegments (oligoVN and oligoBSP) of the cell culture dishes improve to keep pluripotency of hiPSCs on the dishes where hiPSCs were cultured.

T-2065
REGULATION OF TRANSCRIPT SPLICING BY SON IN EMBRYONIC STEM CELLS

Lu, Xinyi, Goeke, Jonathan, Sachs, Friedrich, Ng, Huck Hui
Genome Institute of Singapore, Singapore

Human embryonic stem cells have the ability to differentiate into clinically relevant cell types. In the maintenance of human ES cell pluripotency, transcription factors and epigenetic modifiers play an essential role. However, there is scarce knowledge about the regulation of pluripotency through transcript splicing. In this study, we aim to examine the function of the spliceosome-associated factor SON in human ES cells. We show that SON is upregulated in human ES cells as compared to differentiated cells. In addition, SON is important for both the maintenance and induction of pluripotency in human ES cells and MRC-5, respectively. Furthermore, we identified SON-regulated transcripts through RNA-seq. We also confirmed that SON binds to transcripts encoding for pluripotency regulators and regulates their splicing in human ES cells. Together, our results link splicing regulation of transcript production to self-renewal and pluripotency in human ES cells.

T-2066
A SOX2 DISTAL ENHANCER CLUSTER REQUIRED FOR EMBRYONIC STEM CELL PLURIPOTENCY

Zhou, Harry, Katsman, Yulia, Collura, Felicia, Dhaliwal, Navroop Kaur, Davidson, Scott, Macpherson, Neil N., **Mitchell, Jennifer A.**
Cell and Systems Biology, University of Toronto, Toronto, ON, Canada

SOX2 is part of the core regulatory network of transcription factors required for pluripotency maintenance and cellular reprogramming. Transcription of *Sox2* is complex as the gene is expressed at high levels in pluripotent cells and down-regulated upon differentiation to endoderm or mesoderm while being maintained in the neuroectodermal lineage. Although the role of the SOX2 protein in maintaining pluripotency and contributing to reprogramming has been examined less is known about the sequences regulating the *Sox2* gene at the transcriptional level. Two gene proximal enhancers, *Sox2* regulatory region 1 (SRR1) and SRR2, display enhancer activity in reporter assays, but we show here that they are not sufficient for expression of *Sox2* in embryonic stem (ES) cells. The *Sox2* gene is located in a gene desert yet there is a striking diverse set of occupied transcription factor binding sites in ES cells within a 130 kb region surrounding the *Sox2* gene. The greatest density of transcription factor binding lies between 104 and 112 kb downstream of the gene. This downstream region also recruits the histone acetyl transferase EP300 (p300), which is known to be bound at active tissue specific enhancers. As distal regulatory regions have been shown to be required for the regulation of gene expression over even megabase distances we investigated the role of distal regulatory elements in maintaining transcription of *Sox2* in ES cells. Our data reveal that the distal enhancer cluster located over 100 kb downstream of the *Sox2* transcription start site (TSS) is required for *Sox2* transcription in mouse ES cells and maintenance of the pluripotent phenotype. Chromatin looping brings the distal enhancer cluster into close physical proximity to the *Sox2* promoter in ES cells but not in mouse embryonic fibroblasts (MEFs). Furthermore, reporter analysis of putative enhancer regions surrounding *Sox2* revealed three new classical enhancers active in ES cells but not MEFs: SRR18, SRR107 and SRR111. Using the CRISPR/Cas9 genome editing system we deleted the 7.3 kilobase enhancer cluster containing SRR107 and SRR111 and revealed that these two enhancers are required for *Sox2* expression in ES cells. Deletion of SRR107 and SRR111 on both alleles caused greater than nine fold reduction in *Sox2* mRNA levels, loss of detectable SOX2 protein and differentiation into trophoblast-like cells indicating that the proximal regulatory sequences alone are not sufficient for *Sox2* expression in ES cells. This distal region which we term the Sox2 control region (SCR) is bound by eleven different ES cell expressed transcription factors including OCT4, SOX2, NANOG and KLF4 and requires chromatin loops supported by CTCF, the cohesin complex and bound by mediator to contact the *Sox2* promoter. Together these findings identify a distal enhancer cluster essential for *Sox2* transcription and maintenance of ES cell pluripotency.

T-2067
POLYCOMB GROUP PROTEIN PCGF6 REPRESSES MESODERMAL AND SPERMATOGENESIS SPECIFIC GENES IN ES CELLS AND REPLACES SOX2 IN IPS REPROGRAMMING

Muller, Albrecht[1], Zdzieblo, Daniela[1], Becker, Matthias[1], Lin, Qiong[2], Zenke, Martin[2]
[1]*Institute of Medical Radiology and Cell Research, University of Wuerzburg, Wuerzburg, Germany,* [2]*Institute for Biomedical Engineering - Cell Biology, Aachen, Germany*

Polycomb group (PcG) proteins comprise a large group of evolutionary conserved factors with essential functions for embryonic development and adult stem cell self renewal. PcG proteins constitute two main multiprotein polycomb repressive complexes (PRC1 and PRC2) that function in a hierarchical manner to silence gene expression. The literature describes functionally distinct PRC1 complexes that are defined by Polycomb group RING finger protein (PCGF) paralogs. So far, six PCGF paralogs (PCGF 1-6) have been identified. Paralog-specific functions are not well understood. In our studies, we observed that Pcgf6 showed the highest expression level in undifferentiated mouse ES cells, blastocysts and adult testes. When ES cells differentiated Pcgf6 expression strongly declined. To investigate the function of Pcgf6, we established dox-inducible shRNA knockdown (KD) ES cells. Following Pcgf6 KD the expression of pluripotency genes decreased, while mesodermal genes were de-repressed. Concomitantly, Pcgf6-KD ES cells showed increased hemangioblastic and hematopoietic potential, which is consistent with elevated expression of mesodermal markers. Microarray analysis revealed de-repression of spermatogenesis-specific genes. Finally, iPS reprogramming analysis showed that PCGF6 replaced SOX2 but not OCT4, KLF4 or c-MYC in the generation of germline-competent iPS cells. Together, these analyses show that Pcgf6 is non-redundantly involved in maintaining the pluripotent state.

T-2068
THE ROLE OF PROTEIN-PROTEIN INTERACTIONS IN THE FUNCTION OF NANOG

Mullin, Nick[1], Chambers, Ian[2]
[1]*Scottish Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, United Kingdom,* [2]*University of Edinburgh, Edinburgh, United Kingdom*

The ability of sequence specific transcription factors (TFs) to regulate gene expression relies on their abilities to bind DNA and to interact with partner protein molecules that mediate their downstream effects. Embryonic stem cell (ESC) self-renewal depends on a network of TFs centred on Oct4, Sox2 and Nanog. Using proteomic techniques we have identified an extended Nanog-interactome that contains over 130 proteins with diverse functions. Mutagenesis has been used to identify residues that mediate specific interactions and those mediating Nanog-Nanog homo-dimerization, each of which affects the ability of Nanog to support self-renewal of ES cells. We will present recent data that suggests how the loss of

Nanog-partner protein interaction(s) can affect stem cell function.

T-2069

THE ONCOFETAL PROTEIN SURVIVIN INTERACTS WITH LIN28A AND INHIBITION OF SURVIVIN EXPRESSION RESULTS IN DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

Mull, Amber, **Navara, Christopher**
University of Texas, San Antonio, San Antonio, TX, USA

Human embryonic stem (hES) cells express high levels of the oncofetal protein SURVIVIN. We have previously identified the expression of five SURVIVIN splice variants in hES cells (*SURVIVIN, SURVIVIN-ΔEx3, SURVIVIN-2B, SURVIVIN-3B and SURVIVIN-2α*). SURVIVIN, an inhibitor of apoptosis, is also a vital member of the chromosomal passenger protein complex, required for mitosis. Here we show directed differentiation of hES cells results in significant decrease of SURVIVIN expression. We used inducible shRNA against *SURVIVIN* to inhibit expression of all *SURVIVIN* variants and observed a concomitant reduction of pluripotent transcription factors *OCT4* and *NANOG* mRNA. Furthermore, shRNA inhibition of *SURVIVIN* resulted in a decrease of SURVIVIN, SOX2 and LIN28A protein expression and longer term inhibition of SURVIVIN resulted in cells with a differentiated morphology and loss of NANOG immunoreactivity . Co-immunoprecipitation experiments revealed LIN28A as a novel binding partner of SURVIVIN. Interestingly, both SURVIVIN and LIN28A have been implicated in cell cycle regulation of cancer cells. Tight control of cell cycle regulation is essential for hES cells to maintain pluripotency and it may be through this mechanism that pluripotency is affected in these cells.

T-2070

NEW PROTEOMIC INSIGHTS ON THE ROLE OF NPR-A IN REGULATING SELF-RENEWAL OF EMBRYONIC STEM CELLS

Abdelalim, Essam M.[1], Magdeldin, Sameh[2]
[1]*Qatar Biomedical Research Institute, Qatar Foundation, Doha, Qatar,* [2]*Department of Structural Pathology Institute of Nephrology, Niigata University, Niigata, Japan*

Embryonic stem (ESCs) have unlimited self-renewal potential, and can differentiate into several cell types, which represent ideal sources for cell-based therapy. The Signaling pathways that regulate the self-renewal of ESCs are of great interest. Recently, the NPR-A (natriuretic peptide receptor A) has been recognized as an important regulator for the self-renewal of ESCs. Here, we utilized a comprehensive label-free proteomics to identify new possible protein candidates involved in NPR-A pathway, which may control self-renewal of ESCs. Therefore, NPR-A gene was targeted using small interfering RNA (siRNA). NPR-A knockdown resulted in a loss of ESC self-renewal. Quantitative label-free shotgun proteomic analysis identified differentially expressed proteins related to regulation of pluripotency, proliferation, cell fate specification, chromosome organization, and apoptosis. Intriguingly, we found that in addition to Oct4, Nanog and Sox2, other proteins involved in ESC self-renewal were down-regulated after NPR-A knockdown, such as heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNPA2B1), Non-POU domain-containing octamer-binding protein (NONO), nucleoplasmin (Npm1), SW1/SNF complex, ribosomal proteins, polycomb protein SUZ12, and CDK4. Other proteins related to early differentiation and cell death were regulated as a result of NPR-A knockdown, including importin subunit alpha-4 (IMPα4), importin-5 (IPO5), H3 histones, core histone macro-H2A.1 (H2AY), apurine/apyrimidine endonuclease 1 (Apex1), 78-kDa glucose-regulated protein (GRP78), and programmed cell death 5 (PDCD5). These data offer a comprehensive view to our understanding of the pathways involved in the role of NPR-A in self-renewal of ESCs.

REGENERATION MECHANISMS

T-2071

MELATONIN COUPLING WITH MELATONIN RECEPTOR-2 STIMULATES $G_{\alpha Q}$ INTERACTION TO STIMULATE CYTOSKELETAL REORGANIZATION IN PROMOTING MESENCHYMAL STEM CELLS MOTILITY

Lee, Sei-Jung, Oh, Sang Yub, Suh, Han Na, Ryu, Jung Min, Kim, Dah Ihm, **Han, Ho Jae**
Department of Veterinary Physiology, BK21 PLUS Creative Veterinary Research Center, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

Melatonin (MT), a circadian rhythm-promoting molecule secreted mainly by the pineal gland, has a variety of biological functions and a potent inducer of neuronal cell migration. However, our understanding of the functional roles of MT in human umbilical cord blood derived mesenchymal stem cells (UCB-MSCs) is still fragmentary and incomplete. UCB-MSCs expressed melatonin receptor subtypes 2 (MT-2) only in membrane lipid raft. MT (1 μM) significantly increased UCB-MSCs motility after a 24 h incubation, which was inhibited by MT-2 knockdown. We found that Gαq, but not Gαi and Gα13 co-immunoprecipitated with MT-2, and the binding of Gαq to MT-2 was acutely stimulated by MT. In addition, MT in acting MT-2 stimulated an atypical PKC isoform, PKCζ, but not PKCα, PKCβ, PKCγ, PKCδ, PKCε, and PKCθ. Coupling of MT-2 with Gαq activated the small GTPases, mainly Rac1 and Cdc42, but not RhoA. MT also induced phosphorylation of focal adhesion kinase (FAK) and paxillin, and has ability to stimulate cytoskeletal reorganization-related proteins, such as profilin-1, cofilin-1, α-actinin-4, and filamentous actin (F-actin) in UCB-MSCs. To confirm the functional roles of MT in promoting UCB-MSCs motility, we have further investigated the effect of UCB-MSCs treated with MT on skin wound healing in mice. Topical UCB-MSCs transplantation groups showed an increase of re-epithelialization from mechanical skin wound, but the mice group that received UCB-MSCs treated with MT showed almost complete restoration of epidermis and cornified layer. These results demonstrate that MT stimulated human UCB-MSC motility by increasing cytoskeletal reorganization through a MT-2-dependent PKCζ, Rac1, and Cdc42 pathway, which are critical for providing a suitable microenvironment in MSC transplantation and re-epithelialization of mouse skin wounds.

T-2072

HUMAN LEUKOCYTE ANTIGEN-G PROFILING IN MESENCHYMAL STEM CELLS ISOLATED FROM VARIOUS SOURCES: ROLE IN IMMUNO-MODULATION

Mohanty, Sujata[1], Srivastava, Pallavi[2], Prabha, Punit[1], Sharma, Mukti[1], Teotia, Pooja[1], Kanga, Uma[3], Dahiya, Meetu[1], Kaushal, Namrata[1]
[1]*Stem Cell Facility, All India Institute of Medical Sciences, Delhi, India,* [2]*Department of Biotechnology, All India Institute of Medical Sciences, Delhi, India,* [3]*Department of Transplant Immunology and Immunogentics, All India Institute of Medical Sciences, Delhi, India*

Mesenchymal Stem Cells (MSCs), in addition to tri-lineage differentiation potential, possess remarkable immunosuppressive properties and are known to inhibit the proliferation and function of the immune cells like, T cells, B cells, NK cells and Dendritic Cells (DCs). It has been observed that Human Leukocyte antigen- G (HLA-G)

expression increases when the MSCs are stimulated with various inducers like Progesterone, IFN-γ and LIF (Leukocyte Inhibitory Factor). Here, we aimed to study the role of differential HLA-G expression on MSCs, isolated from different tissue sources and its effect on immune modulation potential of MSCs. MSCs isolated from three different sources; bone marrow, adipose tissue and dental pulp, were evaluated for their HLA-G expression at m-RNA level using qRT-PCR and protein level by Immunofluorescence (IF), Flow Cytometry and Western Blotting. Functional role of HLA-G was further evaluated by mixed-lymphocyte-reaction (MLR). The study was conducted after approval from the IEC (Institute Ethics Committee) and Stem Cell Ethics Committee. Three samples from each of the three sources were used for all the experiments. Transcript level of HLA-G expression from the different sources was evaluated by semi quantitative RT-PCR method. Cytoplasmic and surface expression of HLA-G was evaluated by Immunofluoresence and flow cytometry respectively using anti HLA-G antibody (MEM G/9). HLA-G protein level was quantified by western blot, using anti- HLA-G antibody (Clone 4H84). MLR studies were conducted to study Immunomodulation activity of HLA-G, where MSCs were co-cultured with PBMCs and the proliferation rate of PBMCs (Peripheral Blood Mononuclear cells) was calculated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The results of RT-PCR studies showed that the expression level of HLA-G is higher in Bone Marrow and Adipose derived MSCs in comparison to Dental pulp MSCs. IF study showed that cytoplasmic expression of HLA-G is almost equal in MSCs isolated from all three sources. JEG-3 cell line was used as a positive control. Surface expression of HLA-G was assessed by Flow Cytometry and IF, which revealed almost the same expression of HLA-G in MSCs derived from all the three sources. Western Blotting data revealed the presence of HLA-G protein (32kDa) in MSCs from all the three sources. However, immunomodulatory property was highest in adipose tissue derived MSCs (AD-MSCs), as assessed by MLR study. AD-MSCs showed 73% suppression of lymphocyte proliferation, followed by that by bone marrow and dental pulp derived MSCs, 63% suppression of lymphocytes. Taken together we established the expression of HLA-G in MSCs derived from three different tissue sources and the potential role of HLA-G expression in immunomodulation by these MSCs. Further we also identified that Adipose derived MSCs have better immune suppressive potential, which could be used for immunomodulation, apart from regeneration.

T-2073
THE PARACRINE EFFECT OF MESENCHYMAL HUMAN STEM CELLS RESTORED HEARING IN BETA-TUBULIN INDUCED AUTOIMMUNE SENSORINEURAL HEARING LOSS IN MICE

Zhou, Yixuan[1], Du, Xiaoping[2], Zhou, Bin[1], Cheng, Weihua[3], Yuan, Jindong[4], Di Girolamo, Stefano[5], Barbieri, Marco[6], **Yoo, Tai June**[1]
[1]Yoo Clinic and StemGen Stem Cell Therapy Center, Seoul, Republic of Korea, [2]Hough Ear Intitute, Oklahoma City, OK, USA, [3]Hough Ear Institute, Oklahoma City, OK, USA, [4]Urology, Wuhan First Hospital, Wuhan, China, [5]University of Rome, Rome, Italy, [6]University of Genoa, Genoa, Italy

The para-crine effect of mesenchymal human stem cells restored hearing in β tubulin induced autoimmune sensorineural hearing loss in mice Autoimmune inner ear disease is characterized by progressive, bilateral although asymmetric, sensorineural hearing loss. Patients with autoimmune inner ear disease had higher frequencies of interferon--producing T cells than did control subjects tested. Human adipose-derived mesenchymal stem cells (hASCs) were recently found suppress effector T cells and inflammatory responses and therefore have beneficial effects in various autoimmune diseases. The aim of this study was to examine the immunosuppressive activities of hASCs on autoreactive T cells from the experimental autoimmune hearing loss(EAHL) in murine model and also how human stem cells regenerated mouse cochlea cells. Female BALB/c mice underwent β-tubulin immunization to develop EAHL; mice with EAHL were given hASCs or PBS intraperitoneally once a week for 6 consecutive weeks. Auditory brainstem responses were examined over time. The helper type 1 (Th1)/Th17-mediated autoreactive responses were examined by determining the proliferative response and cytokine profile of splenocytes stimulated with β-tubulin. The frequency of regulatory T(Treg) cells and their suppressive capacity on autoreactive Tcells were also determined. Cochlear tissues were decacificated in 10% EDTA for 10 days, embeded in parafin and sectioned at 6 um. H and E staining or immunostaining with APC conjugated anti-HLA-ABC antibody (BD Pharmingen) were conductedThe organ of Corti, stria vascularis,spiralligament and spiralganglion in stem cell group are normal. In control group, without receiving stem cells, the organ of Corti is replaced by a single layer of cells, atrophy of stria vascularis (and spiral ligament are observed. No neurons are found in the spiral ganglion. The cochleae in the stem cell group (2 out of 6 cochleas or 1 mouse) and 2/3 cochleas in the PBS group (4 out of 6 cochleas or 2 mice) have such abnormal cochlear morphology. Examples of confocal microscopic images: Positive anti-HLA-ABC stained cells are only seen in the stria vascularis (arrows) and in the spiral ligament (arrowhead) of stem cell group (B). All cell nuclei are stained with DAPI (blue). No anti-HLA-ABC positive cells are found in the cochleae of PBS group (A). Systemic infusion of hASCs significantly improved hearing function and protected hair cells in established EAHL. The hASCs decreased the proliferation of antigen-specific Th1/Th17 cells and induced the production of anti-inflammatory cytokine interleukin-10 in splenocytes. They also induced the generation of antigen-specific $CD4^+CD25^+$ $Foxp3^+$T reg cells with the capacity to suppress autoantigen-specific T cell responses. The experiment demonstrated that hASCs are one of the important regulators of immune tolerance with the capacity to suppress effector T cells and to induce the generation of antigen-specific T reg cells and the restoration is due to the paracrine activities human stem cells, since there are newly regenerated mice spiral ganglion cells, not human mesenchymal stem cells given by intra peritoneal transfer.

T-2074
HUMAN MESENCHYMAL STROMAL CELLS PROVIDE PROTECTION THROUGH PARACRINE MECHANISMS IN A HUMANISED MOUSE MODEL OF EMPHYSEMA

Kennelly, Helen, English, Karen
Biology, National University of Ireland, Maynooth, Kildare, Ireland

Mesenchymal stromal cells (MSC) are adult multipotent cells that possess self-renewal and regenerative capacities with the ability to differentiate into a variety of cell types. Originally the differentiation of MSC into specific cells at the injury site was thought to be the important process in MSC therapy. However this differentiation is rare and the beneficial effects of MSC treatment are now thought to be mediated through paracrine effects. One of these effects is profound immune modulation, which facilitates the use of allogeneic MSC as a cellular therapy. This study has examined the use of human MSC for the treatment of Chronic Obstructive Pulmonary Disease (COPD). COPD is a slowly progressive inflammatory disease of the lungs characterised by airflow obstruction that is not fully reversible. Neutrophil mediated inflammation and excessive protease activity is central to pathology, causing narrowing of the small airways and destruction of lung tissue. This results in emphysema and deteriorating

lung function. This study examined the cytoprotective and regenerative mechanisms of MSC treatment both in vitro and in vivo. Utilising a humanised mouse model of emphysema this study demonstrated that administration of MSC exerted beneficial cytoprotective effects in a dose dependent manner, with a significant decrease in inflammatory markers in the lung. Herein, we investigated the role of MSC derived protease inhibitors and growth factors in MSC protection in the COPD model, as well as regeneration of injured epithelium in the lung. In vitro studies demonstrate that MSC express and secrete a range of protease inhibitors and growth factors and influence neutrophil activity. MSC inhibited migration and elastase activity of neutrophils in vitro. These properties potentially explain the cytoprotective effect of MSC observed in vivo. MSC also enhanced epithelial wound repair and inhibited epithelial apoptosis in a Hepatocyte Growth Factor (HGF) dependent manner. These combined effects will potentially halt disease progression while repairing the injured lung tissue, concomitantly highlighting the potential of MSC as a cellular therapy for COPD.

T-2075
CYTOKINE EXPRESSION ANALYSIS OF HUMAN MSC AND INJURED RAT TISSUE IN A MODEL OF STRESS URINARY INCONTINENCE

Kenyon, Jonathan[1], Sadeghi, Zhina[2], Tomechko, Sara[1], Leahy, Patrick[1], Daneshgari, Firouz[1], Caplan, Arnold[3], Hijaz, Adonis[1]
[1]Case Western Reserve University, Cleveland, OH, USA, [2]Urology, Case Western Reserve University, Cleveland, OH, USA, [3]Case Western Reserve University School of Medicine, Cleveland, OH, USA

Stress urinary incontinence (SUI) is a relatively common disorder post-partum and incidence increases with patient age. Human mesenchymal stem cell (hMSC) injections to the periurethral space have shown therapeutic benefit in animal models of SUI. While hMSC improve SUI injury, little is known as to how these cells alter the urethral injury and how injury modulates hMSC function. We hypothesized hMSC and tissue injury crosstalk modulates cytokine expression of both injured urethral tissue as well as the hMSC themselves. To determine cytokine expression changes occurring in injured rat urethral tissue and hMSC, we injected fluorescent labeled hMSC into the periurethral space of rats given vaginal distention. Next, 24h post injury/injection, rats were sacrificed and urethral space was frozen in OTC embedding medium and sectioned. Fluorescent hMSC was easily distinguished from adjacent rat urethral tissue. We then prepared total RNA from laser captured hMSC and adjacent rat urethra tissue. Individual cytokine mRNA was quantified by rat or human specific microarray. The focus of this preliminary experiment was on CC-like, CXC-like, growth factors, BMPs, interferons, interleukins, adhesion molecules, and the various receptors for these cytokines. As expected, we observe dramatic differences in the expression of cytokines between hMSC in culture and hMSC injected into injured rat urethra. Similarly, injured rat tissue treated with hMSC display striking cytokine expressional changes when compared to either normal rat tissue or injured rat tissue not given hMSC. We argue these tissue specific cytokine gene expression modulations are critical for initiation of hMSC response to injury. Further, signaling molecules produced by hMSC are in turn, necessary to promote injury appropriate responses such as suppression of inflammation and apoptosis as well as promoting cell expansion and differentiation.

T-2076
INJURY-INDUCED NEUROGENESIS ENHACED BY VIRAL OVEREXPRESSION OF ANTI-APOPTOTIC GENE BCL-XL IN THE ADULT SUBCALLOSAL ZONE

Kim, Joo Yeon, Kim, Hyun, Sun, Woong
Korea University of Medicine, Seoul, Republic of Korea

Neurogenesis occurs spontaneously in some specific brain regions, including the subventricular zone (SVZ) of the lateral ventricle and subgranular zone (SGZ) of the dentate gyrus in adult brain regions. We recently identified that subcallosal zone (SCZ) also contains neural stem cells and they spontaneously produce neuroblasts but massively eliminated by programmed cell death. Following brain injury, the survival of SCZ-derived neuroblasts was increased, and a subset of neuroblasts migrated toward the injured brain region. In this study, we further characterized their long-term fate, and explored whether promotion of their survival improves the brain repair processes. Within 1 month, most newly produced cells in the injury penumbra differentiated into oligodendrocytes and astrocytes, but neuroblasts were all degenerated by apoptosis and little neurons were produced. On the other hand, overexpression of anti-apoptotic Bcl-xL in the SCZ neural stem cell resulted in progressive increase in the number of neuroblasts. Furthermore, a subset of newly produced neuroblasts differentiated into mature inhibitory neurons exhibiting NeuN. These results indicate that anti-apoptotic therapy may be an efficient means to utilize endogenous neural stem cells for promoting brain repair.

T-2077
HUMAN MESENCHYMAL STEM CELLS-CONDITIONED MEDIUM ENHANCE MUSCLE FUNCTION RECOVERY IN MUSCLE ATROPHY MODELS

Kim, Mi Jin, Kim, Sun-Mi, Yun, Chang-Koo, Lee, Joon Ho, Choi, Yong-Soo
Department of Applied Bioscience, CHA University, Gyeonggi-do, Republic of Korea

Recent many studies have demonstrated that mesenchymal stem cells (MSCs) therapy has the potential to enhance muscular regeneration. We attempted to find new treatments for the muscle atrophy and muscle loss. In short, muscle atrophy-induced rat models were set up and we confirmed the effects of MSCs treatment through the mechanism of muscle atrophy. In this study, we used the human umbilical cord-derived mesenchymal stem cells (UC-MSCs) that promote to muscle regeneration and protect to muscle atrophy. In vivo study, we injected UC-MSCs directly into soleus muscle of muscle atrophy-induced animal, and in vitro study, we confirmed the paracrine effect of UC-MSCs on dexamethasone-induced muscle atrophy cell model. As a results of histological and functional skeletal myogenesis were observed significantly decrease in muscle atrophy-induced group. Importantly, in UC-MSCs-treated group, we confirmed recovery effect that muscle fiber size, soleus muscle mass and muscle-specific gene expression compared to muscle atrophy-induced group. We have also confirmed that UC-MSCs-conditioned medium (CM) contributed to muscle atrophy and regeneration through paracrine mechanisms. Consequently, the mechanism of muscle atrophy occurs via the PI3K-AKT signal pathway, in particular, when the UC-MSCs is used to recover such muscle atrophy can be expected to be a potential therapeutic methods. Also, the results of UC-MSCs-CM showed the same results. Based on these results, we will strengthen the basis of a new medicine for fast rehabilitation and regeneration of atrophied muscles.

T-2078
RECOVERY OF MITOCHONDRIAL FUNCTION AND CELLULAR VIABILITY IN HUMAN MITOCHONDRIA-DEPLETED CELLS BY DIRECT MITOCHONDRIAL TRANSFER IN VITRO

Kitani, Tomoya[1], Kami, Daisuke[2], Kawasaki, Takanori[1], Matoba, Satoaki[1], Gojo, Satoshi[3]
[1]Cardiovascular Medicine, Kyoto Prefectural University of Medicine, Kyoto City, Japan, [2]Kyoto Prefectural University of Medicine, Kyoto, Japan, [3]Regenerative Medicine, Kyoto Prefectural University of Medicine, Kyoto City, Japan

Background: Mitochondrial dysfunction is a potential mechanism for aging and many diseases, including metabolic disorders. Several studies suggested that stem cell transplantation therapy may exert its beneficial effects through intercellular transfer of mitochondria. The objective of this study was to explore therapeutic potential of direct mitochondrial transfer using a multidisciplinary approach in vitro. Methods: Genetically fluorescent-labeled mitochondria were isolated from human uterine endometrial gland-derived mesenchymal cells (human EMCs) carrying DsRed2-labeled mitochondria by differential centrifugation. Rat cardioblast H9c2 was used in xenogeneic transfer. After coincubation of homogeneic or xenogeneic cells with isolated mitochondria, live fluorescence imaging, immunohistochemistry, three-dimensional reconstruction imaging, time-lapse PCR, flow cytometric analysis, and time-lapse microscopy were performed to confirm intracellular localization of exogenous mitochondria. To evaluate the effect of mitochondrial transfer, cellular viability and bioenergetics were assessed in mitochondrial DNA-depleted human EMCs (ρ0 cells) by extracellular flux analysis and resazurin assay. To elucidate the mechanism of direct mitochondrial transfer, effects of various inhibitors of endocytosis were tested. Results: Live fluorescence imaging and immunohistochemical staining with a species-specific mitochondrial antibody demonstrated internalization of exogenous mitochondria in recipient cells after coincubation with isolated mitochondria. The 3D reconstruction images of immunohistochemically stained specimens confirmed that the exogenous mitochondria were located within the cells. Xenogeneic mitochondrial DNA was also detected in recipient cells after coincubation. Flow cytometric analysis showed the percentage of recipient cells containing exogenous mitochondria increased in a dose-dependent manner according to delivered concentrations of isolated mitochondria. The number of cells containing exogenous mitochondria declined over time and disappeared within a week. Time-lapse microscopy revealed that mitochondrial transfer occurred within 1-2 hours after the initiation of coincubation. After coincubation with isolated mitochondria, optimal concentration of isolated mitochondria exhibited a significant recovery in cellular viability and oxygen consumption of ρ0 cells. UV treated mitochondria failed to demonstrate significant increase in cellular viability. Inhibition experiments demonstrated that EIPA, an inhibitor of the Na + /H + exchange, suppressed mitochondrial transfer. Furthermore, EIPA treatment abolished the therapeutic effect of mitochondrial transfer in ρ0 cells Conclusion: Our data suggest a potential application of direct mitochondrial transfer for the treatment of diseases associated with mitochondrial dysfunction.

T-2079
BRAIN CELL REGENERATION BY NON-PLATELET RNA-CONTAINING PARTICLES

Kong, Wuyi
Khasar Medical Technology Co., Beijing, China

Non-platelet RNA-containing particles (NPRCPs) are newly identified subcellular particles in human and mouse blood. In damaged mouse kidneys, NPRCPs can form into nucleated cells after their aggregation, fusion and nuclear formation. These newly formed cells are octamer-binding transcription factor 4 (Oct4)-expressing stem cells. To confirm the function of NPRCPs in brain cell regeneration, we examined the effects of NPRCPs in mouse brains after acute focal cerebral ischemia from day 3 to week 10 with tail-vein transplantation of cultured green fluorescent protein (GFP)-labeled NPRCPs. As early as 3 days after the injection, GFP-labeled NPRCPs appeared in the blood vessels under the pia mater, in the damaged cerebral hemisphere and lateral ventricle areas. Within the first week, numerous NPRCPs had infiltrated into brain tissues in ribbon-like migration or individually infiltration via blood vessels or lateral ventricles. GFP-positive cellular structures with dendrite-like fibers, but without nucleus were found in the brains after 1 week. NPRCPs regenerated brain cells in 3 patterns: 1) aggregation and fusion to form granular brain cells; 2) lineup and fusion to form dendrite-like structures of the new cells; and 3) participating in axon regeneration. Of the 3 regenerative patterns, green fluorescent protein-expressing non-nucleated cellular structures appeared first, which supports our theory that the nucleus of the brain cells can be formed de novo after NPRCP's fusion. Our data provide new evidence that NPRCPs can regenerate stem cells in ischemia-damaged mouse brains after infiltrating into brain tissues via extravasation from blood.

T-2081
XENOPUS LAEVIS: A MODEL ORGANISM TO STUDY SPINAL CORD REGENERATION

Larrain, Juan[1], Lee-Liu, Dasfne[1], Muñoz, Rosana[1], Moreno, Mauricio[1], Edwards, Gabriela[1], Méndez, Emilio[1], Tapia, Víctor[1], Almonacid, Leonardo[2], Faunes, Fernando[1], Melo, Francisco[2]
[1]Center for Aging and Regeneration and Millenium Nucleus in Regenerative Biology, P. Universidad Catolica de Chile, Santiago, Chile, [2]Molecular Bioinformatics Laboratory and Millennium Institute on Immunology and Immunotherapy, P. Universidad Catolica de Chile, Santiago, Chile

In mammals, including humans, spinal cord injury (SCI) results in loss of motor and sensory function leading to paraplegia and quadriplegia. SCI produces massive loss of neurons, oligodendrocytes and astrocytes, and almost no tissue regeneration and functional recovery is observed. Unlike mammals, teleost fish and amphibians such as adult urodeles (e.g. newts) and anuran larvae (e.g. Xenopus) can achieve functional recovery after spinal cord transection. Interestingly, in Xenopus laevis the regenerative ability is restricted to tadpole stages (stage 50-54, R-stages) and is lost at the metamorphic climax (stage 56-66, NR-stages), providing a unique model system to study spinal cord regeneration. Here we will present results from two approaches aiming to understand the genetic and cellular mechanisms of spinal cord regeneration in Xenopus laevis. First, we have studied the role of Sox2+ ependymal cells. We have found that in R-stages Sox2+ cells have a rapid and transient activation in response to injury (1 and 2 days post-transection, dpt), followed by migration of Sox2+ cells into the ablation gap and restoration of the ependymal canal. Importantly, no activation of Sox2+ cells and no migration to the ablation gap occur in NR-stages. Reduction of Sox2 levels by morpholino electroporation diminishes regeneration suggesting that Sox2+ cells are necessary for spinal cord regeneration. We are currently performing experiments to determine if Sox2+ cells activated by injury can make new neurons and also performing transplantation experiments to evaluate their ability to restore regeneration in post-metamorphic frogs. Secondly, to identify biological processes and genes that are required or that inhibit spinal cord regeneration we performed a transcriptomic profile of the response to SCI in R- and NR-stages. Spinal cords from animals at 1, 2 and 6 dpt

or after sham operation were isolated and RNA was sequenced using next generation sequencing. Transcriptional changes were studied comparing transected against sham-operated animals at each time-point. We found extensive changes in the transcriptome of regenerative tadpoles already at 1 day after injury, which was only observed in non-regenerative froglets at 6 days after damage indicating different kinetics of gene regulation in response to injury between these two stages. In addition, we found differential regulation of the following when comparing R- and NR-stages: 1) genes related to neurogenesis and the axonal growth cone; 2) gene ontology enrichment analysis revealed differences in genes from biological processes including cell cycle, response to stress, metabolism, development and immune response and inflammation, and we have validated differential expression of several genes involved in these processes using low-scale validation (RT-qPCR); 3) we have also identified previously uncharacterized transcripts regulated differentially after SCI, providing a subset of genes that can open unexpected venues to understand spinal cord regeneration. In summary we have found extensive differences in the timing and the composition of the transcriptome deployed in response to SCI in regenerative and non-regenerative Xenopus laevis. We envision that introducing Xenopus laevis as a model organism to study spinal cord injury should provide new mechanistic insights in order to learn why mammalian spinal cord has very limited regenerative capacities and how this can be improved.

T-2082
FASN PLAYS A CRITICAL ROLE IN HYPOXIA-INDUCED UMBILICAL CORD BLOOD-DERIVED HUMAN MESENCHYMAL STEM CELLS PROLIFERATION AND MIGRATION VIA MTORC ACTIVATION

Lee, Hyun Jik, Jung, Young Hyun, Oh, Sang Yub, Ryu, Jung Min, Lee, Ki Hoon, Han, Ho Jae
Department of Veterinary Physiology, BK21 PLUS Creative Veterinary Research Center, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

The control of stem cell fate is a main issue for the success of stem cell therapy. In stem cell therapy application, engrafted stem cells are exposed to hypoxic condition. Hypoxia influences stem cell lipid metabolism change, proliferation and migration which are key factors for control of stem cell fate. However, interaction between metabolic and functional change of stem cell on hypoxia remains unclear. The aim of this study was to determine how metabolic change of umbilical cord blood derived-human mesenchymal stem cells (hMSCs) under hypoxic condition induce functional change. In the present study, hypoxia treatment stimulated hMSCs proliferation and migration in a time dependent manner. Also, hypoxia treatment increased expression of major lipogenic enzymes, fatty acid synthase (FASN) and stearoyl-CoA desaturase-1 (SCD-1). To investigate role of these lipogenic enzymes on hypoxia-induced hMSCs proliferation and migration, we used the FASN inhibitor (cerulenin) and SCD-1 inhibitor (CAY10566). Cerulenin but not CAY10566 treatment reduced hypoxia-induced hMSCs proliferation and migration. Interestingly, CAY10566 treatment reduced mouse embryonic stem cells proliferation and migration. It was implicated that hypoxia-induced FASN expression is important for hypoxia-induced hMSCs proliferation and migration. FASN is the key enzyme of *de novo* lipogenesis which mainly produces palmitic acid. Hypoxia treatment also increased expression of hypoxia-inducible factor 1-alpha (HIF-1α) and hypoxia-inducible factor 2-alpha (HIF-2α). Hypoxia-induced FASN expression is inhibited by *HIF-1α* siRNA transfection, but *HIF-2α* siRNA transfection didn't alter hypoxia-induced FASN expression. The mammalian target of rapamycin complex (mTORC) was activated by hypoxia exposure. Cerulenin treatment suppressed mTORC activation on hypoxia and it also reduced hMSCs proliferation and migration which was recovered by palmitic acid treatment. It suggested that FASN expression through HIF-1α pathway on hypoxia is important for hypoxia-induced mTORC activation. In addition, rapamycin, the mTORC inhibitor, pretreatment abolished hypoxia-induced CDK2, CDK4, cyclin D1, cyclin E and F-actin expression. It proposed that hypoxia-induced mTORC activation promotes G1, S phase transition and F-actin reorganization. Furthermore, it has been reported that mTORC pathway may regulate HIF-1α, SREBP-1c expression and fatty acid synthesis. However, we showed that hypoxia-induced mTORC activation didn't affect HIF-1α, SREBP-1c and FASN expression. It was implicated that FASN expression through HIF-1α and SREBP-1c pathway on hypoxia is a upstream component to activate mTORC. In conclusion, our study demonstrated that the lipid metabolism change on hypoxia which promotes FASN expression plays an important role in hypoxia-induced hMSCs proliferation and migration through mTORC activation.

T-2083
QUIESCENT PRIMITIVE NEURAL STEM CELLS REPOPULATE ABLATED DEFINITIVE NEURAL STEM CELLS IN THE ADULT MOUSE BRAIN

Leeder, Rachel, Yammine, Samantha, Xu, Wenjun, Sachewsky, Nadia, Morshead, Cindi M., van der Kooy, Derek J.
University of Toronto, Toronto, ON, Canada

Adult primitive (p)NSCs comprise a rare population of GFAP-, Oct4+, LIF-responsive cells in the periventricular region of the mouse brain. pNSCs can be passaged in vitro to self-renew or give rise to definitive (d)NSCs, which are GFAP+ and EGF- and FGF-dependent. We asked whether pNSCs, which first appear at E5.5 and give rise to dNSCs embryonically become quiescent in the adult mouse brain. We used doxycycline-inducible H2B-GFP mice to perform label retention studies in pNSCs to assess their cell cycle time. We conclude that adult pNSCs are predominantly quiescent and divide approximately once every 2-3 months, in contrast to dNSCs that are known to divide every 15 days. These slow cycling pNSCs express the pluripotency marker Oct4 in the adult brain and we used Oct4fl/fl;Sox1-Cre mice to assess the requirement of Oct4 expression in pNSCs. The forebrain of adult mice homozygous for the excised Oct4 allele no longer gave rise to any pNSC-derived neurospheres, while dNSC-derived neurospheres were not affected by loss of Oct4. This indicates a requirement for Oct4 expression in pNSCs, and provides a key model of a pNSC-null mouse. We investigated whether quiescent pNSCs are recruited for repopulation of dNSCs following ablation of dNSCs and downstream neural progenitors. We ablated dNSCs and downstream progenitors in Oct4fl/fl;Sox1-Cre mice using a 14-day AraC infusion and in Oct4fl/fl;Sox1-Cre;GFAP-tk mice using a 7-day AraC and 3-day GCV infusion. After ablation, we observed a significantly reduced repopulation of dNSCs in Oct4fl/fl;Sox1-Cre as compared to control Oct4wt/fl;Sox1-Cre mice. We observed that following AraC/GCV infusion, dNSC-derived neurospheres had not reappeared by 2 weeks post treatment and after 4 weeks had only returned to 6% naïve dNSC abundance (control mice recovered to 70%). These experiments support the hypothesis that pNSCs are essential for repopulation of dNSCs in the adult mouse brain. We conclude that pNSCs are a predominantly quiescent population upstream of dNSCs in the adult mouse brain, which become activated after removal of the dNSC pool to repopulate the neural lineage.

T-2084

STIMULATION OF EPHB2/EPHRIN-B1 EXPRESSION BY TUMOR NECROSIS FACTOR ALPHA IN HUMAN DENTAL PULP STEM CELLS

Zhu, Lifang[1], Zhang, Chengfei[2]
[1]The University of Hong Kong, Hong Kong, China, [2]Faculty of Dentistry The University of Hong Kong, Hong Kong

Aims: The purpose of this study was to investigate whether in vitro stimulation of dental pulp stem cells (DPSCs) by pro-inflammatory cytokine (tumor necrosis factor alpha, TNF-α) induces secretion of EphB2/ephrin-B1 signaling, and if so, to identify how EphB2/ephrin-B1 are enacted. Methods: Cells isolated from dental pulp were treated with TNF-α,with the concentration ranging from 5 to 100 ng/ml for 2 h and 20 ng/ml over a period ranging from 2 h to 48 h. The mRNA and protein levels of EphB2/ephrin-B1 were measured by real-time polymerase chain reaction (RT-PCR) and western blot analysis, respectively. In addition, TNF-α receptors in the DPSC's were assayed by real-time PCR. The results were analyzed by one-way ANOVA. Results: TNF-α increased EphB2 (TNF-α: 5, 10 and 20 ng/ml) and ephrin-B1 (TNF-α: 5, 10, 20 and 40 ng/ml) mRNA expression in DPSCs ($P < 0.05$). The mRNA expression of EphB2/ephrin-B1 was maximized at 24 h with TNF-α treatment (20 ng/ml) as observed in RT-PCR ($P < 0.05$). The EphB2/ephrin-B1 protein levels were expressed stably at 16 h and 24 h in western blot analysis. Both TNFR1 and TNFR2 receptors were identified as regulators of EphB2/ephrin-B1 expression ($P < 0.05$). Conclusions: In regions of inflammation in pulp tissue, TNF-α may be a principal initiator in regeneration of dental connective tissue by activating a vital signaling pathway in DPSC's; mediated by EphB2/ephrin-B1 gatekeepers. Consequently they may play an important role in recruitment of DPSC's to areas of damage signaled by inflammation.

T-2085

NEURAL PRECURSOR CELL ACTIVATION IN THE AGED MOUSE BRAIN CONTRIBUTES TO FUNCTIONAL RECOVERY AND TISSUE REPAIR FOLLOWING STROKE

Sachewsky, Nadia[1], Zarin, Taraneh[1], Son, Andrey[1], Rahman, Aashiq[1], Hunt, Jessica[1], Kovatcheva, Marta[1], Morshead, Cindi M.[2]
[1]University of Toronto, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada

Stroke is the third leading cause of death and disability worldwide. Currently there are no strategies available to repair the injured brain following stroke. Studies from our group have demonstrated that neural precursor cell (NPC) activation using biologics, such as growth factors and the commonly used immunosuppressant Cyclosporin A (CsA), effectively lead to tissue repair and functional recovery following stroke. These exciting findings support the hypothesis that self-repair strategies using endogenous NPCs hold promise. However, a number of issues need to be addressed before translating these findings to the clinic. It is necessary to demonstrate efficacy in (1) more than one model of stroke and (2) in aged brains, as these are the most clinically relevant population but are rarely used for stroke studies. Indeed, we have published findings revealing that unlike the young brain, the aged NPC niche inhibits NPC activation in old age brains. Hence, we sought to determine if our endogenous repair strategy could be applied to a reperfusion model of stroke and, further, if the strategy was able to promote behavioural recovery in old age mice. We used a focal endothelin-1 model of stroke and administered CsA for NPC activation. CsA has direct pro-survival effects on NPCs in the young adult brain. We first performed in vitro studies to determine the effects of stroke alone and/or CsA in the young versus aged brain. Young adult and aged mice received ET-1 stroke in the motor cortex on day 0 and the neural stem cell colony forming assay (neurosphere assay) was performed 7 days later to assess neural stem cell activation. We observed increased numbers of neurospheres in stroke only and CsA only treatment group in young animals, similar to previous findings. In contrast, there was no expansion in the size of the NPC pool in old age brains in stroke only or CsA only conditions. However, the combination of stroke+CsA was sufficient to significantly expand the NPC pool in the old brain. The additive effect of stroke+CsA was examined in vivo using a NestinCreERT2xROSAyfp reporter mouse. Mice received tamoxifen via chow for two weeks followed by a two week chase period and subsequently received stroke alone, CsA alone, or stroke+CsA for one week. We observed a >4 fold increase in YFP+ NPCs in each of the subependyma, rostral migratory stream, and olfactory bulb in response to stroke or CsA infusion for 7 days and an additive 8-10 fold increase in stroke+CsA conditions. Finally, we looked at behavioural recovery following stroke in young versus aged mice. Young and old age mice were subcutaneously infused with CsA for 32 days starting immediately at time of stroke or 4 days post-stroke. The foot-fault task was used to determine the motor deficits and recovery following treatment. Both young adult and old age mice that received CsA treatment following stroke showed functional recovery and decreased cavity volumes compared to stroke only controls. Moreover, we showed that the functional recovery was not due to neuroprotection, as delaying CsA administration for 4 days post-stroke, a time when neuronal cell death and the maximum cavity volume has occurred, still leads to functional recovery. These findings support the claim that endogenous NPC activation is a promising strategy for the treatment of stroke.

TISSUE ENGINEERING

T-2086

CHONDROITIN SULFATE IMMOBILIZED ON A BIOMIMETIC SCAFFOLD: ITS ROLE IN RECAPITULATING THE CHONDROGENIC NICHE WHILE CONTROLLING INFLAMMATION

Corradetti, Bruna[1], Taraballi, Francesca[1], Minardi, Silvia[1], Balliano, Marta Anna[1], Weiner, Bradley K.[2], Tasciotti, Ennio[1]
[1]Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, USA, [2]Division of Spinal Surgery, Department of Orthopaedic Surgery, Houston Methodist Hospital, Houston, TX, USA

Cartilage loss after trauma or degenerative diseases often results in fibrotic scarring and unsatisfactory joint function. To solve this issue a number of tissue-engineered approaches based on polymeric scaffolds of different nature have been explored. However, despite some promising results in short term recovery, an effective therapy able to consistently repair the damage in the long term remains lacking. The aim of this study was to mimic the extracellular matrix of cartilage and exploit the chondrogenic potential of type I collagen-based scaffolds (CL) functionalized with CS to induce a more physiologic repair of the cartilage injury. We described scaffolds' structure and stability, as well as morphological, physical and chemical properties through scanning electron microscopy, fourier-transform infrared spectroscopy, and thermogravimetry analysis. The influence of the biomimetic environment of scaffolds with CS blended on (CLCS) or crosslinked (CL-LINK-CS) to the collagen on the chondrogenic potential of rat bone marrow-derived mesenchymal stem cells (BM-MSCs) was tested over a 21-day period. Differentiation toward the chondrogenic lineage was assessed through the expression of chondrogenesis-associated genes, such as Sry-related HMG box (Sox9), aggrecan (Acan) and type II collagen (COL2α1). When CS was crosslinked to the collagen, Sox9

expression was found 4.12 (±0.04)-fold increased compared to cells grown on CL scaffolds. Accordingly, Acan expression increased 3.06 (±0.21)-fold when CS was blended and 5.05 (±0.07)-fold when the CS was crosslinked to the collagen even in total absence of chondrogenic supplements. COL2α1 expression was also found 2.54 (0.52)-fold higher in CL-LINK-CS conditions but comparable to the controls in presence of blended CS. We further explored the hypothesis that the immobilized CS could modulate the immune response after the implant and its application in cartilage tissue engineering. Our results support evidences about the role CS plays in modulating inflammation and immunity. To assess the efficacy of the system in helping BM-MSCs retain their immunosuppressive potential, cells grown on CS-CL and CS-LINK-CL scaffolds were stimulated with TNF-α (50 ng/ml) for 48 hrs. A markedly increased prostaglandin E2 secretion was observed when cells were cultured in presence of CS, both blended and crosslinked. Concomitantly, BM-MSCs grown in CL-LINK-CS showed a 32.23(±3.9)-fold increase in the expression of prostaglandin E2 synthetase and a 50.57 (±0.59)-fold increase in COX-2 mRNA levels. At the same time a 567(±67)-fold increase in TGF-β expression was also detected. Interestingly, a statistically significant decrease ($P<0.05$) in the levels of PGE2 released by the cells grown in CL-LINK-CS scaffold was observed in absence of pro-inflammatory cytokines compared to cells grown in CL scaffolds, substantiating the role of the crosslinked CS in reducing stress and stabilizing cells behavior. Our results suggest that CS has an active role on MSC fate and that functionalization with crosslinked CS could be a potential tool for cartilage tissue engineering application.

T-2087
FIBROBLAST GROWTH FACTOR-7 AND HYALURONIC ACID SYNERGISTICALLY ENHANCE SALIVARY GLAND DIFFERENTIATION

Janebodin, Kajohnkiart[1], Bae, Min-Soo[2], Parent, Sara[3], Ieronimakis, Nicholas[1], Monis, Grace[4], Hays, Aislinn[1], Kim, Deok-Ho[2], Reyes, Morayma[1]

[1]Pathology, University of Washington, Seattle, WA, USA, [2]Bioengineering, University of Washington, Seattle, WA, USA, [3]Oral Health Sciences, University of Washington, Seattle, WA, USA, [4]Laboratory Medicine, University of Washington, Seattle, WA, USA

Xerostomia or hyposalivation, is caused by Sjögren's syndrome, radiotherapy in patients with head and neck cancer, medications and aging, resulting in swallowing and speech problems, dental caries, gum disease, and oral infection. Tissue engineering is a promising strategy for functional salivary gland regeneration, but it is limited by the availability of a good source of stem cells, inducers, and biodegradable scaffolds. Fibroblast Growth Factor-7, FGF-7 (Keratinocyte Growth Factor, KGF) is secreted by ecto-mesenchyme, and has been shown beneficial for salivary gland restoration. Hyaluronic acid (HA) is one of the major extracellular matrix components of the developing and adult salivary gland, which may play an important role in control of salivary gland function. We proposed to study the effect of FGF-7 and HA on the generation of a functional salivary gland tissue by primary salivary gland epithelial cells. Primary salivary gland cells were isolated and characterized from murine Sca1-GFP derived submandibular salivary glands. Sca1-GFP+ salivary gland were ex vivo expanded for 10 passages. The ability to expand salivary gland epithelial cells in our long-term culture was demonstrated by cell morphology, salivary gland gene and protein expression. Then, we conducted in vitro salivary gland differentiation in three different conditions, matrigel as a positive control, HA-hydrogel without and with FGF-7 (200ng/ml). After 1 day in differentiation, salivary gland cells started to form acinar-like structures. After 5 days in differentiation, morphological, histological and molecular analyses were performed. Interestingly, cells in HA-hydrogel with FGF-7 up-regulated the expression of salivary gland epithelial markers in both gene and protein levels, compared to that in matrigel and HA-hydrogel alone. We are currently pursuing the in vivo model by transplanting salivary gland cells subcutaneously with HA-hydrogel without and with FGF-7 (200ng/ml) into immuno-compromised mice for 4 weeks and we will examine whether Sca1-GFP+ cells can form functional salivary gland tissue transplants, compared between HA hydrogel alone and that with the presence of FGF-7. Then the next step we will apply this approach into a mouse model with salivary gland injury and determine whether or not using HA hydrogel with FGF-7 may enhance the capacity of salivary gland regeneration. In summary we have developed an in vitro system to expand salivary gland Sca1+GFP stem cells for in vitro and in vivo differentiation. These cells hold great promise for salivary gland tissue engineering approaches.

T-2088
STRUCTURE-BASED INVESTIGATION OF WNT SIGNALING MODULATORS

Zheng, Jie

Structural Biology, St Jude Children's Research Hospital, Memphis, TN, USA

The canonical Wnt signaling pathway plays critical regulatory roles in development, stem cells and cancer. Aiming to further understand the stepwise signaling cascade in the Wnt pathways and to develop potential novel "tool box" for stem cell regulation through mediating the Wnt signaling pathway, we have taken a systems biology approach by developing small molecules that modulate the protein-protein interaction at different signaling steps in the Wnt pathways. While Wnt inhibitors have therapeutic potential in anti-cancer therapy, Wnt signaling activators may be useful in maintaining stem cell pluripotency, regulating cell regeneration. Based on the concept of "inhibitor of an inhibitor is an activator", we hypothesize that compounds that can interrupt the functions of Wnt antagonists would function as Wnt activators. Initial hits for the inhibitors of Wnt antagonists sFRPs and DKKs have been obtained. The functions of these activators are being evaluated. We have also developed small molecules that can reverse the DKK antagonistic effect by specifically binding to the DKK-binding cavity of the third β-propeller domain of LRP5/6 and block the interaction.

T-2089
CONSEQUENCE OF METABOLIC SWITCHING IN 3-D EMBRYONIC STEM CELL CULTURE

Alsobaie, Sarah

Imperial College London, London, United Kingdom

In vitro 3D culture offers both a model to understand self-renewal and stem cell behaviour in-vivo as a route to industrial production. We have used a variety of analytical tool including proteomics and quantitative RT-PCR to compare 3D (both static and dynamic) with 2D cultures. ESC's were encapsulated in alginate beads and shortly after encapsulation cells in 3D (both static and dynamic) showed high oxygen uptake rates compared to 2D culture. However, after 9 days of encapsulation glucose uptake increased in both static and dynamic 3D cultures and was combined with a pronounced increase in cell density and up-regulation of hypoxia induce-factors (HIf-1α and Hif-2α). This shift in metabolism toward anaerobic glycolysis is associated with high expression of hexokinas2 (Hk2) and increased lactate production. Interestingly, despite extensive cell proliferation at day 18, where cell numbers reached up to 80k/ bead, cells in dynamic culture

switched back from anaerobic to aerobic metabolism. Additionally, this alteration in energy consumption was not associated with nutrient depletion since both glucose and glutamate were not depleted in the culture medium. We further show that 3D dynamic culture increases expression of the master gene regulators (Oct4, Nanog ans Sox2) and decreases de novo methyltransferase (Dnmt3b and Dnmt3a) expression. Consistent with this Rex1, an inner-cell mass (ICM) associated marker, is over-expressed in 3D dynamic culture whereas Fgf5, an epiblast transition marker, is down-regulated. These results suggest that using 3D dynamic culture may suppress spontaneous differentiation and enhance naive pluripotent state. Interestingly, pluripotency is sustained during prolonged culture despite the switching in cell metabolism. Using SILAC-based proteomics to compare 2D vs. 3D dynamic culture, we show that encapsulated stem cell are characterized by down-regulation of the glycolysis enzymes and increased mitochondrial respiratory proteins, oxidative stress and antioxidant defence markers. Additionally, ECM protein expression (laminin, fibronectin, heparan sulfate (HS) proteoglycans, agrin, and nidogen-2) increased at least 20 fold. By monitoring the oxygen consumption, hypoxia-induced factors expression as well as Oct4 levels at different time points, we demonstrate that the stem cell's self-renewal status is regulated despite the metabolism transitions. We postulate that the 3D culture environment provides the niche required sensing and responding to external and internal stimuli and this provide novel look of *in vitro* embryonic stem cell behaviour. Thus, exposing embryonic stem cells to different stimuli in 3D may trigger self-renewal acquisition as has been recently suggested by using mechanical stimuli and acidic environments to enable somatic cells reprogramming. This idea is further supported by as recently published paper shows that 3D encapsulation helps fibroblast reprogramming and the acquisition of stemness without transcription factors transition.

T-2090
INTEGRIN EXPRESSION IN HUMAN PERIODONTAL LIGAMENT STEM CELLS CULTURED ON TWO DIFFERENT TITANIUM SURFACES

Barreto, Mardem Portela e Vasconcelo[1], Ginani, Fernanda[1], Queiroz-Neto, Moacir F.[2], Barboza, Carlos A.[1], Moura, Carlos E.[1]
[1]Department of Morphology, Federal University of Rio Grande do Norte, Natal, Brazil, [2]Department of Biochemistry, Federal University of Rio Grande do Norte, Natal, Brazil

The initial interaction of cells with biomaterials involve their anchoring and spreading over the surface of the material, and in this context integrins play an important role in the establishment of cellular contacts with the biomaterial. With surface treatment, it is possible to modify the characteristics of implants, such as chemical composition, morphology, roughness and topography, all influencing the molecular and cellular events that drive the early stages of biointegration. Previous *in vitro* experimental studies have shown that ion nitriding produces nanometric roughness on the titanium surface and increases cell adhesion and proliferation in nitrided surfaces compared to non-treated surfaces, but little is known about the expression of integrins in stem cells cultured on these surfaces. The aim of this study was to evaluate the expression of integrins α_2 and β_1 in human periodontal ligament stem cells cultured on titanium disks with polished surfaces or with nanometric roughness produced by plasma nitriding. Sixteen grade II ASTM F86 titanium disks, 14 mm in diameter and 1.5 mm thick, were polished with silicon carbide sandpaper in running water and polished in colloidal silica suspension. The samples were later divided into two groups, according to the surface produced; Polished - untreated and Nitrided - bombarded surfaces were obtained by treatment of unloading a planar cathode in a vacuumed atmosphere in the presence of nitrogen. Human periodontal ligament stem cells were obtained from two healthy permanent third molars extracted due to surgical indication. The multipotential nature of the cells was confirmed by expression of stem cell surface markers by flow cytometry and analysis of the osteoblastic and adipose differentiation potential. On third passage, the cells were cultured for 48 hours on the surfaces of the two groups and also on the surface of plastic tissue culture plates as a positive control for cell growth. The quantitative analysis of the expression of integrins α_2 and β_1 cells was performed by flow cytometry. An increased expression of integrin α_2 (36.05%) was observed in cells cultured on the polished surface, with lower expression (28.28%) in cells cultured on the nitrided surface. Conversely, the expression of integrin β_1 was lower in cells cultured on discs with a polished surface (29.19%), and was considerably higher in cells cultured on discs with a surface treated by plasma nitriding (37.09%). It can be concluded that the modification of titanium surfaces by planar cathode nitriding alters the integrin expression in human periodontal ligament stem cells. Further studies are needed to identify the molecular mechanism by which these features can influence the adhesion of periodontal ligament stem cells to the biomaterial.

T-2091
DYNAMIC BIODISTRIBUTION OF THERAPEUTICALLY BIOACTIVE SELECTED RENAL CELLS IN LARGE ANIMAL PRECLINICAL MODEL

Basu, Joydeep[1], Sangha, Namrata[1], Bertram, Timothy[2], Jain, Deepak[2]
[1]Tengion, Inc, Winston-Salem, NC, USA, [2]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 stem 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 renal cells", or SRC), and have demonstrated that SRC can attenuate the progression of chronic kidney disease within rodent models of renal insufficiency. SRC mechanism of action (MOA) may be contingent upon secretion of therapeutically relevant trophic factors and micro-RNAs and activation of host-specific stem cell populations. SRC is formulated within a hydrogel biomaterial matrix to create a Neo-Kidney Augment (NKA). NKA is currently in Phase I clinical trials in patients presenting with end-stage renal disease. Delivery of NKA is mediated through 16 gauge cannula injected directly into the kidney cortex. As with other cell-based therapies targeting soft organs, data on product bio-distribution post-implantation has been limited. To this end, we have developed methodologies for longitudinally tracking the bio-distribution of SPIO-labelled SRC upon delivery to porcine kidney using magnetic resonance imaging (MRI) in living swine, from immediately subsequent to SRC delivery, to 30 days post-delivery. These methodologies have been leveraged to evaluate bio-distribution of a clinically relevant dose of SRC in living swine. We provide evidence that SPIO-labelled SRC are retained within the renal medulla of living swine at up to 30 days post-implantation. These data further support our paracrine-based working hypothesis for SRC mechanism of action and may be of relevance to cellular therapeutics targeting other soft organs.

T-2092
COMBINATORIAL CASSETTE FOR SCREENING OSTEOGENIC POTENCY OF TRANSPLANTED HUMAN BONE MARROW STROMAL CELLS

Bodhak, Subhadip[1], Fernandez de Castro Diaz, Luis[2], Kuznetsov, Sergei A.[2], Kilts, Tina[2], Young, Marian F.[2], Lin-Gibson, Sheng[1], Simon, Jr., Carl G.[1], Robey, Pamela G.[2]

[1]Biomaterials Group, Biosystems and Biomaterials Division, National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA, [2]Craniofacial and Skeletal Diseases Branch, National Institute of Dental and Craniofacial Research (NIDCR), National Institutes of Health (NIH), Bethesda, MD, USA

The use of in vivo transplantation assays has become a valuable standard for evaluating the osteogenic potency of stem cell populations. However, traditional in vivo measurements suffer from high variability and low throughput. For example, a traditional mouse subcutaneous model allows performing 2 to 4 transplants per mouse and there is great variability between individual mice. To address these needs, we have developed an in vivo combinatorial transplant model that uses a combi-cassette with multiple slots for screening osteogenic potency of transplanted human bone marrow stromal cells (hBMSCs) in the subcutaneous pockets of immunodeficient mice. The combi-cassette approach was tested using a series of positive and negative controls with different combinations of hBMSCs loaded into hydroxyapatite/tricalcium phosphate (HA/TCP) or Gelfoam scaffolds. We have fabricated combi-cassettes with 7 wells from Teflon. The cassettes were 3 mm thick and each microwell was 4.3 mm in diameter with a 44 µL volume. Three of the wells were loaded with a HA/TCP-hBMSC formulation (60:40 wt% HA:TCP powder, Zimmer Inc.) which previously formed bone and supported host hematopoiesis in mouse subcutaneous model. Three wells were loaded with a negative control Gelfoam-hBMSC formulation (Gelatin Sponge, Pfizer Inc.) which does not induce osteogenesis. The last well was loaded with HA/TCP without hBMSCs. Each mouse received one combi-cassette plus two traditional "non-combi" transplants, a positive control HA/TCP-hBMSCs and a negative control Gelfoam-hBMSCs. Immunodeficient NOD.Cg-Prkdcscid female mice (age 8 weeks) served as transplant recipients. Transplants from 3 mice were recovered after 4, 8 and 14 weeks from transplantations (3 mice/time point). White field and fluorescent microscopy from histology (H and E staining) revealed similar and abundant bone formation in positive controls (HA/TCP-hBMSCs) at both the combi-cassettes and the traditional "non-combi" transplants at all time points. Bone morphology was comparable between combi-cassette and "non-combi" positive controls and good vascularization was found in each condition. In contrast, no bone formation appeared in the Gelfoam-hBMSC negative control samples for either the combi-cassette or the traditional "non-combi" transplants at any timepoint. HA/TCP transplants without hBMSCs also failed to show bone formation, demonstrating that hBMSCs did not migrate between the wells in the combi-cassettes. We have developed a combinatorial platform for high-throughput in vivo screening of osteogenic potency of hBMSCs on various scaffold formulations, improving efficiency and reducing animal to animal variability. In addition, each well in the combi-cassette uses 66% less material (cells and scaffolds) than the traditional "non-combi" transplants. The combi-cassette also makes for efficient sample handling since the whole cassette can be fixed, embedded and stained at once allowing the staining and comparition of all 7 transplants on the same slide. This approach also has the ethical benefit of reducing the number of animals required for each test. Disclaimer: Contribution of NIST, not subject to copyright in the United States. Certain equipment, instruments or materials are identified in this paper in order to adequately specify the experimental details. Such identification does not imply recommendation by the NIST nor does it imply the materials are necessarily the best available for the purpose.

T-2093
TISSUE ENGINEERED BONE CONSTRUCTS FOR THE MODELING OF PROSTATE CANCER METASTASIS TO BONE

Chong, Mark[1], Lim, Jing[1], Tamilselvan, Sahaana[1], Sia, Ming Wei[1], Lee, Lui Shiong[2], Chan, Jerry[3], Teoh, Swee Hin[1]

[1]Nanyang Technological University, Singapore, [2]Singapore General Hospital, Singapore, [3]KK Women's and Children's Hospital, Singapore

Co-cultures of human mesenchymal stem cells (MSC) and endothelial cells have been extensively studied for the engineering of bone grafts for regenerative application. These constructs have been shown to undergo osteogenesis and vasculogenesis, giving rise to vascularised osseous tissue following implantation in various animal models. The ability to recapitulate such physiological processes in vitro have led to suggestions that tissue engineered bone (TEB) constructs may be useful for the modelling of skeletal diseases, such as bone metastasis. In particular, prostate cancer metastatic formation and progression in bone is shown to be heavily influenced by interactions with osseous and vascular cells within the bone marrow niche. Here, we studied the use of TEB as a model of the bone metastatic environment. We hypothesised prostate cancer cells to interact with osteogenic and vasculogenic pathways with TEB, leading to tumour cell activation and proliferation. To test this hypothesis, cells were fluorescence-labelled and imaged in real time to obtain live data on vascular morphometry, as well as cancer cell proliferation and migration. Osteogenic induction assays indicated endothelial and prostate cancer cells to potentiate osteogenesis of MSC, as evaluated by qPCR of osteogenic genes (ALP, RUNX2, COL1A1, OCN) and quantitative analysis of mineral content by Alizarin Red S. When co-cultured with MSC, EC were found to spontaneously form networks in dose-dependent fashion, with a minimum 1:1 MSC:EC ratio required for the formation of stable networks and used in subsequent experiments. Following the assembly of endothelial cells and MSC into vascularised bone constructs, Histone H2B-RFP fusion protein-labelled prostate cancer cells (PC3) were added at a 1:100 PC3: MSC ratio. Compact clusters of PC3 cells reminiscent of micrometastatic formations could be found organised in the vascularised bone niche by day three, but failed to proliferate significantly over the next 11 days. Cell proliferation data was obtained non-destructively by imaging and quantification of cell nuclei. It was found that, while PC3 cells were able to attach to MSCs, proliferation was found to be suppressed 99.3 fold in the presence of MSC alone ($p<0.001$), and 8.7 fold ($p<0.001$) on the endothelial-MSC co-cultured constructs. Taken together, these results suggest that thus-cultured TEB were able to create a quiescent bone marrow niche in which prostate cancer cells were able to attach but remained dormant. Current work in progress includes studying the effects of pro-inflammatory cues on the vascular and osteogenic properties of the TEB, and consequent effects on tumour cell activation.

T-2094
MODULATION OF ADIPOSE STEM CELL DIFFERENTIATION IN 3D SCAFFOLDS

Clevenger, Tracy[1], Burke, Daniel[2], Ashley, Rebekah[3], Rowland, Teisha[4], Hinman, Cassidy[1], Xu, Zhuojin[1], Hawker, Craig[2], Clegg, Dennis O.[1]
[1]Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA, USA, [2]Chemistry, University of California, Santa Barbara, Santa Barbara, CA, USA, [3]Molecular, Cellular and Developmental Biology, UCSB, Santa Barbara, CA, USA, [4]University of California, Santa Barbara, Santa Barbara, CA, USA

Soft tissue defects can result from tumor removal, trauma, deep burns and liposarcomas. Currently reconstruction approaches using fat grafting have variable efficacy, and there is an unmet medical need for better treatments. Adipose-derived stem cells (ASCs) are capable of differentiating into various cell types including; adipocytes, chondrocytes, osteoblasts, and endothelial cells. These cells are easily obtained through commonly employed liposuction procedures, they are highly abundant, robust and capable of self-renewal. These characteristics make ASCs an ideal candidate for treatments of soft tissue defects. The development of a synthetic scaffold, supporting survival and differentiation of ASCs would help to establish a predictable, stable treatment. Adhesive extracellular matrix molecules are known to be important in the proliferation and differentiation of cells, and many matrix proteins contain Arg-Gly-Asp (RGD) attachment sites, which have been found to play a large role in the adhesion of cells through integrins. It has been previously shown that the RGD motif can be manipulated to provide varying degrees of binding affinity for cells, and adhesion can be modulated by other peptides. We have tested RGD peptides that support a range of adhesivity by incorporating 3D hydrogel scaffolds seeded with ASCs. We measured the ability of these scaffolds to support adhesion, survival, proliferation and differentiation. Differentiation was monitored by assaying changes in gene expression, morphology and integrin expression. Results show that while more adhesive substrates afforded better attachment, proliferation and survival, slightly less adhesive peptides yielded more extensive differentiation of ASCs to adipocytes. Discovering the ideal scaffold composition for differentiation of ASCs to adipocytes will have future applications in the clinic for treatment of soft tissue defects.

T-2095
INVESTIGATE OF DIFFERENTIATION OF HUMAN ADIPOSE DERIVED STEM CELLS (ADSCS) INTO NEURAL LIKE-CELLS ON 3-D ALIGNED NANOFIBER SCAFFOLDS

Esfandiari, Behnaz[1], Soleimani, Masoud[2], Foroughi Abari, Mohamad Ali[3]
[1]Biology, Olom Tahghighat Islamic Azad University, Tehran, Iran,
[2]Hematology, Modaress University, Tehran, Iran, [3]Biology, Ferdowsi University of Mashhad, Mashhad, Cyprus

Nerve regeneration is a complex biological phenomenon. Neural tissue repair and regeneration strategies have received a great deal with attention because is directly affects the quality of the patient's life. There are many scientific challenges to regenerate while using conventional autologous nerve grafts and from the newly developed therapeutic strategies for the reconstruction of damaged nerves.Recent advancements in nerve regeneration have involved the application of tissue engineering principles and this has involved a new perspective to neural therapy. The success of neural tissue engineering is mainly based on the regulation of cell behavior and tissue progression through the development of a synthetic scaffold that is analogous to the natural extracellular matrix and can support three-dimensional cell cultures. In this study ,the potential of human adipose derived stem cells (ADSCs) for neuronal differentiation in vitro on poly-(ϵ-caprolactone) (PCL) nanofibrous scaffolds were investigated. Aligned PCL nanofibrous scaffolds were fabricated by electros pinning process and their surface was modified with O2 plasma treatment to enhance the ADSCs proliferation, adhesion and interaction with nanofibrous scaffold and their chemical characterization were carried out using SEM, contact angel and tensile test. Plasma treated (p-PCL) nanofiber scaffold was found to exhibit the most balanced properties and was used for culture of ADSCs. The proliferation of ADSCs appraised by MTT assay showed that the grown on PCL nanofibrous scaffold were comparatively higher than those on tissue culture plate (TCP). The differentiation of ADSCs was carried out using neuronal inducing factors including bFGF, forskolin and Retinoic acid in DMEM/F12 media. The expression of MAP2, β Tubulin and NSE were confirmed by immunocytochemistry. It was found that the direction of cells elongation and neuritis outgrowth on Aligned nanofibrous scaffolds is parallel to the direction of fibers. Our results on the differentiation of ADSCs to neuronal cells on nanofibrous scaffolds propose that PCL is cost-effective material for nerve regeneration and hADSCs can be differentiation in to neuron like cells.

T-2096
A COMPARATIVE ANALYSIS OF COMPACT BONE, ADIPOSE, AND BONE MARROW DERIVED MESENCHYMAL STEM CELLS AND EFFECTS OF HYPOXIC PRECONDITIONING ON OSTEOGENIC POTENTIAL

Fernandez-Moure, Joseph[1], Corradetti, Bruna[1], Van Eps, Jeffrey[1], Weiner, Bradley[2], Rameshwar, Pranela[3], Tasciotti, Ennio[1]
[1]Nanomedicine, Houston Methodist Research Institute, Houston, TX, USA, [2]Orthopedic Surgery, Houston Methodist Hospital, Houston, TX, USA, [3]University of Medicine and Dentistry New Jersey, Newark, NJ, USA

Introduction:Mesenchymal stem cells (MSCs), readily harvested from bone marrow and adipose tissue, hold great promise for regenerative therapies in the musculoskeletal system. While particular sources of MSCs are predisposed to immune functions, other sources are excellent for tissue regeneration. Bone marrow and adipose derived MSCs have been . To this end, we sought for a novel source of MSCs in humans, the bone. We isolated and charterized compact bone MSCs (cbMSCs) from human spinal lamina and then studied their in vitro osteogenic potential compared with adipose-derived MSCs (adMSCs) and bone marrow derived MSCs (bmMSCs). These studies were conducted with the hypothesis that, in osteogenic conditions, cbMSCs would have a greater biosynthetic and transcriptional activity as compared to adMSCs and bmMSCs. Methods:Bone fragments from patients undergoing laminectomy were subjected to enzymatic processing. Adherent cells then underwent flow-assisted cell sorting (FACS) using markers CD34, CD 45, HLA-DR, CD11b, CD19, CD90, CD105, CD 44, CD29, and CD73. These cells were then subjected to trilineage differentiation. AdMSCs and bmMSCs were obtained from age matched independent donors and similarly characterized. All cell types underwent colony forming unit analysis. Expanded MSCs were subjected to osteogeneic differentiation for 2 and 4 weeks in hypoxic and normoxic conditions. Biosynthetic activity was quantified histologically using Von Kossa staining for calcium. Transcriptional activity was assessed by real-time PCR (RT-PCR) for genes linked to osteodifferentiation : RUNX2, ALP, osteopontin, and osteocalcin,. Results:Adherent cells from the bone were found to be negative for markers CD34, CD45, HLA-DR, CD11b, CD19 and positive for CD105, CD44, CD29, CD90, and CD73. This population of cells was capable of tri-lineage differentiation and formation of colonies. Compared ot bmMSC and adMSC, cbMSC had the least number of colonies, but had a greater number of cells per colony. We observed the greatest calcium deposited by cbMSCs at

wks 2 and 4 in both hypoxic and normoxic conditions. The calcium deposition was greater for all groups in hypoxic conditions These functional observations correlated with the RTPCR for markers of osteodifferentiation. In normoxic conditions cbMSC the greatest increase was seen in SPP1 and RUNX2. In hypoxic conditions all cell types demonstrated an increase in osteogenic marker expression. cbMSC demonstrated an even greater increase in osteogenic marker expression with marked increases in SPP and ALP. Conclusion:In conclusion, cortical bone contains a population of compact bone stromal cells with defined MSC surface markers capable of tri-lineage differentiation. This population of MSCs showed greater biosynthetic activity in vitro. This finding correlated to with the PTPCR for markers of osteodifferentiation. Hypoxic preconditioning increased calcium deposition and gene expression increased across all groups. cbMSC, though, demonstrated the greatest increases with RUNX2 and ALP having marked upregultion. These findings show that for application so of bone regeneration the compact bone as a source harbors a cell population that is potentially superior in osteoregenerative potential when compared to age matched bmMSCs and adMSCs. This finding adds to our understanding of the therapeutic potential for this novel source of MSCs and seems to poise cbMSCs as the preferred cell type for orthopedic engineering applications.

T-2097
HYDROGELS INCREASE THE SURVIVAL OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED OLIGODENDROCYTE PRECURSOR CELLS AFTER TRANSPLANTATION INTO THE INJURED SPINAL CORD

Fuehrmann, Tobias[1], Tam, Roger[1], Coles-Takabe, Brenda[1], Van Der Kooy, Dereck[1], Morshead, Cindi M.[1], Nagy, Andras[2], Mothe, Andrea[3], Tator, Charles[3], Shoichet, Molly S.[1]
[1]Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada, [2]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada, [3]Krembil Neuroscience Centre, University Health Network, Toronto, ON, Canada

The loss of function after traumatic spinal cord injury (SCI) is due to the primary mechanical injury, and the secondary injury, which results in a cascade of cellular and molecular events, leading to axonal degeneration, demyelination of spared axons, inflammation, expression and/or release of axon-growth inhibitory/repulsive molecules at and around the lesion site, and glial scarring. Cell transplantation-based treatment strategies positively influence a number of these targets. Transplantation of embryonic- or foetus-derived neural stem/progenitor cells into different experimental animal models of SCI has generally resulted in some degree of functional improvement. However, cell fate of donor cells was only poorly controlled. Furthermore, remyelination of spared, demyelinated axons after spinal cord injury by grafted cells has the potential to preserve functional pathways and improve function. Interestingly, transplantation of neural stem/progenitor-derived oligodendrocytes has been shown to be more efficient for remyelination mediated-repair than grafting non-differentiated or uncommitted cells. Nevertheless, survival of the cells upon transplantation is limited and cell-delivery systems aiming to enhance the survival and integration of grafted cells might lead to greater beneficial effects. We differentiated human induced pluripotent stem cells into oligodendrocyte precursor cells (hOPCs), as identified by the expression of OLIG2, NKX2.2, SOX10, and PDGF-receptor alpha. As a cell delivery vehicle a minimally invasive, injectable hydrogel comprised of hyaluronan and methylcellulose (HAMC) was used. In a pilot study, hOPCs were transplanted into experimental animals receiving a moderate clip compression injury (24g) at level T2. Cells were transplanted with and without HAMC 7 days after injury at 4 sites rostral and caudal to the lesion. Surviving cells were found at the injury/injection site 7 days after transplantation (14 days after injury), but only when transplanted in HAMC. These surviving hOPCs expressed SOX10, a marker for oligodendrocytes and oligodendrocyte precursor cells. No surviving cells were found in animals receiving hOPCs in media. To further increase hOPCs survival within the HAMC hydrogel, we modified MC with adhesive peptides and tested cell survival in vitro. Cells were cultured in HAMC, peptide-modified HAMC and tissue culture polystyrene for 1 week. HOPCs demonstrated greater cell survival when cultured in peptide modified HAMC compared to HAMC and cell culture media. The peptide sequence also promoted greater cell-substrate (HAMC) interaction than HAMC alone, as indicated by a greater hOPC migration within the peptide modified HAMC. These data indicate the feasibility of our approach to promote graft cell survival with hydrogels.

T-2098
DECELLULARIZED AND RECELLULARIZED KIDNEY AS A THREE-DIMENSIONAL SCAFFOLD FOR RENAL TISSUE ENGINEERING

Goldenberg, Regina, Martins, Andreza Bastos, Silva-Mendez, Bernardo Jorge, Nascimento, Juliana Silva, Fonseca, Rodrigo Nunes da, Silva, José Roberto, Barros, Cintia Monteiro de, Campos de Carvalho, Antonio Carlos, Souza-Menezes, Jackson
Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Aim: The aim of this study was to decellularize intact rat kidneys in a manner that preserved the intricate architecture properties necessary to reconstitute basic renal structures in vitro. Methods and Results: All animal procedures were approved by the Institutional Animal Care and Use Committee (CCS/ UFRJ - MACAÉ019). The rats were sedated and anesthetized. A laparotomy was performed for kidney excision. The kidneys were washed twice with PBS followed by decellularization with 1% SDS for 15 h followed by perfusion for 15 minutes PBS. The kidney extracellular matrix (ECM) was fixed in 4% PF at room temperature and embedded in paraffin or stored in antibiotic-antimitotic solution in PBS to use as scaffolds for cell culture. The porcine proximal tubule cell line (LLC-PK1) was plated in ECM for 3, 7 and 14 days after culture initiation, and partially recellularized ECM were fixed in 4% PF and histochemical analyses with H and E, picrosirius and PAS were performed. After 15h of decellularization process, the kidneys became translucid. Qualitative analysis of decellularized tissues revealed well-preserved architecture. The detergent-based perfusion protocol successfully produced acellular kidneys that retained the web-like basement membrane. HE and Picrosirius staining confirmed the removal of all cellular material. After LLC-PK1 recellularization procedure cell adhesion on renal ECM was observed. PAS staining showed cell adhesion to the decellularized kidney ECM. Conclusion: These findings demonstrate that decellularized kidney sections retain critical structural properties necessary to use as a three-dimensional scaffold and promote recellularization. This study provides the initial steps in developing strategies for renal tissue engineering and repair.

T-2099
SKIN SUBSTITUTE PRODUCED WITH ALLOGENEIC OR XENOGENEIC DERMIS AND A SYNGENEIC EPIDERMIS ARE NOT REJECTED

Goyer, Benjamin, Auger, François A., Germain, Lucie
Laval University, Quebec, QC, Canada

The transplantation of our autologous skin substitute, developed in the LOEX center, for the treatment of severely burned patients allowed to save several lives. However, the major inconvenience

remains in the time required for their production. To overcome this problem, the possibility of using an allogeneic dermis associated with an autologous epidermis without rejection was assessed in the present study. Allogeneic epidermis is known to be rejected. Thus, various substitutes (allogeneic, xenogeneic, chimeric and autologous) were tested according to the self-assembly method. Fibroblasts and keratinocytes were extracted from adult skin of BALB/c and C3H/HeN mice, and normal human skin. To reconstruct the dermis, dermal fibroblasts were cultivated in the presence of ascorbic acid, which promotes extracellular matrix assembly and allows the formation of thick sheets of collagenous tissue. Epithelial cells (keratinocyte) were then added in order to reconstruct the epidermis. Following epidermal proliferation and differentiation, these substitutes were transplanted on a syngenic mouse C3H/HeN model in triplicate to estimate their functionalities. Allogeneic, autologous, xenogeneic and chimeric skin substitutes were grafted and compared according to rejection criteria 19 and 35 days after grafting. Immunohistochemical analysis suggests the establishment of a tolerance of the allogeneic and the xenogeneic dermis associated with a syngeneic epidermis 35 days post grafting. In contrast, we observed a systematic rejection of all skin substitutes produced with allogeneic or xenogeneic epidermis 19 days after grafting. In conclusion, this work is a new method that could decrease the waiting time of the patient for the production of skin substitutes.

T-2100
PHYSICAL CUES OF BIOMATERIALS GUIDE STEM CELL FATE OF PROLIFERATION AND DIFFERENTIATION

Higuchi, Akon[1], Ling, Qing-Dong[2]
[1]*Department of Chemical and Materials Engineering, National Central University and RIKEN Advanced Science Institute and King Saud University, Jhong-li, Taiwan,* [2]*Cathay General Hospital, New Taipei, Taiwan*

Increasing evidence suggests that the physical microenvironments of stem cells, in addition to soluble biological factors, help to direct stem cell fate during proliferation and differentiation. Little is known about the effect of substrate stiffness on the pluripotency fate and proliferation of hematopoietic stem and progenitor cells (HSPCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs). Therefore, we investigated ESCs (H9) culture as well as the ex vivo expansion of HSPCs cultured on biomaterials with different elasticities and grafted with different nanosegments. We prepared dishes coated with polyvinylalcohol-co-itaconic acid (PVA-IA) films having different elasticity ranging from a 3.7 kPa to 30.4 kPa storage modulus by controlling the crosslinking time in crosslinking solution containing glutaraldehyde, and grafted with several ECM-derived cell-adhesion peptides though N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) chemistry in an aqueous solution. Fibronectin or an oligopeptide (CS1 [EILDVPST] and cell binding domain of vitronectin) was grafted onto the PVA-IA substrates. High ex vivo fold expansion of HSPCs was observed in the PVA-IA dishes grafted with fibronectin or CS1, which displayed an intermediate stiffness ranging from 12.2 kPa to 30.4 kPa. Furthermore, HSPCs cultured in fibronectin or CS1-grafted PVA-IA-coated dishes with a stiffness of 12.2 to 30.4 kPa generated more pluripotent colony-forming units (CFU-GM and CFU-GEMM) than those in TCPS dishes. It is also found that the presentation of specific cell-binding domains of CS1 at high concentrations (relative to the FN density) appears to be important for the signal transduction pathways that promote the ex vivo expansion of HSPCs and maintain their pluripotency. Thus, both the physical cues (i.e., the elasticity of the cell culture substrate) and the amounts of specific biological cues (i.e., CS1 or FN) that are grafted onto cell culture substrates represent important factors that determine the ex vivo expansion of HSPCs and maintain their pluripotency. hESCs (H9) were cultured in a chemically defined medium of mTeSR1 on several grades of stiffness of PVA-IA hydrogels grafted with oligopeptide derived from vitronectin (KGGPQVTRGDVFTMP). The hESCs cultured on relatively stiffer substrates (e.g., storage moduli of 25 kPa and 30 kPa) tended to differentiate after five days of culture, whereas the hESCs cultured on relatively softer substrates of 12-16 kPa maintained their pluripotency based on the morphology of hESC colony. Only a few small or no colonies of hESCs were observed on the softest substrates (10 kPa). Therefore, these results indicate that cell culture substrates with the optimal elasticity can maintain the pluripotency of an hESC culture. It is concluded that both the physical and biological properties of biomaterials affect the ex vivo expansion of HSPCs as well as culture of ESCs and iPSCs.

TECHNOLOGIES FOR STEM CELL RESEARCH

T-2101
SINGLE USE EXPANSION AND HARVEST OF ADULT STEM CELLS SUPPORTS LARGE SCALE MANUFACTURING

Murrell, Julie, Punreddy, Sandhya, Kehoe, Daniel
EMD Millipore, Bedford, MA, USA

As more stem cell therapeutics progress through clinical testing, current in vitro culture methods are cumbersome to scale. We previously demonstrated an expansion paradigm that uses a scalable, single use, stirred tank bioreactor with microcarrier scaffold for bone marrow derived human mesenchymal stem cell (hMSC) culture that enables direct monitoring for the specific characteristics of hMSCs at any point during the culture, thus assuring product quality and consistency. In addition, a bioreactor provides ease of use in handling and lower medium volume requirements. Here we describe expansion, harvest and characterization of the bioreactor system for adipose-derived hMSCs. The cells were evaluated using flow cytometry to assess a variety of markers that confirm identity and purity. Differentiation potential was confirmed using traditional methods. Finally, we evaluated harvest technologies and found that they provide good recovery of viable cells. In this work, we verified that cells expanded in the single use stirred tank bioreactor and subsequently harvested were identical in phenotypic profile in comparison to flat culture and maintained the desired cell characteristics of hMSCs, thereby confirming the consistency, quality and reproducibility of large scale in vitro systems for stem cell expansion.

T-2102
NONINVASIVE IN VIVO IMAGING OF MESENCHYMAL STEM CELLS USING NIS

Peng, Kah-Whye[1], Suksanpaisan, Lukkana[1], Degrado, Timothy[2], Russell, Stephen J.[1]
[1]*Molecular Medicine, Mayo Clinic, Rochester, MN, USA,* [2]*Radiology, Mayo Clinic, Rochester, MN, USA*

There is a need for robust noninvasive imaging technologies to monitor the in vivo fate of transplanted stem cells. Optical imaging modalities that rely on the GFP and firefly luciferase reporter gene are widely adopted but are restricted to small rodents such as mice. We have been developing nuclear imaging modalities for monitoring the fate of mesenchymal stem cells. Mesenchymal stem cells (MSC) are endowed with immune suppressive properties and are capable of differentiating into osteocytes, chondrocytes and adipocytes. Currently, there are more than 200 clinical trials worldwide investigating the safety and

efficacy of MSC for tissue repair or immune modulation in a variety of conditions, including joint and tendon repair or healing. However, it remains unknown for how long the cells survive post-transplantation, if they proliferate, engraft or die. The thyroidal sodium iodide symporter (NIS) is a non-immunogenic protein expressed on the surface of thyroid follicular cells that mediates the uptake of iodide. NIS expression can be monitored noninvasively, quantitatively and repeatedly by imaging the biodistribution of various radioactive isotopes (I-125, I-123, I-124, Tc-99m pertechnetate) using gamma camera, SPECT or PET imaging modalities in small and large animals. In this study, we evaluated 1) the feasibility and sensitivity of NIS as a reporter gene for noninvasive imaging of MSC in vivo and 2) resolution using SPECT or PET tracers, 3) the in vivo biodistribution of MSC post administration into mice and rats, 4) the long term durability of the stem cells in these animal models. Results from our study will be presented at the meeting.

T-2103
BIOENGINEERING A CHROMOSOME-CENTERED DELIVERY SYSTEM FOR THERAPEUTIC INTERVENTION OF GENETICALLY-BASED DISEASES

Perkins, Edward[1], Rose-Hellekant, Teresa[2], Holy, Jon[2], Drewes, Lester[2], Anderson, Grant[3], Greene, Amy[1]
[1]Biomedical Sciences, Mercer University School of Medicine, Savannah, GA, USA, [2]Biomedical Sciences, University of Minnesota School of Medicine, Duluth, MN, USA, [3]College of Pharmacy, University of Minnesota, Duluth, MN, USA

Bioengineered stem cells offer a unique, gene-based, therapeutic approach applicable to an increasing variety of proposed clinical applications. To meet this end, flexible, and safe methodologies are required for consistent and efficient cellular bioengineering devoid of potentially harmful cellular alterations. Our long-range goal is to actualize a tractable, versatile, synthetic chromosome-based platform (termed ACE chromosome), onto which multiple genetic factors may be placed for directing a multi-targeted approach to cancer therapy. The ACE chromosome is an autonomous synthetic chromosome "circuit board" designed to contain approximately 70 site-specific, recombination acceptor sites that can carry single or multiple copies of genes of interest. Bioengineered ACE chromosomes are readily purified by flow cytometry and chromosome sorting, making the ACE system amenable to stem cell bioengineering. To date, the ACE system has been used to bioengineer a variety of stem cell types including human mesenchymal stem cells (hMSC), CD34+ human hematopoietic stem cells (hHSC), murine embryonic stem cells and other primary and transformed cells. An essential component of the ACE platform bioengineering system consists of designed ACE targeting vectors (termed pAPP vectors), along with a unidirectional, site-specific integrase derived from bacteriophage lambda (ACE integrase) for delivery and targeting of multiple genetic factors to the platform. As an initial proof-of-principle of our synthetic chromosome system's ability to produce anti-cancer agents, we have utilized the ACE system to produce decorin (DCN), a small pericellular matrix proteoglycan that modulates collagen fibrillogenesis and tumor cell proliferation. Based upon our earlier demonstration of ACE platform delivery to hMSCs, our long-range goal is to generate DCN producing hMSCs for localized delivery of this tumoricidal factor to inhibit cancer growth. To further demonstrate the utility of the ACE platform system for genome-scale applications, BAC clones spanning genomic regions of interest can be retrofitted and targeted to the ACE platform. Two off-the-shelf BAC clones, spanning the 33kb genomic GLUT-1 gene and adjacent regulatory sequences, were retrofitted to contain the targeting sequence required for integration onto the ACE chromosome. Confirmed, retrofitted BACs were then integrated onto the platform using the ACE integrase. Retrofitted clones were quality controlled by FISH and PCR to confirm maintenance of all GLUT-1 exons (10 exons) and targeting to the ACE chromosome. Our results further delineate the ACE system as a novel cytoreagent-centered, bioengineering strategy amenable to a variety of stem cell-based therapeutic applications founded upon the following salient features: 1.ACE platform chromosomes can be generated and stably maintained in a wide variety of mammalian cells including stem cells. 2.ACE platform chromosomes can be tractably engineered via site-specific, uni-directional recombination to express single, multiple or large therapeutic factors. 3.Engineering of ACE chromosomes circumvents insertional mutagenesis of the host chromosomes. Results from our studies illustrate the flexibility and utility of this nonintegrating, transportable, synthetic delivery system and underscore its potential application in stem cell-based therapies and disease model systems.

T-2104
INTER-ALPHA TRYPSIN INHIBITOR IS PRESENT IN PLURIPOTENT STEM CELL EXTRACELLULAR MATRIX AND HAS A DIRECT EFFECT ON ATTACHMENT AND SIGNALING

Pijuan-Galitó, Sara, Tamm, Christoffer, Annerén, Cecilia
Department of Medical Biochemistry and Microbiology (IMBIM), Uppsala University, Uppsala, Sweden

Pluripotent stem (PS) cells, such as embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, have traditionally been grown *in vitro* using media containing either serum or serum-derivatives such as albumin. These additives improve attachment and proliferation rates. However, they are very complex, undefined supplements that contain a plethora of growth factors and extracellular matrix proteins, making these media formulations incompatible with standardized and controlled culture processes. We have previously described a novel LIF (leukemia inhibitory factor)-induced pathway, including the Src family kinase Yes, the Yes-associated protein (YAP) and the TEAD family of transcription factors, which contributes to mouse embryonic stem cell self-renewal. In the present study we show that fetal bovine serum (FBS) consistently activates this pathway to a similar extent as LIF. Through a series of fractionating techniques, inter-alpha trypsin inhibitor (IαI) is identified as a distinct component in serum capable of activating the Yes/YAP/TEAD2 pathway in mouse embryonic stem cells. Addition of purified IαI to FBS- and LIF-starved mouse ES cells confirms it capable of inducing YAP nuclear translocation and TEAD2-dependent transcription in a dose-dependent manner, suggesting that this serum protein specifically activates the YAP/TEAD2 pathway. IαI is a family of protein-glycosaminoglycan-protein complexes that are present in plasma at high concentrations in mammals (0.6-1.2 mg/ml). They consist of combinations of heavy chain domains (HC1-5) and a light domain (Bikunin, Bk) covalently linked together by a chondroitin sulphate chain. The most common form of IαI in human serum contains HC1, HC2 and Bk. IαI is predominantly produced by the liver and remains inactive until processed in the target tissue by TSG-6 (tumor necrosis factor stimulated gene 6 protein). The HC domains are then transferred and covalently bound to hyaluronan, while the Bk domain exhibits protease inhibitory activity when released from the HCs. The HC domains have primarily been described as extracellular matrix remodeling proteins. However, recent studies indicate that IαI also plays a role in metastatic processes and increased HC production in certain cancer cell lines has been linked to a decreased migration capacity. In this study we show that both human and mouse PS cells stain positive for HC1 and HC2 in *in vitro* culture. However, no HC1 or HC2 mRNA transcripts are detected in PS cells, indicating that IαI HC molecules from serum are transferred to the pluripotent stem

cell extracellular matrix. Interestingly, addition of IαI to serum-free medium promotes attachment of both mouse and human PS cells to non-coated tissue-culture treated plastic. We demonstrate for the first time that human ES and iPS cells can attach and self-renew through at least 10 passages on uncoated plastic in a serum-free medium supplemented with purified IαI. These PS cells retain their expression of typical pluripotency markers and colony morphology. HC2 appears to be the IαI domain responsible for these effects since HC2 alone activates TEAD2, and HC2-coating promotes PS attachment; while neither Bk nor HC1 are able to induce similar effects. In conclusion, this study identifies IαI as a component of serum capable of signaling and inducing attachment of pluripotent stem cells, and suggests that the HC2 domain is responsible for this effect.

T-2105
DEVELOPING COCLINICAL TRIALS WITH STEM LIKE TUMOR INITIATING CELL BASED HUMAN PATIENT DERIVED XENOGRAFT CANCER MODELS

Rajasekhar, Vinagolu K.[1], Scher, Howard I.[1], Heller, Daniel[1], Tabar, Viviane[1], Kim, Kitai[1], Healey, John H.[1], Studer, Lorenz[2]
[1]Memorial Sloan Kettering Cancer Center, New York, NY, USA, [2]Sloan-Kettering Institute for Cancer Research, New York, NY, USA

In contrast to genetically engineered animal cancer models or cancer cell line derived xenograft models, human patient tumors are composed of dynamically heterogenous tissues representing different cell types with a spectrum of mutations. Same type of cells within a single tumor may also contain a variety of mutations, and some of these mutant cells may pose no threat to become an aggressive tumor. Tumors also contain a minor population of stem-like tumor-initiating cells (TIC), which can recreate the entire parent tumor heterogeneity when transplanted into immunocompromised animals. Because of their minority and subtypes within the total bulk tumors, the genetic/epigenetic, transcriptome and proteome signatures of these TICs are likely to become less significant in the total bulk tumor tissue analysis. Moreover, tumor heterogeneity is also a dynamic process during development and has thus been one of the fundamental hurdles in identifying functional targets in cancers. Therefore, purified and functionally characterized TICs, would offer an excellent platform for unveiling the precise cancer therapeutics. Towards this goal, we are developing patient derived xenograft (PDX) models and organoids/organotypic explants of human cancers in order to first obtain a large amount of bulk tumor tissues and then isolate sufficient cell numbers of TICs. Utilizing purified TICs, we are developing and banking live PDX models (PDX^{TICs}) which are sequentially transplantable over many generations with the same fidelity. Our work also relied on orthotopic PDX models of cancer in order to maintain their parent tumor microenvironment and relevant signaling cross talks. Exploiting similar principles of the in vivo-imaging and nanotechnologies available to our patients in clinic, we have succeeded in tracking metastatic abilities of TICs in vivo in these PDX^{TICs} models. We have also identified novel markers (eg. SOX9), and functionally relevant signaling pathways (eg. NF-kB) in the purified TICs. We are validating a set of these and additional new markers in the TICs of many different human cancers. Additional experiments on the molecular genetic and functional properties of the TICs identified clinical relevance to therapy resistant patient tumors. We have also developed novel screening strategies for identifying compounds to target their metastatic ability and these screening strategies are also expandable to the discovery of new anti-cancer compounds. Thus, our approaches aiming to develop specifically the TIC-based mouse hospital for human cancers will facilitate real-time patient-specific co-clinical trials for the first time towards combating cancers at their cell of origin. These models are harmonized and will be open to all those interested in collaborating.

T-2107
REPLICATIVE STRESS DURING SOMATIC CELL REPROGRAMMING

Ruiz, Sergio[1], López-Contreras, Andrés[1], Batada, Nizar[2], Fernández-Capetillo, Óscar[1]
[1]CNIO, Madrid, Spain, [2]Ontario Institute for Cancer Research, Toronto, ON, Canada

Reprogramming of somatic cells into induced pluripotent stem (iPS) cells has been achieved by the expression of defined sets of transcription factors (e.g. OCT4, SOX2, KLF4 and cMYC). Recent reports have demonstrated that hiPS cells contain genomic structural variations. In fact, it has been observed an increased overall frequency of copy number variations (CNVs) in human iPS cells relative to their corresponding non-pluripotent samples as well as to human embryonic stem (hES) cells. Interestingly, the group of de novo-CNVs that arise during the reprogramming process was detected frequently associated to common fragile sites (CFSs). This suggests that replicative stress (RS), a type of DNA-damage characterized by perturbed DNA replication and a well-known source of CNVs, might occur at the reprogramming process. Among the factors that cause RS include down-regulation of replication factors, decrease in the pool of deoxynucleotides (dNTPs) or alteration in the levels of proteins involved in the RS response such as ATR and CHK1 kinases. Importantly, RS is boosted by the expression of certain oncogenes, such as cMYC. In this work, we sough to provide evidence for RS occurring at the reprogramming process, to understand the mechanisms underlying this RS and to evaluate the consequences of modifying the levels of RS during reprogramming. Our results and ideas in this topic will be presented.

T-2109
DEVELOPMENT OF A NOVEL XENO-FREE MEDIUM, STEMFIT™, FOR FEEDER-FREE CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELLS

Senda, Sho[1], Okamoto, Satoru[2], Taniguchi, Yukimasa[3], Ozawa, Hiroki[1], Ando, Yumi[1], Yokoyama, Mizuho[1], Matsumoto, Takuya[1], Kobayashi, Tsuyoshi[1], Takizawa, Nanako[4], Ichisaka, Tomoko[4], Asano, Kanako[4], Morizane, Asuka[4], Doi, Daisuke[4], Takahashi, Jun[4], Yoshida, Yoshinori[4], Toyoda, Taro[4], Osafune, Kenji[4], Itoga, Jyunko[3], Yagi, Emiko[3], Nishizawa, Masatoshi[4], Yamanaka, Shinya[4], Sekiguchi, Kiyotoshi[3], Nakagawa, Masato[4]
[1]Institute for Innovation, Ajinomoto CO., Inc., Kawasaki, Japan, [2]Research Institute for Bioscience Products and Fine Chemicals, Ajinomoto CO., Inc., Kawasaki, Japan, [3]Institute for Protein Research, Osaka University, Osaka, Japan, [4]Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan

In order to apply human pluripotent stem cells to regenerative medicine, the cells should be produced under restricted conditions conforming to GMP guidelines. Feeder-free (Ff) and xeno-free (Xf) conditions are preferable to achieve this goal. To realize stable and efficient Ff /Xf culture, we developed a novel Xf medium, StemFit™. Using StemFit™, in combination with recombinant human laminin-511 E8 fragment as matrix, human embryonic stem cells (hESCs) and induced pluripotent cells (hiPSCs) can be easily and stably passaged by dissociating the cells into single cells for long periods, without any karyotype abnormalities. Furthermore, we have succeeded to replace all the protein components of StemFit™, including human albumin, to recombinant proteins, greatly reducing the risk of contaminations of infectious pathogens.

T-2110

IDENTIFICATION OF COMPOUNDS REGULATING NEURITE OUTGROWTH AND RETRACTION IN HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS USING HIGH CONTENT SCREENING

Sherman, Sean[1], Bang, Anne[2]
[1]Conrad Prebys Center for Chemical Genomics, Sanford-Burnham Medical Research Institute, La Jolla, CA, USA, [2]Conrad Prebys Center for Chemical Genomics, Sanford Burnham Medical Research Institute, La Jolla, CA, USA

Human induced pluripotent stem cell (hiPSC) derived neurons are powerful tools for *in vitro* modeling of neurological disease, providing a source of patient specific cells that would otherwise be unobtainable. We have developed a high content screening assay measuring the effects of compounds on the growth and branching of neurites in hiPSC derived neurons. Neurite formation plays a fundamental role in regeneration, and in formation of functional neuronal networks. Development of technology platforms that can be used to perform such compound screens against hiPSC derived neurons with relatively high throughput will be essential to realize the potential that hiPSC hold for disease modeling and drug discovery. In the current work we have screened a reference set of approximately 5,400 compounds comprising a broad set of known drugs, kinase inhibitors, and well-characterized modulators of specific signaling pathways. Our immunofluorescence-based screen evaluates multiple factors of neurite outgrowth including neurite length, number, and branching as well as nucleus-based measures of overall cell health. Within this set we have identified multiple compounds that alter neurite morphology. These include compounds that increase or decrease overall neurite outgrowth as well as those that specifically affect neurite length or branching. The primary screen identified small molecules targeting pathways known to regulate neurite PKC, cytoskeleton dynamics, or estrogen receptor activity. In addition, several novel compounds were identified that have not been previously associated with regulating neurite outgrowth. Our results demonstrate the feasibility of performing higher throughput drug screens on hiPSC derived neurons, establish a platform for future screens using patient specific cells, and provide a comprehensive data set describing toxicity and modulation of neurite growth by over 5000 bioactive compounds, primarily known drugs and well-characterized modulators of known targets.

T-2111

DIFFERENTIATING HUMAN PLURIPOTENT STEM CELLS TO NEURONS - APPROACHES IN MEDIA DEVELOPMENT

Shin, Soojung, Cokonis, Melanie, Kaur, Navjot, Yan, Yiping, Newman, Rhonda, Vemuri, Mohan C., Kuninger, David
Thermo Fisher Scientific, Frederick, MD, USA

Specialized cell culture media is a foundational tool for researchers working in diverse areas, from basic and applied research to biopharmaceutical applications. Thermo Fisher Scientific offers media systems for culture of human and rodent (primary) neural cell types and more recently has focused on identifying conditions that drive stem cell differentiation toward specific neural lineages. AIM: To develop new cell culture systems that enable robust differentiation of human pluripotent stem cells (PSCs) to distinct neuronal subtypes. METHODS: We have adopted a multifaceted approach for driving PSC to neuronal differentiation- 1. Disconnecting specification/ regionalization studies from maturation, allowing experimental time line compression and enabling parallel development activities. 2. Utilizing complex Design of Experiment (DOE) approaches and mathematical modeling paired with validated endpoint assays; 3. Incorporating small molecule chemical library screening to identify compounds with desired properties. RESULTS: We demonstrate the feasibility of distinguishing PSC specification from neuronal maturation by utilizing banks of neural stem cells (NSCs), produced in 7 days using GIBCO® Neural Induction Medium. The NSCs provide a good model to screen and optimize conditions driving neural differentiation and maturation. Additional results of definitive screening DOEs as well as modeling predictions are described. CONCLUSIONS: In the last several years significant advances in stem cell biology have enabled broader adoption of these cells and provided deeper insight into the mechanisms which regulate their growth and specific cell fate determination. In this work we present our approach to harness this insight to develop next generation culture systems to create useful neuronal cell models from PSCs.

T-2112

IDENTIFYING ADULT STEM CELLS RESIDING IN THE BULGE AREA OF HAIR FOLLICLES USING MICRO-RAMAN SPECTROSCOPY

Short, Michael[1], Tsai, Tsung-Hua (Martin)[2], Lui, Harvey[3], Zeng, Haishan[1]
[1]Integrative Oncology, BCCRC, Vancouver, BC, Canada, [2]The Department of Dermatology and Skin Science, University of British Columbia, Vancouver, BC, Canada, [3]The Department of Dermatology and Skin Science, University of British Columbia, Vancouver, BC, Canada

Identifying and monitoring Adult stem cells (ASCs) in tissue without damaging them is a major challenge. The objective of this study was to determine whether the minimally invasive method of micro-Raman spectroscopy (mRS) can be used to identify ASCs *in situ*. Thin sections of murine skin were used to test this idea on ASCs in the bulge area of hair follicles. Two sequential thin sections of each sample were used, one section in the sequence was stained with CD-34 to identify hair follicles with a significant population of ASCs, and the other left unstained for mRS measurements. Raman spectra were acquired from multiple locations within cells of the hair follicle by focussing 515 nm excitation light onto the sample with a 100x objective lens. Clear Raman spectra were obtained in few seconds. Altogether Raman spectra were obtained from 100 cells: 52 were identified as putative ASCs and 48 as putative differentiated cells by their correlation to stained and unstained regions in the matching thin section. The Raman spectra from each cell were averaged, and multivariate statistical analyzes performed on these average spectra with a leave-one-out cross validation. Using this method ASCs and differentiated cells could be separated with 98% sensitivity and 94% specificity. Changes in nucleic acids, lipids and protein were evident between the two groups *in situ*. Furthermore analyses of the spectral variance indicated differences in cellular dynamics between the two cell groups. This study has shown that Raman micro-spectroscopy has a potential role in identifying adult follicle stem cells, laying the groundwork for future applications of hair follicle stem cells and other somatic stem cells *in situ*.

T-2113

MAPPING THE EMBRYO FOR STEM CELLS RESEARCH; LIFEMAP DISCOVERY AS THE ROAD MAP TO CELL DIFFERENTIATION

Shtrichman, Ronit[1], Edgar, Ron[1], Nazor, Yaron[1], Rinon, Ariel[1], Blumental, Jacob[1], Livnat, Idit[1], Ben Ari, Shani[1], Buzhor, Ella[1], Ohana, Reut[1], Leshansky, Lucy[1], Shraga, Netta[1], Bogoch, Yoel[1], West, Mike[2], Golan, Yaron[1], Warshawsky, David[1]
[1]LifeMap Sciences, Tel Aviv, Israel, [2]BioTime Inc., Alameda, CA, USA

Understanding how cells differentiate during embryonic development

is invaluable for the derivation of functional cells from stem cells in-vitro. Mapping the human embryo is an overwhelming challenge which includes characterization of all the cell types that make up the developing and mature human body, including embryonic progenitor cell types that occur in between states. The LifeMap Discovery database (http://discovery.lifemapsc.com) is taking the leading role towards this effort, providing the research community with an easy to use data portal describing embryonic development along with substantial information about stem and progenitor cells, their differentiation protocols and cell therapy applications. The database has been designed to integrate data from the in vivo and the in vitro, including gene expression and signaling information in developing cells. We will demonstrate how this integrated knowledge is used for identification and classification of derived stem cells, for matching derived stem cells to similar in vivo cells, and its value during the development of novel differentiation protocols and therapeutic products. Recently developed GeneAnalytics, a novel powerful gene expression analysis tool, can integrate and cluster data extracted from numerous resources. It supports analysis of multiple genes and applies a novel algorithm to match gene sets to tissues, anatomical compartments and cells within LifeMap Discovery. The matching score is calculated based on gene selectivity, uniqueness and enrichment in each specific entity above and takes into account the type of data source and technology used. The GeneAnalytics application is the most comprehensive analysis tool available today for modeling gene expression data in the embryo and the adult body. The value provided by LifeMap Discovery originates from the combined power its available data, which enables identifying, predicting and indicating possible differentiation paths and future regenerative medicine applications. LifeMap Discovery integrates with the database GeneCards - the human genome compendium, and MalaCards, the human diseases compendium and is provided free of charge for academic institutions.

T-2114
CLINICAL SCALE PRODUCTION OF HUMAN MESENCHYMAL STEM CELLS USING AN INDUSTRIAL SINGLE-USE BIOREACTOR

Siddiquee, Khandaker, Sha, Ma
Eppendorf, Enfield, CT, USA

Stem cell based regenerative medicine has great potential to revolutionize human disease treatments. Among the various stem cell platforms, mesenchymal stem cell (MSC) poses one of the highest potential as evident by clinical trial activities. Currently, there are over 400 clinical trials based on MSC registered at clinicaltrials.gov. Although successful expansion of MSC culture in vitro has been well established, the large clinical scale production of MSC remains to be a bottle neck, potentially limiting the immediate clinical applications should some of the trials receive FDA approval. Here in this study, we demonstrate the success of large clinical scale culture of human adipose derived mesenchymal stem cells (AdMSCs) in an industrial single-use bioreactor at 3.75 liter scale. The bioreactor offers a precision controlled environment for the ideal growth of stem cells under simulated physiological conditions. Stem cells and culture media were monitored, analyzed and controlled (e.g., cell count, cell viability, dissolved oxygen level, pH, temperature, glucose, glutamine, lactate, and ammonia levels etc.), thus allowing us to produce AdMSCs in large clinical scale quantities while maintaining healthy stem cell properties evident by stem cell marker assays and differentiation assays performed at end of the culture. Furthermore, with clinical relevance in mind, every cell culture step, from T-flask to shake flask to bioreactor vessel was conducted strictly using single-use consumables. AdMSCs were first scaled-up using the microcarrier based shake flask method previously described at ISSCR 2013 (Poster Presentation: T-2027). After a 15-day expansion period, the microcarriers containing stem cells were used to inoculate bioreactor with 3.75 liter volume, at a final cell concentration of 17,000 cells/ml with microcarriers concentration of 17 g/l. The agitation speed was set at 25 rpm; the temperature of the culture was controlled at 37 °C; the DO level was set to 15%. The control was set to 4-gas mode to maintain the DO set point using automated 4-gas mixing (air, CO2, N2 and O2) through overlay (vessel head space) instead of the typical sparger to minimizing foaming and to achieve dissolved Oxygen level closer to hypoxic physiological conditions. The pH of bioreactor was controlled by CO2 and 10% NaHCO3 solution. The gas flow rate was set to 0.1 SLPM during first 4 days and was increased to 0.3 SLPM thereafter. The agitation speed was also increased to 30 rpm from 25 rpm. In addition, 50% media exchanges were performed every 4 days; AdMSCs achieved ~14 fold expansion in the bioreactor, reaching high cell density of 0.23 million cells/ml at day 14. AdMSCs expanded using the single-use bioreactor retained their stem cell properties as evident by CD44, CD105 and CD90 immuno fluorescence stem cell marker assays, RT-PCR of CD105, OCT4, CD45, CD44, SOX2 genes and their ability to differentiate into either adipocytes or osteocytes.

T-2115
GENE TARGETING IN RAT EMBRYONIC STEM CELLS USING GENE EDITING TECHNOLOGY

Smith, J. Robert[1], He, Hong[1], Rajanahalli, Pavan[1], Ostertag, Eric[2], Vivian, Jay[3], **Weiss, Mark[1]**
[1]Anatomy and Physiology, Kansas State University, Manhattan, KS, USA, [2]Anatomy and Physiology, Transposagen Biopharmaceuticals Inc, Lexington, KY, USA, [3]University of Kansas Medical Center, Kansas City, KS, USA

Targeting gene modification in rat embryonic stem cells (ESC) has been difficult to accomplish following transfection and selection to identify on-target homologous recombination (HR) compared to mouse ESCs; the impact on research is that it is more difficult to develop rat models of human disease. We chose to determine whether the use of two new gene editing technologies, Transcription Activator Like Effector Nucleases (TALEN) or Clustered Regularly Interspaced Short Palindromic Reports (CRISPR)-Cas9 nuclease, which provide for targeted gene editing, could enable on-target HR in rat ESCs. Use of either gene editing technology enabled targeted HR in rat ESCs, as validated by fluorescence, PCR, and Southern Blotting. TALENs increased the efficiency of creating conditional p53 knockout ESCs using a vector that contained both positive and negative selection components so it could be compared directly with on-target HR following transfection without TALENs. After TALEN plus vector transfection and selection, 25% of the tested clones (10 out of 40 clones) were positive for targeted insertion, which was approximately 100 times more efficient than vector transfection and selection without TALEN (1 targeted clone out of 410 clones screened). Using TALENs also resulted in insertion into both p53 copies; e.g., both DNA copies targeted in ~ 5% of the clones (2 out of 40). The CRISPR-Cas9 system has been reported to be more efficient and more selective than TALENs in mouse ESCs. Therefore, using the CRISPR-Cas9 system, we wished to determine whether gene-editing would permit on-target HR without selection. Here, we used a CAG-RFP reporter vector that was targeted the rat Rosa26 locus. Following transfection with Cas9, guide RNA and vector, targeted insertion was present in 50% of the randomly selected clonal lines expressing RFP (8 out of 16). Transfections with a guide RNA modified at one base, as a specificity control, reduced on-target insertion efficiency. In summary, gene editing technologies improve the efficiency of on-target HR modification in rat ESCs by greater than 100 fold, and increased HR efficiency without antibiotic selection.

Thus, these methods reduce stress and decrease time to identify gene targeted rat ESCs.

T-2116
ESTABLISHMENT OF A NONINVASIVE METHOD FOR COUNTING HUMAN PLURIPOTENT STEM CELL NUMBERS BY LIVE CELL IMAGING

Suga, Mika[1], Kii, Hiroaki[2], Uozumi, Takayuki[2], Kiyota, Yasujiro[3], K. Furue, Miho[1]
[1]National Institute of Biomedical Innovation, Osaka, Japan, [2]Instruments Company, Nikon Corporation, Yokohama, Japan, [3]Instruments Company, Nikon Corporation, Tokyo, Japan

Background: Cell density is a critical factor for controlling both growth and differentiation of human pluripotent stem (hPS) cells including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. Despite the fact of the evolution of hPS cell culture techniques, counting cell numbers is still problematic. In this study, we have developed a noninvasive cell counting method for hPS cells through analyzing live cell images. Materials and Methods: Two human iPS cell lines, iPS-TIG114-4f1 (JCRB1437, JCRB Cell Bank, Osaka Japan) and Tic (JCRB 1331) were cultured on feeders or without feeders in a multi-well plate. After the cells were stained with a cell-permeant SYTO24 green fluorescent nucleic acid stain, phase contrast and fluorescent images of the cells were obtained intermittently in a cell incubator observation system. Immediately after imaging, cell numbers were counted by hemocytometer. Area of the cell colony coverage was measured from phase contrast images and nuclei numbers was counted from green fluorescent images by image analysis software. Results and Discussion: The nuclei numbers counted by fluorescent image analysis were similar with the cell numbers counting by hemocytometer in the case of total cell numbers above 1x 10^4. Analysis of phase contrast and fluorescent images revealed that there was a significant correlation between the colony coverage area and nucleus numbers, indicating that numbers of hPS cells can be estimated from the total colony coverage area through phase contrast imaging. Obtaining time-lapse phase contrast images enables us to monitor growth rate of hPS cells. Because the correlation between the cell numbers and colony area were specific for each hPS cell line, the correlation ratio might be characterized as the property of each hPS cell line. Our noninvasive cell counting technique could be useful for quality control or high-throughput screening analysis without wasting or damaging the cells.

T-2117
TRANSCRIPTIONAL BURSTING KINETICS OF ENDOGENOUS GENES IN EMBRYONIC STEM CELLS

Suter, David M., Mandic, Aleksandra, Strebinger, Daniel P.
IBI, EPFL, Lausanne, Switzerland

We recently demonstrated that mammalian genes are transcribed with widely different burting kinetics. Here we show that transcriptional bursting also occurs in embryonic stem cells, and we analyze how bursting kinetics are modulated upon cellular differentiation.

T-2118
ENHANCEMENT OF SDF-MEDIATED GENOMIC EDITING THROUGH THE INTRODUCTION OF SEQUENCE-SPECIFIC DOUBLE STRAND DNA BREAKS (DSBS)

Suzuki, Shingo[1], Tasto, Carissa[1], Sargent, R Geoffrey[1], Lee, Albert[1], Yezzi, Michael J.[1], Guimarães, Alessander O.[2], Muench, Marcus[2], Porteus, Matthew H.[3], Gruenert, Dieter C.[1]
[1]University of California, San Francisco, San Francisco, CA, USA, [2]Blood Systems Research Institute, San Francisco, CA, USA, [3]Stanford University, Stanford, CA, USA

Sequence-specific modification of mammalian genomic DNA has been achieved through homologous recombination (HR) with plasmids that contain donor sequences homologous to the genomic target. While that event occurs at a low frequency (<10^-6), recent studies with oligo/polynucleotide small/short DNA fragments (SDFs) have shown that the efficiency of homologous exchange can be enhanced. In addition, studies with sequence-specific homing nucleases, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPRs), have indicated that the efficiency of homologous exchange between a plasmid carrying donor targeting sequences and a genomic target can be further enhanced through the introduction of double strand breaks (DSBs) within the genomic target. To more readily quantify and an optimize what parameters would increase the efficiency of SDF-mediated HR, a HEK293 cell line carrying a stably integrated mutant GFP gene was used as a target. These studies showed that the size and amount of transfected SDFs were one of the primary factors that could modulate the efficiency of TALEN-enhanced SFHR. By increasing the initial correction efficiency, it will be easier and quicker to isolate clones of cells that have been corrected. In that context, this study also describes a method for the isolation of clones of cystic fibrosis (CF) induced pluripotent stem (iPS) (CF-iPS) cells in which the disease causing delF508 mutation in the CF transmembrane conductance regulator (CFTR) gene was corrected. Clones of corrected cells are isolated from a mixed population of corrected and un-corrected CF-iPS cells present after transfection with the donor wild-type CFTR-SDFs and the homing TALENs through a cyclic series of PCR screening and enrichment that eventually results in the isolation of an individual clone. Because this approach does not rely on the insertion of selectable markers, further disruption of the genomic DNA is minimized. Combined with the GFP optimization analysis, it should now be possible to rapidly isolate clonal populations of corrected cells in the development of comprehensive cellular and genetic regenerative therapy for CF.

T-2119
HIGH FREQUENCY OF INTRACELLULAR ICE FORMATION DURING SLOW COOLING IN COLONIES OF HUMAN EMBRYONIC STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS: COMPARISON TO DISSOCIATED SINGLE CELLS

Takada, Kei[1], Hirai, Masako[1], Hamao, Mari[1], Kashigi, Fumi[1], Kawase, Eihachiro[1], Suemori, Hirofumi[1], Nakatsuji, Norio[2], Takahashi, Tsuneo A.[1]
[1]Embryonic Stem Cell Research, Institute. for Frontier Med. Sciences, Kyoto University, Kyoto, Japan, [2]Institute for Intergrated Cell-Material Sciences, Kyoto University, Kyoto, Japan

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) have potential to provide an unlimited source of cells and tissues for regenerative medicine. Efficient cryopreservation method for these cells is necessary in the cell processing and banking. However these cells are known to be susceptible to freezing injury, and post-thaw recovery of these cells is very low as compared to other cell types. Current protocols for cryopreservation of these pluripotent stem cells, the conventional slow freezing and vitrification method, causes excessive loss of cell recovery, proliferation and pluripotency after freeze-thawing. Rho-associated kinase inhibitor (ROCKi) made it possible to maintain the dissociated single cells and these single cells could be cryopreserved better than those frozen as colonies. Still, the reasons for the high survival of dissociated single cells compared to the

colonies are not well understood. To investigate the cause of cell death during cryopreservation, we observed the cells during freeze-thawing using a cryomicroscope connected with video image recording. We found that colonies were subject to intracellular ice formation (IIF) at slow cooling rates which was associated with cell death. The IIF propagated through adjacent cells composing colonies. The IIF in colonies equilibrated with 10% DMSO was observed frequently during the cooling at -5 °C /min, and still at -2 °C /min. The cells having ice crystals formed intracellularly lost their proliferation ability, though the propidium iodine viability assay did not show good correlation with cell death. The IIF at -20 °C /min rapidly cooling was confirmed by heat diffusion by differential scanning calorimetry. The dissociated single cells were more resistant to IIF compared to the colonies. The cells frozen and thawed on a cryostage in the microscope were collected and cultured. The dissociated single cells frozen without IIF at -2°C /min and cultured for 30 passages kept their pluripotency and differentiation ability into three germ layers. Slow cooling of dissociated single cells with low concentration of cryoprotectant is recommended for cryopreservation of hESCs and hiPSCs to avoid consecutive IIF that occurs in the colonies with high frequency.

T-2120
A NEW METHOD FOR THE DETECTION OF RESIDUAL MOUSE FEEDER CELLS CONTAMINATED IN THE PRODUCTS OF HUMAN PLURIPOTENT STEM CELLS

Takahashi, Tsuneo A.[1], Tanaka, Keiji[2], Okuda, Shinji[2], Hirai, Masako[1], Takada, Kei[1], Kawase, Eihachiro[1], Suemori, Hirofumi[1], Nakatsuji, Norio[3], Kitagawa, Masanari[2]

[1]Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan, [2]Biotechnology Research Laboratories, Takara Bio Inc., Otsu, Japan, [3]Institute for Intergrated Cell-Material Sciences, Kyoto University, Kyoto, Japan

Mouse feeder cells have been used to support the growth and cultivation of various stem cells, including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). For stem cell applications such as regenerative medicine, the mouse feeder cells, such as mouse embryonic fibroblasts (MEFs), should be removed completely. We have been developing a method to produce a clinical grade hESCs by the clean-up method and detection of residual MEFs is critical to insure the quality and safety of the product. The conventional antibody staining method is not sensitive enough to deny the existence of MEFs in the hESC product. Therefore, we developed a new sensitive PCR method for the detection of residual mouse feeder cells in the hESC products with high specificity and sensitivity. Antibody immunofluorescence staining (IFS) method was used to detect the same samples for comparison. The mouse feeder cell primer set was designed to facilitate highly sensitive detection and quantification of genomic DNA derived from residual MEFs using real-time PCR to analyze genomic DNA extracted from the hESCs. The primers were designed to detect mouse mitochondrial DNA sequence. The MEFs and the hESC cells were mixed with serial dilution, from 0.3 to 0.0001%. In the IFS method using monoclonal anti-feeder antibodies conjugated to APC (Milteny Biotec), the sensitivity for detection of mouse feeder cells was 5% in hESCs. The hESCs were cultured in a defined culture medium without feeder cells for multiple passages, then the remaining MEF were detected by the IFS and the real-time PCR method. In the product of clean-up hESCs (working cell bank), the contamination of MEF was less than 0.001%. This new real-timePCR method to detect mouse feeder cells should be useful for establishment of clinical grade hESCsand other cells cultured for advanced cell therapy including hiPSCs.

T-2121
GENERATION OF PAX7+ MUSCLE PROGENITOR CELLS FROM HUMAN EMBRYONIC STEM CELLS AND THEIR USE IN REGENERATIVE DRUG DISCOVERY APPLICATIONS

Tarunina, Marina[1], Cerqueira, Antonio[2], Chapman, Charlotte[1], Watson, Thomas[1], Ramathas, Vidya[1], Hernandez, Diana[1], Jeyakumar, Jey[1], Cerletti, Massimiliano[2], Choo, Yen[2]

[1]Plasticell Limited, Stevenage, United Kingdom, [2]Progenitor Labs Limited, Stevenage, United Kingdom

Muscle regeneration and repair is mediated by a rare specialized population of progenitor cells known as satellite cells. These cells have the ability to self-renew, expand and proliferate as myoblasts or undergo myogenic differentiation to fuse and restore damaged muscle. Muscle progenitor cells derived from human embryonic stem (hES) cells represent a renewable source of material for high throughput drug screening and regenerative medicine applications. Here we describe the discovery of several novel serum-free protocols for the generation of myogenic progenitors from hES cells, characterized by the expression of canonical satellite cell marker Pax7 and their ability to differentiate into fusion-competent myoblasts. The new myogenic protocols were discovered using a high-throughput combinatorial platform, termed Combicult®, which is capable of multiplexing very large numbers of cell differentiation protocols to identify critical combinations that result in high efficiency differentiation to a given phenotype. More than 5000 combinations of distinct cell culture media compositions were screened to discover 10 optimal conditions for the induction of hES cells into Pax7+ muscle progenitor cells. We then characterized these cells for differentiation potential, cell surface markers expression, phenotype stability and suitability for compound screening. An enriched population of hES derived Pax7+ progenitor cells was then used to develop a high content screening system based on the expression of mature skeletal muscle markers. High throughput phenotypic screening of several highly annotated chemical libraries including FDA approved drug library revealed a number of hit compounds that promote myogenesis in vitro. The regenerative properties of these small molecule drugs will be further tested in animal models of neuromuscular disorders for future therapeutic applications.

T-2122
CONDITIONED MEDIA OF HUMAN IMMORTALIZED MESENCHYMAL STEM CELL CLONES AS A NOVEL THERAPEUTIC TOOL TO INDUCE TISSUE REGENERATION AND TO REPAIR DAMAGED TISSUES

Terunuma, Hiroshi[1], Ashiba, Keisuke[1], Deng, Xuewen[1], Terunuma, Atsushi[1], Takane, Tsubasa[1], Yamashita, Yasuhiro[2]

[1]Biotherapy Institute of Japan, Tokyo, Japan, [2]Geneticfactor inc., Tokyo, Japan

Transplantation of mesenchymal stem cells (MSCs) has become available for repairing tissue damage. However, the use of MSCs requires invasive cell collection maneuvers and elaborate cell culture procedures. There also is a potential risk of tumorigenesis of transplanted MSCs. Because transplanted MSCs exert their ability in tissue repair by secreting various soluble factors, the issues related to MSC transplantation can be circumvented by using MSC-derived conditioned media (MSC-CM) without transplanting MSCs. While recent studies demonstrated the utility of MSC-CM in repairing tissue damage, MSC-CM should become even more useful in clinical applications when it becomes possible to maintain the constantly high quality of MSC-CM through repeated passages of MSCs. Therefore, we developed immortalized MSC clones from human dental pulp-derived primary MSCs and characterized their molecular profiles. To generate

immortalized MSCs, we transduced human dental pulp-derived primary MSCs with immortalizing genes and isolated immortalized MSC clones. We characterized the individual MSC clones for the gene expression profiles on more than 20,000 mRNA using microarrays. We also determined the levels of near 100 soluble factors in the MSC-CM of individual MSC clones using antibody arrays. We found that the MSCs generate and secrete a broad spectrum of bioactive macromolecules, some of which are immunoregulatory and others contribute to the microenvironments to promote tissue regeneration. Although the absolute amounts of the bioactive factors varied from clone to clone, each immortalized MSC clone produced stable amounts of the bioactive factors even after multiple passages in cell culture. Thus, the immortalized MSC clones serve as stable sources of large amounts of soluble factors. These data demonstrate that MSC-CM of immortalized MSC clones may allow the development of novel therapeutic strategies for inducing tissue regeneration and repairing damaged tissues.

T-2123
ANTIBODY-BASED TOOLS AND PROTOCOLS FOR IMPROVING STEM CELL CHARACTERIZATION WORKFLOWS

Tieberg, Deborah[1], Hancock, Michael[1], Asprer, Joanna[2], Honeyager, Shawn[1], Goossens, Tony[1], Chen, Anne[3]
[1]Thermo Fisher Scientific, Madison, WI, USA, [2]Thermo Fisher Scientific, Carlsbad, CA, USA, [3]Thermo Fisher Scientific, Boston, MA, USA

Stem cell biology constitutes one of the fastest growing areas in the life sciences. Accordingly, there is strong demand for improving the characterization tools and protocols available to stem cell researchers. We report here the development of a series of antibody-based tool sets and protocols that facilitate detection of important cellular markers of pluripotent stem cells and the differentiated cell types that can be derived from them (e.g., three germ layers, neural stem cells, cardiomyocytes). First, optimized immunocytochemistry reagent sets were identified by screening panels of validated primary antibodies against established stem cell markers and matching them up with appropriate dye-conjugated secondary antibodies and optimized fixative, permeabilization, blocking, and wash buffer systems. We demonstrate how these reagent sets can be applied to simplify traditional fixed-cell immunocytochemistry workflows and enable more information per sample via multiplex staining strategies that are compatible with a variety of imaging platforms. A second series of live-cell imaging reagents was generated to improve culture characterization and clone selection during cellular reprogramming workflows that are used to generate induced pluripotent stem cells. These tool sets are composed of dye-conjugated primary antibodies against select cell surface markers that are paired together with an imaging medium specifically designed to maximize fluorescence signal detection while maintaining cell health. We anticipate that this series of protocol and technology improvements will significantly augment the current characterization approaches available to stem cell researchers.

T-2124
DEVELOPMENT AND IMPLEMENTATION OF A STEM CELL INFORMATION MANAGEMENT SYSTEM (STIMS) FOR THE GERMAN CENTER FOR CARDIOVASCULAR RESEARCH

Umbach, Nadine[1], Friede, Tim[2], Rienhoff, Otto[3], Zimmermann, Wolfram-Hubertus[4]
[1]University Medical Center Göttingen, Institute of Medical Informatics, German Center for Cardiovascular Research (DZHK), partner site Göttingen, Göttingen, Germany, [2]University Medical Center Göttingen, Institute of Medical Statistics, German Center for Cardiovascular Research (DZHK), partner site Göttingen, Göttingen, Germany, [3]University Medical Center Göttingen, Institute of Medical Informatics, Göttingen, Germany, [4]University Medical Center Göttingen, Department of Pharmacology, German Center for Cardiovascular Research (DZHK), partner site Göttingen, Göttingen, Germany

Setting up stem cell repositories with transparent use/access rules, unambiguous stem cell quality annotation, and complete as well as traceable process documentation is essential for exploitation of the full potential of cellular reprogramming. Here, we report how the German Center for Cardiovascular Research (DZHK), a network of major cardiovascular research institutions and clinical heart centers in Germany, addressed this challenge. A core element was the development of an information management system (IMS) for documentation of the whole work process for the generation of human induced pluripotent stem cell (iPSC) models, including somatic cell collection, processing, cultivation, reprogramming, quality control, storage, and distribution to collaborators. Also, a major challenge was the establishment of technical, legal, and ethical prerequisites to link individual experimental cell data with phenotypic data from individual patients enrolled in ongoing DZHK clinical trials. An interdisciplinary team of stem cell researchers, clinicians, and information scientists (DZHK-Stem Cell Working Group) screened available IMS for their ability in comprehensive, accurate, and traceable documentation of iPSC generation and stem cell biobanking. For implementation of a stem cell IMS (StIMS) 115 software requirements were identified and classified in essential, conditional or optional demands. In a comparative market analysis, 16 IMS were tested and evaluated according to regulatory compliance, software flexibility to integrate complex stem cell processes, management of analytical results and sample storage, integration of lab instruments, costs, customer support, and user friendliness. After selecting an IT solution, the StIMS was customized for a multi-center pilot project. We identified a commercially available IMS and customized it according the demands of the DZHK-Stem Cell Working Group. The user-configured StIMS was successfully implemented first locally at the DZHK-partner site Göttingen and is presently being rolled-out to all DZHK partners (which include 14 research institutions and clinics at 7 locations in Germany as well as external partners). Role-based access control to the web-based tool with password-protection restricts access only to registered and approved users. To track changes all data modifications in the system are logged in audit trails. First patient material from TransitionCHF-Cohort study (aiming to recruit n=3,000 patients with heart failure) will be included with the first patient recruited in Q2/2014. The iPSC generation process, collected experimental data, and phenotypic data from the clinical study will be linked in a de-coded and privacy-conform manner. The DZHK StIMS was locally validated and is presently being rolled-out on national level within the framework of the DZHK. It primarily serves stem cell researchers in the cardiovascular field and will provide transparent access to quality controlled iPSC models with patient data for academic research. We anticipate that the StIMS can be easily adopted by other stem cell laboratories or biobanks also with different research interests.

Moreover, we advocate connecting established and developing stem cell infrastructures to provide controlled and high quality access to the growing body of iPSC models for translational research and here especially for the simulation of complex genetic diseases such as heart failure.

T-2125
MICROFLUIDIC CULTURE SYSTEMS TO STUDY EARLY MAMMALIAN MORPHOGENESIS USING PLURIPOTENT STEM CELLS

Razian, Golsa, **Ungrin, Mark**
Comparative Biology and Experimental Medicine, University of Calgary, Calgary, AB, Canada

The progression from a fertilized oocyte to a fully developed newborn animal involves multiple cell divisions and developmental transitions, as initially pluripotent stem cells gradually commit to distinct cell lineages. A key developmental stage is the blastocyst stage - a time when embryonic (epiblast) and extra-embryonic (hypoblast) lineages are specified and segregate into distinct tissues. The epiblast, which gives rise to the embryo proper, is a structured pluripotent epithelium - a micro-scale tissue with tremendous developmental potential. The epiblast undergoes gastrulation to give rise to the three canonical germ layers of definitive endoderm, ectoderm, and mesoderm, which in turn generate all the tissues and organs in the developing embryo. Understanding this stage of development is thus of great importance to both developmental biology and tissue engineering. The majority of our present understanding of mammalian embryology has been obtained in mouse, much of it using in-vivo-isolated blastocysts. This is a very powerful system, but it can also be labour intensive and expensive, with single experiments requiring several hundred embryos in some cases. There is therefore a significant need for in vitro model systems that will allow precise recapitulation of the earliest stages of embryogenesis. To mimic early developmental stages, investigators have used embryoid bodies (EBs), which are aggregates of embryonic stem cells (ESCs) that form three dimensional structures that can be induced to differentiate into different cell lineages. While EBs have differences in geometry from the embryonic bilaminar disc, they have been widely and successfully used as a model system for this stage of development. While this work has primarily focused on murine cells, we have previously demonstrated that human ESCs exhibit similar potential, extending the applicability of this approach into other species where developmental studies have previously been limited for both ethical and practical reasons. Subsequent induction of tissue-level gastrulation behaviour has proved more challenging. This challenge is not unexpected, due to the well defined and oriented gradients of signalling molecules that are known to play a major role in embryonic axis formation and cell fate specification. There is thus a significant need for culture systems that permit recapitulation of the microenvironment of graded signals that is native to the bilaminar disc. I will present progress towards the development of microfluidic devices that will allow us to culture and manipulate individual colonies of pluripotent stem cells and provide a model system for the study of peri-implantation morphogenesis.

T-2126
MULTIPARAMETRIC FLOW CYTOMETRY ASSAYS FOR THE EVALUATION OF MESENCHYMAL STROMAL CELL IMMUNOMODULATORY ACTIVITY

Vidal, Jason G.[1], Corselli, Mirko[1], Hingorani, Ravi[1], Carson, Christian[2], Emre, Nil[1]
[1]BD Biosciences, San Diego, CA, USA, [2]BD Biosciences, La Jolla, CA, USA

Although many studies have documented the immunomodulatory ability of mesenchymal stromal cells (MSCs), factors like the tissue of origin, time in culture and culture conditions are responsible for high batch-to-batch variability. Reproducible assays to determine the phenotype and potency of MSCs are needed to obtain consistent results. We show how multicolor flow cytometry can be used to i) assess the immunophenotype of resting and licensed MSCs and ii) measure the inhibition of T-cell activation. Multiparametric flow cytometry was used to investigate the co-expression of known surface and intracellular immunomodulatory molecules by resting or licensed MSCs. Resting adipose derived MSCs (ADSCs) displayed a more licensed phenotype as compared to bone marrow derived MSCs (BM-MSCs), based on higher expression of adhesion molecule CD54, IL-6 and PGE2-producing enzyme COX2. Upon licensing with IFNγ +/- TNFα, ADSCs and BM-MSCs similarly up-regulated IL-6, IL-8, CXCL-10, CCL-2, CCL-5, COX2, CD54 and CD274. We then optimized a multiparametric flow cytometry potency assay to simultaneously investigate activation of both T-cells and resting MSCs in co-culture. T-cells and MSC were discriminated based on size and surface marker signature. Cell proliferation analysis revealed that the activation of T-cells was significantly reduced in the presence of BM-MSCs and completely abrogated by ADSCs, thus suggesting a possible correlation between licensed phenotype and immunomodulatory activity. In the presence of activated T-cells, MSCs increased the production of IL-6, COX2, CD54 and CD274. In conclusion, multiparametric flow cytometry enables the simultaneous analysis of intracellular and surface markers to i) determine the licensed status of different batches of MSCs, ii) measure the level of immunomodulatory activity, iii) identify the source of cytokines in co-culture systems and iv) define biomarkers predicting MSC potency.

T-2127
SUPER RESOLUTION MICROSCOPY AND SMALL PROBE 3D FISH ALLOW VISUALIZATION OF NUCLEAR ARCHITECTURE, AND INSIGHTS INTO EPIGENETICS AND CHROMATIN REGULATION OF STEM CELLS

Watters, Robin Ann[1], Hu, Ying Samuel[2]
[1]Waitt Biophotonics, Salk Institute, La Jolla, CA, USA, [2]Waitt Advanced Biophotonics Center, Salk Institute, La Jolla, CA, USA

Visualizing the nanometer-scale architecture of gene organization is invaluable for understanding the developmental potential of human embryonic stem cells (hESCs). To achieve this imaging capability, we combined fluorescent in situ hybridization (FISH) using approximately 200-basepair small DNA probes with single molecule super-resolution microscopy. As a proof-of-principle study, we designed 20 small DNA probes covering the beginning and end regions of the HER2 locus. We labeled the probes with small molecule dyes, performed FISH and singe-molecule imaging using stochastic optical reconstruction microscopy (STORM). The sub-diffraction-limited resolution allowed us to measure the nanometer-scale distance between the beginning and end regions of the HER2 locus. Since the DNA organization depends on the cell cycle, we hypothesize that the measured distance from the HER2 locus forms a cell-cycle-dependent distribution. We

further investigate this dependence by labeling the centromeric region of chromosome 17 on which the HER2 locus is located. Small probe, 3D Fluorescent in situ hybridization allows spatial investigation of three dimensionally preserved nuclei using fluorescently labeled, nucleic acid probes. The probe design is facilitated by the web site www.hdfish.eu (Bienko, et al, 2012). Stochastic Optical Reconstruction Microscopy (STORM): STORM is a super resolution microscopy technique that affords resolution of approximately 20nm, well below the diffraction limit, and allows molecules and subcellular structures to be individually identified, mapped and analyzed topographically. Fluorophores attached to molecules are turned on and off by laser excitation. Light Sheet Microscopy for Super Resolution: Lasers excite and relax fluorophores attached to single molecules while time lapsed images are taken. The images are reconstructed with Image J software with the QuickPalm plug in, to obtain information about the size, shape and location of the DNA. The control, a BAC probe for the centromere of chromosomes 12 and 17 hybridized with H1 ESCs, as evidenced by confocal microscopy. We do not expect the structure or architecture of this control probe to change. HER2 probes, designed for super resolution STORM and light sheet microscopy, were also hybridized with H1 ESCs, as evidenced by light sheet microscopy. Five reconstructions (images) of the HER2 gene, in hESCs have been made providing nanoscopic structural information of the HER2 gene in its natural physiological environment. We were able to measure the start and end of the HER2 gene, a scale that is below the diffraction limit. The protocol we have established works for super resolution microscopy and 3D FISH. This technique can be used for visualization and mapping of subnuclear structures in stem cells. For example, the technique can be used to compare and contrast chromosomal regions of transcriptional factors such as Sox2 or C-Myc in ESC cells, iPS cells, normal somatic cells, and cancer cells in which these factors are present. It can also be used to examine the evolution of the binding sites of these transcriptional factors, as cells differentiate. Future work includes the design of probes that will target the sox2, c-myc, her2/3 and klf4 transcriptional factors DNA sites.

T-2128
SINGLE-CELL ANALYSIS OF LIPID NANOPARTICLE DELIVERY OF SIRNA TO MANIPULATE GENE EXPRESSION IN EMBRYONIC STEM CELLS

White, Adam K.[1], Sikorski, Darek[2], Zwaenepoel, David[3], Ansari, Aysha[3], Walsh, Colin[3], Taylor, Robert J.[3], Ramsay, Euan[3], Piret, James[4], Cullis, Pieter[5], Hansen, Carl[6]

[1]Genome Science and Technology, University of British Columbia, Vancouver, BC, Canada, [2]Biomedical Engineering, University of British Columbia, Vancouver, BC, Canada, [3]Precision Nanosystems Inc., Vancouver, BC, Canada, [4]Chemical and Biological Engineering, University of British Columbia, Vancouver, BC, Canada, [5]University of British Columbia, Vancouver, BC, Canada, [6]Physics and Astronomy, University of British Columbia, Vancouver, BC, Canada

Manipulation of gene expression is fundamental for understanding the function of proteins involved in maintaining and differentiating embryonic stem cells. Here we show a method using lipid nanoparticles (LNP) to deliver small interfering RNA (siRNA) to efficiently silence gene expression in a human embryonic stem cell line. siRNA-LNP were prepared using the NanoAssemblr™ microfluidic-based nanoparticle manufacturing platform . LNPs prepared using the NanoAssemblr™ instrument mimic the neutral, cholesterol containing structure of low-density-lipoproteins (LDL), which are taken up by cells though the LDL-receptor (LDLR) in presence of Apolipoprotein E4 (ApoE4). We show that uptake of siRNA-LNP in embryonic stem cells was enhanced by the presence of ApoE4, resulting in highly efficient uptake (>80% after 24 hours) with little apparent toxicity. We characterize the siRNA-LNP by measuring the dependence of particle uptake as a function of ApoE, siRNA concentration and incubation time. siRNA-LNP uptake is correlated with target gene knockdown in established and difficult-to-transfect primary cell lines. Additionally, we use a microfluidic device for performing high-throughput single-cell digital PCR to precisely quantify knockdown of gene expression in hundreds of individual embryonic stem cells. By examining the cell-to-cell variability, kinetics, and efficiency of using this lipid nanoparticle technology for nucleic acid delivery, we provide an informed basis for optimizing the manipulation of gene expression in embryonic stem cells.

T-2129
STANDARDIZATION OF PLURIPOTENT STEM CELLS AND DIFFERENTIATED CELLS USING SINGLE-CELL ANALYSIS

Yamane, Junko, Maruyama, Toru, Kobayashi, Kenta, Fujibuchi, Wataru

Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

There are a number of human iPS cell lines in the world. Thus it is urgently needed to standardize those cell lines for safer use in the application phase. To describe cell features at the highest resolution, we used the multiplexed single-cell mRNA-seq and carried out human transcriptome analysis for one ES cell line, three iPS cell lines, and primary cell lines of four normal differentiated cells. The iPS cell lines were established by different methods such as RV, LV, and EV. Firstly, we examined the differentially expressed genes (DEGs) between every pair of cell lines using Bioconductor. However, we observed only few DEGs among ES and iPS cell lines with FDR < 0.01. Secondly, we performed principal component analysis at single-cell resolution, observing that each cell line is well-clustered in the first three principal component space. Thirdly, in order to evaluate the fluctuation of gene expression, we analysed coefficients of variation, which corresponds to the biological fluctuations of genes, displaying that several house keeping genes are relatively stable than other genes. These genes could be used as stable marker for checking technical errors in single-cell experiments. Lastly, we examined distributions of gene expression levels within the same cell line and compared among all the cell lines. As a result, we found that some remarkable genes such as DNA (cytosine-5-)-methyltransferase 3 beta gene are specifically up-regulated in ES and iPS cell lines but down-regulated in differentiated cells. Importantly, some gene expressions are distributed in bimodal thus highly-variant even in the same cell line, which is the advantage of single-cell analysis and cannot be detected by standard cell population analysis.

T-2130
A NOVEL BFGF/TGF-BETA-FREE DEFINED CULTURE SYSTEM FOR HUMAN PLURIPOTENT STEM CELLS

Yasuda, Shinya, Shahsavarani, Hosein, Yoshida, Noriko, Hasegawa, Kouichi

Institute for Integrated Cell-Material Science (iCeMS), Kyoto University, Kyoto, Japan

Although Xeno-free chemically defined culture systems has been attracted as ideal media for application of human pluripotent stem cells (hPSCs) in regenerative medicine, their affordable production is not yet an industrial reality due to the use of purified or recombinant proteins such as cytokines/growth factors and the lack of the knowledge on hPSC self-renewal. Moreover, concerns on high-cost quality control of recombinant growth factors such as bFGF or TGFβ in the culture media is hindering progress towards well-defined media regarding the large amount use of them in scalable hPSC culture systems. To date, the

lack of chemical compounds to replace bFGF or TGFβ is a main barrier to develop further cost-effective chemically defined culture medium. We have recently developed a defined hPSC culture system dependent of Wnt ligand and a small molecule DYRKs inhibitor, ID-8, without bFGF/TGFβ supplementation (Hasegawa et al., 2012). Exploiting a screening approach, a chemical compound has been identified which can replace Wnt ligand from the system. We next screen a library of diverse chemical compounds for their ability to promote hPSC proliferation and identified a component, which can enhance hPSC growth. For further development, combinatorial re-optimization of identified components of medium, extracellular matrix, passaging methods and corresponding reagents have been examined in various hPSC (hESC and hiPSC) lines. The hPSCs cultured on newly developed culture system maintained their characteristic morphology, expressed high levels of markers of pluripotency, and retained a normal karyotype for long term (>passage 50 at least). These remarkable properties of this system, turn it into the most attractive media for expansion of hPSCs over 1200 times per month without bFGF/TGFβ as similar expansion as hPSCs on feeder layer. We are currently pursuing the elucidation of the underlying molecular mechanisms that identified chemical compounds affect hPSCs self-renewal. This is a first report of bFGF/TGFβ-free robust culture system for hPSCs propagation, which is chemically defined, cost-effective, and devoid of animal-derived components. Such culture system that supports long-term self-renewal of hPSCs will accelerate and generalize the translational perspectives of hPSCs through production of GMP-quality hPSC lines in near future.

T-2131
THE IDENTIFICATION OF AMINO ACID BASED POLYMERS FOR CULTURE OF HUMAN MESENCHYMAL STEM CELLS UNDER SERUM-FREE CONDITIONS

Zhang, Rong
Changzhou University, Changzhou, China

Human mesenchymal stem cells (hMSCs) have been found to differentiate into not only adipocytes, chondrocytes and osteoblasts, but also muscle cells, hepatocytes, skin cells, pancreatic cells as well as neurons. In addition, they can modulate the immune response in life threatening diseases such as graft verses host disease. The broad applications of this cell type has made them very attractive to researchers and clinicians for cell therapy and tissue regeneration applications. To achieve their full potential, hMSCs need to be cultured and processed in serum-free, defined medium and synthetic substrates that avoid batch-to-batch variation and potential pathogen transfer. Here we report the identification of amino acid based polymers which were identified through the high-throughput fabrication and serum free-screening of inkjet printed amino acid based polymer microarrays of 2072 different materials. Identified candidates were up-scaled (in 24 well plates) and analysed for the culture of hMSCs. The top polymer was applied for long-term culture (over 5 passages) of hMSCs from fat and bone marrow respectively. The cultured hMSCs were characterized via flow cytometry with +CD105,+ CD73, +CD90, -CD34 and -CD14 respectively. The expanded hMSCs on the amino acid-based polymer were differentiated to adipocytes, chondrocytes and osteoblasts in specific differentiation medium and showed that the multipotency of hMSCs were maintained after long-term culture.

T-2132
CONTROLLING EXPANSION AND CARDIOMYOGENIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS IN SCALABLE SUSPENSION CULTURE

Kempf, Henning[1], Olmer, Ruth[1], Kropp, Christina[1], Rückert, Michael[1], Jara-Avaca, Monica[1], Robles-Diaz, Diana[1], Franke, Annika[1], Elliott, David[2], Roa Lara, Angelica[1], Kensah, George[1], Gruh, Ina[1], Haverich, Axel[1], Martin, Ulrich[1], **Zweigerdt, Robert**[1]
[1]REBIRTH-Cluster of Excellence, Hannover Medical School, Hannover, Germany, [2]Murdoch Childrens Research Institute, Parkville, Australia

To harness the potential of hPSCs, abundant provision of their differentiated progenies is required. We have recently established single cell inoculated suspension cultures of hPSCs (Zweigerdt et al., Nature Prot. 2011) which form aggregates in stirred tank reactors (Olmer et al., Tissue Eng. 2012) in the defined medium mTeSRTM (STEMCELL Technologies). This work enabled the translation of conventional, adherence-dependent "2D" culture of hPSC to "3D" suspension culture. Since stirred tank bioreactors allow straightforward up scaling and comprehensive monitoring of process parameters these systems are widely used for the mass culture of conventional mammalian cell lines. Application of stirred reactors to hPSC culture, however, is in its infancy. Aiming at low medium consumption but integration of all probes relevant for process monitoring (pO2, pH, biomass) a parallel "mini bioreactor system" consisting of individually controlled vessels (DASGIP / Eppendorf) was utilized. hPSC expansion as matrix-independent aggregates in suspension culture was combined with cardiomyogenic differentiation using chemical Wnt-Pathway modulators. A multi-well screening approach was up-scaled to stirred tank bioreactors applying controlled feeding strategies (Batch and Cyclic Perfusion) followed by pre-optimized differentiation. We found that the size of aggregates is not the prevailing factor regarding divergent differentiation outcomes, but physical and physiological culture parameters that shape aggregate development in the expansion phase. Global profiling revealed culture-dependent expression of BMP agonists/antagonists, suggesting their decisive role on cell fate determination. Furthermore, metallothionein was discovered as a potentially stress-related marker in hPSC culture. Optimized condition in 100 ml bioreactor scale enabled the production of 40 million CMs at >80% purity, which were directly applicable for bioartificial cardiac tissue formation, a potential strategy for heart repair, drug discovery and safety pharmacology.

REPROGRAMMING

T-2133
CONTROL OF INNATE IMMUNITY ENHANCES MRNA-MEDIATED REPROGRAMMING OF SOMATIC CELLS

Min, Chang-Woo, Kim, Ah-Young, Lee, Eun-Mi, Lee, Eun-Joo, Kang, Kyung-Ku, Lee, Myeong-Mi, Kim, Sang-Hyeob, Sung, Soo-Eun, Ghim, Soong-Koo, Hwang, Meeyul, Jeong, Kyu-Shik
Department of Patholgy, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea

Direct introducing of mRNA encoding for Oct4, Sox2, Klf4 and c-Myc, mRNA-based approach, could be appropriate method because of its non-integrating characteristics. This approach requires repetitive induction of mRNA to maintain high level of pluripotency-associated genes. However, repetitive transfections cause innate immune response which result in diverse defensive reactions of target cells. Due to immunological advantage of adipose-derived stem cells (ASCs) compared to mouse embryonic fibroblasts (MEFs), we hypothesized

that the use of modified mRNA which consist of immune response free NTP and less immunologic cell, ASCs, instead of MEFs could enhance reprogramming of somatic cells. MEFs and ASCs were isolated from C57BL/6 mouse and passage 1~2 cells were used. We transfected each normal mRNA and modified mRNA to MEFs and ASCs every two days. On sixth day, cells were collected for molecular analysis or sub-cultured onto MEFs feeder cells inactivated with mitomycin C to observing morphological change. We observed morphological changes, cell viability and expression of innate immune associated genes (RIG-I, STAT1, IFIT1, OAS1) of each groups. Cell viability was measured by MTT assay and gene expressions were measured by quantitative real-time PCR. ASCs transfected with normal and modified mRNA showed morphological changes but MEFs did not. The cell viability was good in ASCs groups. MEFs transfected with normal mRNA showed sharp decrease of cell number and viability. But ASCs transfected with modified mRNA showed similar cell viability to non-transfected ASCs. Innate immunity associated gene expression correspond to cell viability test. MEFs transfected with normal mRNA express high level of innate immunity associated genes and ASCs transfected with modified mRNA showed low level of innate immunity associated genes.Avoidance from innate immunity with diverse method improves cell viability through reducing activation of immune response associated genes. We suggest that from the present study, ASCs can be great source of iPS cells from the immunological point of view.

T-2134
ENHANCED REPROGRAMMING OF "PRIVILEGED" HUMAN MYELOID PROGENITORS VIA STABLE INDUCTION OF A CELL CONTACT-DEPENDENT MYELOID NF-KAPPA-B-STAT3 SIGNALING AXIS

Agarwal, Jasmin R.[1], Huo, Jeffrey[2], Park, Tea Soon[3], Zambidis, Elias[4]
[1]Institute for Cell Engineering, Johns Hopkins University, Baltimore, MD, USA, [2]Johns Hopkins School of Medicine, Baltimore, MD, USA, [3]Johns Hopkins School of Medicine, Baltimore, MD, USA, [4]Johns Hopkins University School of Medicine, Baltimore, MD, USA

Although fibroblasts are the most commonly reprogrammed human donor cell type, extremely low reprogramming efficiencies of <0.1% are typically achieved. Additionally, human induced pluripotent stem cells (hiPSC) derived from fibroblast donor cells typically possess incompletely reprogrammed states with aberrant epigenetic patterns. Recent studies have revealed that murine myeloid progenitors represent a "privileged" donor cell type capable of highly efficient non-stochastic reprogramming rates. In agreement with such findings, we first reported a non-integrating human episomal reprogramming system that generates high-quality myeloid-hiPSC from mesenchymal stromal cell (MSC)-primed myeloid progenitors with unprecedented efficiencies of up to 50% of input cells. We have subsequently discovered that these myeloid-iPSC were molecularly indistinguishable from human embryonic stem cells (hESC), and possessed superior multi-lineage differentiation potencies, compared to hiPSC derived via standard methods. The key step in our reprogramming method is the MSC priming of cord blood (CB)-, bone marrow (BM)- or peripheral blood (PB)-derived myeloid cells prior to the expression of episomal Yamanaka factors. Soluble and contact-dependent signals were found to facilitate a rapid and high-fidelity conversion to pluripotency. Bioinformatics analyses identified a major role of inflammatory Toll-like receptor-NFκB and STAT3 signaling. NFκB signaling is known to be a critical signaling pathway for the maintenance and differentiation of hESC. Here we show that growth factor (GF)-activated CB cells showed strong NFκB signaling at the time of episomal transfection in contrast to fibroblasts (99% vs. 26%, respectively, p<0.00001). Additionally, we discovered a major and rapid initial STAT3 activation in GF-activated CB cells, which was maintained by subsequent co-culture on MSC and MEF (mouse embryonic fibroblasts) but lost without MSC (70% vs. 28% STAT3 activation, respectively, p=0.0001). STAT3 signaling in primed myeloid cells was cell contact-dependent. The master reprogramming factor STAT3 is known to be required for the acquisition of pluripotency and is an important epigenetic regulator in myeloid lineages, together with NFκB signaling. Myeloid cells are professional inflammatory cells that possess a unique epigenetic plasticity and are especially receptive to the induction of an open chromatin state. We propose that the activation of myeloid inflammatory STAT3-NFκB pathways induces an open chromatin state that permits the Yamanaka factors to bind to promoters of pluripotency genes resulting in highly efficient reprogramming. Our hypothesis is that major myeloid-specific STAT3-mediated epigenetic events play critical roles in the erasure of the donor epigenetic memory and the establishment of high quality ESC-like hiPSC.

T-2135
GENERATION OF INDUCED NEURAL PRECURSOR CELLS FROM HUMAN CORD BLOOD

Evers, Daniela[1], Kesavan, Jaideep[1], Koegler, Gesine[2], Peitz, Michael[1], Bruestle, Oliver[1]
[1]Institute of Reconstructive Neurobiology, Bonn, Germany, [2]Heinrich-Heine University, Institute for Transplantation Diagnostics and Cell Therapeutics, Duesseldorf, Germany

Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) represents an attractive approach for generating donor cells for neuro-restorative approaches employing cell transplantation. However, the validation and neural differentiation of human iPSCs is time-consuming, and extended culture periods may favour the acquisition of mutations. First evidence indicates that ectopic expression of SOX2 and c-MYC is sufficient to program human cord blood (CB) CD34+ cells into induced neuronal-like cells, a heterogeneous population that comprises different levels of neuronal maturation and displays incomplete transgene silencing (Giorgetti et al., 2012). Here we set out to directly convert CB cells into a homogenous and transgene-free population of induced neural precursor cells (iNPCs). Upon transduction of CD34+ cells with non-integrating Sendai viruses coding for SOX2 and c-MYC we were able to obtain primary iNPC colonies within 20 days. After serial passaging clonal iNPC lines were found to be free of viral replicons and to express typical neural progenitor markers such as PAX6, SOX2, NESTIN and PLZF. INPCs could be expanded for more than 30 passages and were able to differentiate into derivatives of the central as well as the peripheral nervous system, yielding various neuronal and glial subtypes. Neurons derived from these iNPCs were able to fire action potentials upon current injection and exhibited spontaneous postsynaptic currents indicating formation of neuronal circuits. Conversion of cord blood-derived cells into transgene-free iNPCs may enable the establishment of a patient-specific cellular resource for neural cell replacement and other biomedical applications.

T-2136
STRUCTURE FUNCTION ANALYSIS OF HIF1 AND HIF2 AND THEIR DIFFERENCES IN PROTEIN COMPLEX IN REPROGRAMMING

Ferreccio, Amy[1], Mathieu, Julie[2], Robitaille, Aaron[1], Moon, Randall T.[2], Ruohola-Baker, Hannele[3]
[1]Biochemistry, University of Washington, Seattle, WA, USA, [2]University of Washington, Seattle, WA, USA, [3]Institute for Stem Cell and Regenerative Medicine University of Washington, Seattle, WA, USA

The emerging role of hypoxia and the hypoxia inducible transcription factors (HIFs) in the acquisition of stemness emphasizes the importance of metabolic context in cell fate. We have recently shown that hypoxia can induce the reversal of human ESC early differentiation and that HIFs are important for the metabolism of primed stem cell state (Zhou et al., 2012; Mathieu et al., 2013; Mathieu et al., 2014). Previous studies show that aggressive cancers display gene expression signatures characteristic of ESCs and are commonly exposed to hypoxic environments. HIFs may connect these two facts: hypoxia, through HIFs can induce a human embryonic stem cell-like transcriptional program in cancer cells (Mathieu et al., 2011). We have recently used the reprogramming assay to analyze HIF1alpha and HIF2alpha function in stem cell acquisition. HIF1alpha and HIF2alpha, homologous proteins with comparable functional domains are both required for reprogramming. We are now in process of analyzing the key targets of these transcription factors and their mechanism of action in metabolism. Surprisingly, constitutive activation of HIF1alpha and HIF2alpha result in opposite outcomes during reprogramming; while constitutive activation of HIF1alpha is beneficial, constitutive activation of HIF2alpha is inhibitory for iPSC induction (Mathieu et al., 2014). We will now, through structure-function analysis identify the critical regions that convey the opposite functions of these two transcription factors in the reprogramming assay. Further, we have performed Mass Spectrometry analysis of the nuclear HIF complexes to dissect the differences between HIF1alpha and HIF2alpha complexes in reprogramming context. We will test the function of the candidate differentially interacting molecules in the reprogramming assay by overexpression and loss-of-function experiments. These studies will be essential for future HIF2-based therapeutic discoveries.

T-2137
PROGRAMMABLE DNA-BASED EPIGENETIC SWITCHES TRIGGER TRANSCRIPTIONAL ACTIVATION OF DEVELOPMENTAL GENES IN SOMATIC FIBROBLASTS

Ganesh Pandian, Namasivayam, Le, Han, Taniguchi, Junichi, Shinsuke, Sato, Sugiyama, Hiroshi
Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Kyoto, Japan

Introduction: Artificial induction of pluripotency in somatic cells through transcriptional reprogramming has changed the scientific view that the fate of the specialized cells is irreversible. Sequential and/or parallel modifications in the chromatin modifying enzymes turn `ON` and `OFF` the transcriptional machinery at the right place and time to regulate cell fate. Since the epigenome is inherently flexible, it could be modulated through pharmacological interventions. Accordingly, several small molecules targeting the epigenetic enzymes or key transcription factors were shown to enhance the somatic cell reprogramming. However, these effectors artificially alter the epigenome in a sequence independent manner. Recently, cellular reprogramming of mouse embryonic fibroblasts (MEFs) into induced pluripotent stem cells got achieved with a cocktail of seven small molecules alone. However, requirement of several small molecules and time taken to achieve completely reprogrammed cell line are the major concern. Since cellular reprogramming is multi-factorial in nature, there is a demand for versatile small molecules capable of modulating the complicated gene networks associated with pluripotency. Results and Discussion: As a novel chemical approach to control cell fate *via* site-specific chromatin modifications, we synthesized a new class of dual-functional small molecule termed, SAHA-PIP containing sequence-specific pyrrole-imidazole polyamides (PIPs) and chromatin modifying histone deacetylase (HDAC) inhibitor like SAHA. Screening studies carried out to evaluate the effect of SAHA-PIPs on iPSC factors (*Oct-3/4*, *Nanog*, *Sox2*, *Klf4* and *c-Myc*) in MEFs indicated that SAHA-PIPs distinctively activate the iPSC factors by triggering epigenetic marks that are associated with transcriptionally permissive chromatin. The scope of improving SAHA-PIPs as efficient genetic switches was further substantiated with the identification of a new SAHA-PIP `**δ**` that could dramatically induce *Oct-3/4* and *Nanog* by about 30 to 40-fold. Genome-wide gene analysis revealed that **δ** induced multiple pluripotency genes in MEF by more than ten-fold to initiate cellular reprogramming in just 24 h. Evaluation of the effect of SAHA-PIPs on genome-wide gene expression in human fibroblasts divulged that each SAHA-PIP activated their own unique set of developmental genes and some therapeutically important genes like *KSR2* (the obesity gene), *MX2* (HIV antagonist), *SEMA6A* (the retinal 'ON' circuit factor) and *TET1*. Interestingly, a SAHA-PIP `I` remarkably switched `ON` the core pluripotency genes circuitry. Likewise, SAHA-PIP K enforced the transcriptional activation of germ cell genes, which are typically conserved in a somatic cell. Summary and Outlook:We have successfully developed targeting small molecules that could trigger cellular reprogramming by switching "ON" the transcriptional machinery conferring to pluripotency. Unlike other small molecules currently employed to improve reprogramming efficiency, PIP conjugates can be tailored to bind predetermined DNA sequences. Therefore, strategies to expand our tunable epigenetic switches could create an epoch-making approach in cellular reprogramming as they may precisely coax the somatic cells into pluripotent stem cells and/or a totally new type of cells.

T-2139
SMALL COMPOUNDS FACILITATE RAPID AND SYNCHRONOUS REPROGRAMMING OF SOMATIC CELLS INTO IPS CELLS BY DEFINED FACTORS

Brumbaugh, Justin[1], Bar-Nur, Ori[1], Verheul, Cassandra[2], Apostolou, Eftychia[1], Walsh, Ryan[1], Hochedlinger, Konrad[3]
[1]Harvard Stem Cell Institute, Massachusetts General Hospital/Harvard Medical School, Boston, MA, USA, [2]Massachusetts General Hospital/ Harvard Medical School, Boston, MA, USA, [3]Howard Hughes Medical Institute and Harvard Medical School, Boston, MA, USA

Reprogramming somatic cells into induced pluripotent stem cells (iPSCs) by defined factors such as Oct4, Klf4, Sox2 and c-Myc (OKSM) is an inefficient and lengthy process. Recently, loss of Mbd3 was reported to endow almost every somatic cell with induced pluripotency after 6-8 days of OKSM expression. Conversely, transient activation of C/EBPα prior to OKSM expression in B cells is sufficient to generate Oct4-GFP+ cells with a short latency and at high efficiency. Together, these findings support the view that OKSM-induced iPSC formation is a stochastic process that can be rendered deterministic upon genetic manipulation. Here, we tested whether efficient and synchronous iPSC formation could be induced from somatic cells without further genetic manipulation. We screened a variety of small molecules, individually or in combination, for their effect on iPSC generation and identified ascorbic acid (AA) and the GSK3-beta inhibitor CHIR-99021 as the most effective treatments. Combined supplementation (termed "AGi")

had a synergistic effect on the derivation of iPSCs from different cell types (GMPs, proB cells, IgM+ B cells and fibroblasts), enhancing reprogramming greater than an order of magnitude. Moreover, AGi exposure yielded stable IPSC clones from GMPs after as little as 48 hours of factor expression. To accurately quantify reprogramming efficiency and kinetics, we sorted single GMPs carrying an inducible OKSM cassette and *Oct4-GFP* knock-in reporter. Remarkably, exposure to AGi yielded over 95% Oct4-GFP$^+$ wells by day 5. Consistent with the synchronous emergence of iPSCs, the majority of cells within individual wells exhibited homogeneous Oct4-GFP$^+$ activation (90-100% by day 5) in the presence of AGi whereas control cultures remained highly heterogeneous until day 30. To dissect the molecular consequences of AGi treatment, we performed expression profiling. Reprogrammable MEFs exposed to AGi for only 48 hours were already distinguishable from MEFs exposed to doxycycline alone. Somatic transcripts (e.g. *Prrx1, Fbln5, miR-143*) were downregulated rapidly in AGi-treated cells while key pluripotency transcripts including *Nanog*, *Utf1, Sall4* and *miR-290-295* were upregulated in AGi-exposed cells. Further, AGi abolished the transient upregulation of differentiation-associated genes such as *Prx, Bmp2* and *Cxcr4*, suggesting that these molecular changes normally resist iPSC formation. Lastly, we examined whether AGi treatment enhances cell fate transitions in the context of transdifferentiation of fibroblasts into induced neural stem cells (iNSCs). Whereas control cultures gave rise to iNSCs at the expected low efficiency, AGi increased the recovery of iNSC colonies by 6-fold. In this study, we provided molecular and cellular evidence that AGi induces near-homogeneous reprogramming of different somatic cells into iPSCs at efficiencies and kinetics that have so far only been achieved with genetic manipulation. From a practical point of view, AGi-mediated reprogramming enables molecular studies of the reprogramming process in bulk cultures and is expected to streamline integration-free iPSC generation systems that have thus far been hampered by low efficiencies.

T-2140
RNA-MEDIATED GENERATION OF INTEGRATION-FREE IPS CELL LINES FROM LATE-OUTGROWTH ENDOTHELIAL PROGENITOR CELLS (L-EPCS) DERIVED FROM HUMAN BLOOD

Eminli-Meissner, Sarah[1], Moon, Jung-Il[1], Poleganov, Marco[2], Beissert, Tim[2], Sahin, Ugur[2], Morrell, Nick[3], Huang, Chris[3], Rana, Amer[3], **Hamilton, Brad**[1]
[1]Stemgent, Cambridge, MA, USA, [2]TRON-Translational Oncology at University Medical Center Mainz, Mainz, Germany, [3]Department of Medicine, University of Cambridge, Cambridge, United Kingdom

In 2010, it was first published that repeated transfection of fibroblasts with a cocktail of reprogramming mRNAs resulted in the generation of stable, integration-free human iPS cells. While many advancements have been made to refine this process on fibroblasts, to date no group has been able to demonstrate RNA-based reprogramming of a blood derived cell type. This limitation has been primarily due to the inability to efficiently and repeatedly deliver mRNA to cells originating from blood without inducing cytotoxicity. Peripheral blood provides easy access to adult human cell types for reprogramming purposes. As a result there are numerous studies utilizing blood derived cell types to generate patient and disease specific iPS cell lines for both research and clinical applications. Notably, late-outgrowth endothelial progenitor cells (L-EPCs) can be clonally isolated from both human peripheral blood and cord blood. The L-EPCs adherent nature and high proliferative capacity while maintaining their cell identity makes them highly desirable for repeated transfection with RNA, as non-adherent hematopoietic cells typically have limited expansion potential and exhibit maturation in culture. Lastly, the ability to generate clinical grade iPS cells from L-EPCs using RNA reprogramming technologies presents a unique therapeutic opportunity to treat myeloproliferative disorders in which the disease-causing somatic mutations are restricted to cells in the hematopoietic lineage. Here we present data demonstrating the cellular reprogramming of human L-EPC lines into stable and fully reprogrammed iPS cells via transfection with novel non-modified reprogramming mRNAs and reprogramming associated miRNAs.

T-2141
TRANSCRIPTOME ANALYSIS OF THE PHASES IN HUMAN SOMATIC CELL REPROGRAMMING

Hernandez, Javier, Liu, Yu, Jayakumaran, Gowtham, Hirsch, Calley, Wrana, Jeffrey L.
Mount Sinai - Lunenfeld-Tanenbaum Research Institute, Toronto, ON, Canada

Reprogramming of somatic cells to pluripotency is accompanied by extensive changes at the molecular and cellular level. In mice, cells transition through a sequence of transcriptional and epigenetic events in three stepped phases, where somatic cells first undergo a mesenchymal to epithelial transition (initiation), prior to activating the first pluripotency markers (maturation), and finally establishing their own self-sustaining transcriptional network to regulate stemness (stabilization). Although common core factors govern stem cell identity in mouse and human pluripotent cells, different signaling pathways and culture conditions regulate them. Therefore understanding how pluripotency is acquired in human cells, and exploring differences from the mouse system is critical to fully harness the potentials of induced pluripotent stem (iPS) cell technology. To study the cellular events and mechanisms of human somatic cell reprogramming, we established a robust human reprogramming system. Using this system, we have characterized the phases of human somatic cell reprogramming by profiling both the coding and non-coding transcriptome. Our preliminary results reveal altered kinetics of the initiation, maturation and stabilization phases, compared to mouse reprogramming. In addition to a large array of genes that are essential for both human and mouse reprogramming, our findings also implicate a unique set of genes specific to generating human iPS cells. Further functional analyses will validate the mechanisms regulated by signaling events specific to human reprogramming. Together, our results contribute to a better understanding of how human somatic cells acquire pluripotency and facilitate major transitions of cell fate.

T-2142
MANIPULATION OF KLF4 EXPRESSION GENERATES PARTIALLY REPROGRAMMED IPSCS PAUSED AT SUCCESSIVE STAGES OF REPROGRAMMING

Hisatake, Koji[1], Kato, Tetsuro[1], Ayakawa, Daisuke[1], Oinam, Lalhaba[1], Ohtaka, Manami[2], Fukuda, Aya[1], Nakanishi, Mahito[2], Nishimura, Ken[1]
[1]Faculty of Medicine, University of Tsukuba, Tsukuba, Japan, [2]Research Center for Stem Cell Engineering, National Institute of Advanced Industry, Tsukuba, Japan

The detailed mechanism of reprogramming somatic cells into induced pluripotent stem cells (iPSCs) remains largely unknown. Partially reprogrammed iPSCs are informative and useful for understanding the mechanism of reprogramming, but it remains technically difficult to produce them in a predictable and reproducible manner because it is not straighforward to control the expression levels and stoichiometry of reprogramming factors, which affect the efficiency of iPSC generation and the quality of generated iPSCs. We have developed a unique gene

transfer system, named SeVdp vectors, which remain persistently in the cytoplasm without integrating into the host genome and also enable expression of multiples genes with a constant stoichiometry from a single vector genome. SeVdp vectors harboring four reprogramming factors (Oct4, Sox2, Klf4 and c-myc) reprogram mouse and human somatic cells very efficiently. Here, we analyzed the effect of reduced expression of the four reprogramming factors using SeVdp vectors, and found that reduction of Klf4 expression reproducibly generates a homogeneous population of partially reprogrammed iPSCs. Up-regulation of Klf4 enable these cells to resume reprogramming, indicating that they are "paused iPSCs" that are stalled on the path towards iPSCs. Moreover, the different level of Klf4 expression allowed generation of many types of paused iPSCs that have paused at distinct intermediate stages of reprogramming. Whole genome analyses suggested that low Klf4 expression is sufficient to repress somatic cell-specific genes but not for activating pluripotency-related genes. The analyses also showed that there are at least two distinct modes of response by which pluripotency genes are activated in a Klf4-dependent manner. Finally, in addition to mouse cells, this system allowed generation of partially reprogrammed iPSCs from human cells. These results indicate that paused iPSCs produced by our system will facilitate the mechanistic analysis of various stages of reprogramming in both mouse and human cells.

T-2143
SMALL MOLECULES INDUCE DIRECT DIFFERENTIATION OF HUMAN DERMAL FIBROBLAST AND HUMAN MESENCHYMAL STEM CELLS INTO HEPATOCYTE-LIKE CELLS

Choi, Eunhyun[1], Ham, Onju[1], Lee, Se-Yeon[1], Lee, Chang Youn[2], Park, Jun-Hee[2], Lee, Jiyun[1], Seo, Hyang-Hee[1], Seung, Minji[1], Yun, INa[1], Han, Sun M.[1], **Hwang, Ki Chul**[3]

[1]Yonsei University College of Medicine, Brain Korea 21 Plus Project for Medical Science, Seoul, Republic of Korea, [2]Yonsei University, Department of Integrated Omics for Biomedical Sciences, Seoul, Republic of Korea, [3]Yonsei University College of Medicine, Severance Biomedical Science Institute, Seoul, Republic of Korea

Direct differentiation of fibroblast or stem cells into hepatocyte-like cells can be induced by regulating various transcription factors using viral vectors. Although genetic modulation through differentiation using viral vectors is beneficial, a strategy for differentiation without the use of viral vectors is required for clinical application. Since small molecules modulate cell fate by regulating multiple cellular signaling processes, their use has arisen as a possible strategy for modulating differentiation. In our previous study, we identified that small molecules have the potential to drive differentiation of rat bone marrow-derived mesenchymal stem cells (rBMMSCs) into hepatocyte-like cells.. Thus, forward from this study, we tested direct differentiation of human dermal fibroblast (HDF) or human BMMSCs (hBMMSCs) into hepatocytes by regulating transcriptional levels using a combination of three small molecules: inhibitors of CKD (1,2), PRKDC, and PKC.. The small molecules were treated onto HDF and hBMMSCs to regulate the expression of transcription factors including Hnf4α, GATA4, Hnf1α, and FOXO3.. Regulation of these transcription factors caused the expressions of hepatocyte markers including CK18, Alb, Ttr, Hnf4α, CK8, and CK19 to be up-regulated in the cells treated with small molecules. In addition, we detected the involvement of microRNA (miR), as a regulator of biological function, in inducing differentiation into hepatocytes. We measured the expression levels of miR-200b,c, miR-203, miR-181, miR-1, miR-182, and miR-34 and found that they were regulated in the small molecules-treated cells. These results indicated that the chosen combination of small molecules induced differentiation into hepatocyte-like cells in HDF and BMMSCs.

T-2144
REFINING MODELS OF SOMATIC REPROGRAMMING

Jayakumaran, Gowtham[1], Hirsch, Calley[2], Trcka, Daniel[3], Hernandez, Javier[1], Wrana, Jeffrey[4]

[1]Mount Sinai - Lunenfeld Tanenbaum Research Institute, Toronto, ON, Canada, [2]Mount Sinai - Samuel Lunenfeld Research Institute, Toronto, ON, Canada, [3]Samuel Lunenfeld Research Centre, Toronto, ON, Canada, [4]Mount Sinai Hospital, Samuel Lunenfeld Research Institute, Toronto, ON, Canada

Reprogramming of somatic cells to pluripotency demonstrates the remarkable plasticity of cells and serves as a unique model to understand the molecular mechanisms governing cell fate choices. In reprogramming, plasticity is not observed to the same extent for all cells and cellular transition to pluripotency only occurs in few reprogramming cells. One model fitting this observation is the 'stochastic' model, whereby a small fraction of reprogramming cells initially acquire competency probabilistically at varying latencies and later undergo a sequence of defined molecular events to become pluripotent. A contrasting model supports an 'elite' cell model, where specific cells in the somatic cell population are predisposed to acquire pluripotency upon reprogramming factor expression. Here, we test the validity of these contrasting models by combining a highly efficient piggybac primary reprogramming system with a DNA barcoding strategy. Our results reveal the existence of unique parent cells in the starting somatic cell population that upon factor expression and continued culture, proliferate and account for the bulk of reprogramming cell population. Notably, progenies of the same parent cells dominate reprogramming cultures suggesting an inherent competency in these 'elite' somatic parent cells to reprogram and acquire pluripotency. Our approach refines the prevailing models of somatic reprogramming and enhances current understanding of cell plasticity in reprogramming cell cultures.

T-2145
CHROMATIN REMODELING MEDIATED ATM ACTIVATION PREVENTS HEPATIC LINEAGE REPROGRAMMING

Ji, Shuyi, Zhu, Linying, Yu, Zhiyong, Cen, Jin, Hui, Lijian

Shanghai Institute of Biochemistry and Cell Biology, Shanghai, China

Direct lineage reprogramming by ectopic expression of lineage-specific transcription factors is a process of epigenetic remodeling but with low efficiency. It is poorly understood by which mechanisms a cell resists epigenetic remodeling during lineage reprogramming. Here we show that forced expression of hepatic-specific transcription factors Foxa3, Hnf1α and Gata4 induces a strong activation of the tumor suppressor p53, which further leads to proliferation arrest and cell death and prevents hepatic lineage reprogramming. Remarkably, previous studies have revealed that p53 is also a key barrier for the iPS cell induction. We found that the activation of p53 is mediated by ATM in a DNA-damage-independent manner. By characterizing the molecular events during the early stage of hepatic lineage reprogramming, we found that ATM activation is subsequently activated after chromatin remodeling. To reveal whether ATM is activated by chromatin remodeling, we set off to identify the key chromatin remodeler involved in hepatic lineage conversion. We identified a member of SWI/SNF complex as a key barrier of hepatic lineage conversion. Inactivating this SWI/SNF complex member affects the recruitment of ATM and reduces the activation of ATM and p53. These findings therefore shed light on how cells response to epigenetic remodeling and identify a novel function of ATM as a sensor of chromatin remodeling in cell lineage reprogramming.

T-2146

POSITIVE COACTIVATOR 4 (PC4) IS REQUIRED MAINTENANCE OF PLURIPOTENCY IN ES CELLS AND ENHANCE EFFICIENCY OF REPROGRAMMING FIBROBLAST TO IPS CELLS

Jo, Junghyun[1], Hong, Soomin[1], Kim, Hyung Joon[2], Lee, Jeoung Eun[3], Lee, Sung-Geum[3], Baek, Ahmi[2], Hwang, Sohyun[4], Lee, Insuk[4], Lee, Dong Ryul[1]

[1]Department of Biomedical Science, College of Life Science, CHA University, Seoul, Republic of Korea, [2]Fertility Center of CHA Gangnam Medical Center, CHA University, Seoul, Republic of Korea, [3]CHA Stem Cell Institute, CHA University, Seoul, Republic of Korea, [4]Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul, Republic of Korea

Introduction: Currently, our group reported that multipotent spermatogonial stem cells (mSSCs) were established from SSCs by in vitro culture induction method. We found that intermediate state SSCs (iSSCs) existed in this process, and screened differentially expressed genes (DEGs) in three types of cells. Among these DEGs, expression of PC4 as known to transcriptional coactivator was significantly increased during reprogramming. PC4, which is known to an ortholog of yeast Sub1, enhances RNA polymerase (RNAP) II transcription, and is involved in various DNA dependent programs such as DNA repair, replication, and transcription interacting between transcriptional activators and components of the RNAPII basal transcription machinery. PC4 has been well studied in terms of molecular functions,however, the role of PC4 has not been studied in embryonic stem cells (ESCs). We hypothesized that molecular and physiological function of PC4 may act an important role in ESCs. So, in this study, we have investigated whether PC4 can increase for transcriptional activity of the pluripotency-related genes in ESCs for maintenance of pluripotency, and enhance somatic cell reprogramming into pluripotent state by a gain-of-function assay. Materials and Methods: For gain-of-function assay, *Pc4* over-expression (OE) ESCs were established by lentiviral transfection that inserted VENUS (YFP) as reporter system. We also performed transfection of *Pc4*-siRNA into ESCs to knock-down (KD) *Pc4* expression as a loss-of-function assay using Lipofectamine 2000. We confirmed OE and KD level of *Pc4* by RT-PCR, qRT-PCR, and Western blot analysis. After establishment of *Pc4* OE and KD ESCs, alteration of pluripotency-related genes expression was examined by RT-PCR and qRT-PCR. To analyze the phenotypic change of ESCs by *Pc4* OE, proliferation assay was performed counting of colonies and cell numbers. Recombinant R7-conjugated PC4 protein was purified, and delivered into ESCs as supplementation in growth medium. Additionally, reprogramming efficiency was examined by generation of iPSCs that transfected OSKM along with *Pc4* or R7-PC4 protein. Results: *Pc4* OE ESCs were increased by 2-fold of *Pc4* expression level than control ESCs, and expression level of *Oct4*, *Sox2*, and *Klf4* showed a significant increase. According to alteration of these gene expressions, proliferation of ESCs was slightly higher in *Pc4* OE ESCs. On the other hand, KD *Pc4* level was shown that decreased up to 80 %. After 48 h, *Sox2* and *Klf4* level was significantly decreased by *Pc4* KD. Additionally, R7-PC4 protein delivery was performed as supplementation in growth medium, and we obtained similar results with that of *Pc4* OE ESCs. *Pc4* viral transfection and R7-PC4 protein delivery nearly doubled in efficiency of reprogramming of fibroblast into iPSCs, respectively. Conclusion: In this study, we found that PC4 enhance the transcription of pluripotency-related genes and cellular proliferation of ESCs by *Pc4* gain- and loss-of-function assay. R7-PC4 protein transiently increased expression levels of the pluripotency-related genes, and proliferation in ESCs corresponding to *Pc4* OE ESC lines. Moreover, PC4 can accelerate reprogramming of fibroblast into iPSCs via transcriptional activation. Therefore, we suggest that PC4 was required maintenance of pluripotency in ESCs and enhanced reprogramming efficiency, and this study may contribute to improve the method and understand the mechanisms of somatic cell reprogramming.

T-2147

THE RB TUMOR SUPRESSOR RESTRICTS REPROGRAMMING BY DIRECTLY SILENCING PLURIPOTENCY GENES

Kareta, Michael[1], Gorges, Laura[2], Hafeez, Sana[1], Zmoos, Anne-Flore[1], Cecchini, Matthew J.[3], Spacek, Damek[1], Batista, Luis[1], O'Brian, Megan[1], Ng, Yi-Han[1], Ang, Cheen Euong[1], Vaka, Dedeepya[1], Artandi, Steven[4], Dick, Frederick A.[5], Sage, Julien[6], Wernig, Marius[7]

[1]Stanford University, Stanford, CA, USA, [2]Monsanto Company, Saint Louis, MO, USA, [3]London Regional Cancer Program, Western University, London, ON, Canada, [4]Stanford University School of Medicine, Stanford, CA, USA, [5]7London Regional Cancer Program, Western University, London, ON, Canada, [6]Stanford School of Medicine, Stanford, CA, USA, [7]Stanford University, Palo Alto, CA, USA

Reprogramming of differentiated cells to induced pluripotent stem (iPS) cells is a fascinating yet poorly understood process. Based on the similarities between reprogramming and cancer, we investigated the tumor suppressor Rb in the induction of pluripotency. Here, we show that loss of Rb increases the efficiency of iPS cell reprogramming. Surprisingly, Rb inactivation does not enhance reprogramming by accelerating the cell cycle. Instead, we found that Rb directly binds regulatory regions of many pluripotency genes, including Sox2. Consequently, loss of Rb leads to a slight but widespread de-repression of the pluripotency program, rendering Rb-deficient cells reprogrammable without exogenous Sox2. Moreover, Sox2 was found to critically mediate Rb-induced tumorigenesis. These results identify Rb as a global transcriptional repressor of the pluripotency network, explaining previous reports about Rb's involvement in cell fate pliability. Moreover, our observations implicate factors involved in pluripotency such as Sox2 in cancers driven by loss of Rb.

T-2148

DOWN-REGULATION OF THE MAIN GENES AND MIRNAS INVOLVED IN REPROGRAMMING IN GASTRIC ADENOCARCINOMA REVEALS NEW INSIGHT INTO GASTRIC CANCER

Khalili, Mitra[1], Mowla, Seyed Javad[2], Sadeghizadeh, Mjid[2], Vasei, Mohammad[3], Alimoghaddam, Kamran[4], Davood, Khalili[5]

[1]Medical Genetics and Biotechnology, Zanjan University of Medical Sciences, zanjan, Iran, [2]Molecular Genetics, Tarbiat Modares University (TMU), Tehran, Iran, [3]Pathology, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran, [4]Hematology-Oncology and Stem Cell Research Center, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran, [5]Prevention of Metabolic Disorders Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Introduction: Induced expression of a defined number of embryonic stem cell (ESC)-specific genes, mainly OCT4 and SOX2, could reprogram a differentiated cell into an induced pluripotency stem (iPS) cell. The program is also inhibited by the action of some tumor suppressor genes, most importantly P53. There is a possibility that the re-expression of iPS genes, i.e. OCT-4 and Sox2, and diminished expression of p53 and its main down-stream targets, i.e. P21 and miR-145, contributes also to tumorigenesis. Materials and Methods: A total number of 34 tumor and their matched non-tumor (as control) gastric surgical specimens were obtained. The expression of the candidate genes were evaluated

by using real-time PCR and immunohistochemistry (IHC). Results: Our data revealed a significant down-regulation of P21, miR-145 and miR-302b genes in intestinal type of adenocarcinoma samples versus their matched non tumor samples. The same finding was obtained for Sox2 expression; however, a significant down-regulation was confined only to the diffuse type of tumors. By performing receiver-operating characteristic (ROC) analyses, the highest total area under the curve (AUC) was found for SOX2 (AUC=82%, p= 0.000). So it indicates that SOX2 has a good ability to discriminate correctly between tumor and non-tumor samples. In coordination to real-time PCR data, immunohistochemistry confirmed the expression of Sox2 protein in related samples. Discussion: Despite the fact that some hESC-specific genes are up-regulated in tumors, our data revealed down-regulation of miR-302b, OCT4 and SOX2, in high grade tumors. We also found that the expression of all genes is significantly varied between diffuse vs. intestinal types of gastric cancer. It supposed that down-regulation of miR-145 and P21, might be required for tumor progression in its course towards a more malignant behavior. Based on ROC curves, it seems that evaluation the expression of is informative diagnostic tools for gastric tumors.

T-2149
REPROGRAMMING OF EPITHALIAL CANCER CELLS THROUGH ADMINISTRATION OF TRADITIONAL MEDICINAL HERBS

Kim, Sang-Gyung, Kang, Bomi, Lee, A-jin, Jang, Haebong
Laboratory Medicine, Catholic University of Daegu, School of Medicine, CIMI, Daegu, Republic of Korea

In vitro culture of cancer cells isolated from patient suffered from various types of cancer, although not straightforward, often considered as a necessary model for genome characterization of such cancers and drug screening for anti-cancer treatment. It has become evident that Rho-associated protein kinase (ROCK) inhibitor administration and culture of cancer cells on embryonic feeder cell layer promote the survival of dissociated cancer cells through cellular reprogramming *in vitro*. Traditional medicinal herbs have known as anti-inflammatory, anti-thrombotic and anti-oxidant effects etc. These traditional medicinal herbs have been considered as anti-cancer treatment. However their mechanisms of action in cancer cells have not been defined well. MCF-7 cells (breast cancer cell line) were cultured onγ-irradiatedNIH3T3feeders(mouse embryonic fibroblasts). Twomedicinal herbsA (100μg/mL), and B(10μg/mL) were administered to cultures and their effects on cellular reprogramming were measured. Also, a well-known ROCK inhibitor, Y-27632 (10μg/mL) was administered to culture for 24 hours to compare and contrast the role of these medicinal herbs of cellular reprogramming of cancer cells. To be more specific, expression of Notch-1 and Notch ICD protein and epithelial stem cell markers, Np63a, integrin a6 and CD44 were tested by western immunoblotting and also their mRNA levels were measured by quantitative RT-PCR (qRT-PCR)after 24 hours of each herbs or Y27632 administrations. Notch-1 and notch ICD protein level were decreased whereas Np63a and Integrin a6 expression were increased with Y-27632 treatment. Interestingly, medicinal herb B treatment on the culture showed similar pattern as Y-27632 treatment of the protein expression of Notch-1, Notch ICD, Np63a and Integrin a6. Co-administration of Y-27632 and medicinal herbA showed no difference in Notch-1 mRNA expression which may indicate medicinal herb A possess opposite effects compared with Y-27632 at least on Notch1 mRNA expression. Furthermore, both protein and mRNA levels of CD44 was increased by Y-27632,. These increment was partially reverted by co-administration of Y27632 with herbA and B. These data suggest that, medicinal herb B may have a similar role in cancer cells as ROCK inhibitor at least in regulation of both protein and mRNA expression that related to Notch signaling pathway and epithelial stem cells. On the other hand, medicinal herb A seems to have a opposite effect in cancer cells as ROCK inhibitor at least in regulation of both protein and mRNA expression that related to Notch signaling pathway and epithelial stem cells. Therefore, medicinal herb B can be a potential candidate as a cellular reprogramming reagent in cancer cells.

T-2150
FUNCTIONAL CHARACTERIZATION OF DIRECTLY REPROGRAMMED NEURONS WITH SELF-REGULATING VECTORS

Lau, Shong, Rylander, Daniella, Jakobsson, Johan, Parmar, Malin
Lund University, Lund, Sweden

We have generated human induced neurons (iNs) from fetal lung fibroblasts with microRNA-regulated lentiviral vectors. The iN reprogramming factors (Ascl1, Brn2 and Myt1l) are followed by the target sequences of microRNA-124, an endogenous neuron specific microRNA. This allows expression of reprogramming factors in fibroblasts and down-regulation of the transgene specifically in cells that have converted into neurons. The conversion was successful with both doxycycline-regulated and housekeeping promoters driven reprogramming factors. Interestingly, this approach could also be combined with integration-deficient lentiviral vectors. Using whole-cell patch clamp technique to measure the electrophysiological properties of iN (obtained using both PGK or doxycycline-regulated promoter), we show that the resulting iN cells are able to fire action potentials and also show spontaneous and post-synaptic activities in co-culture with mouse primary glial cells. The ability to form synaptic connections with primary neurons was further determined using mono-synaptic tracing using modified rabies virus. We are currently comparing the functional properties of the iN cells generated with microRNA-regulated vectors using the same techniques. In summary, human iNs generated with self-regulating transgenes enables robust generation of functional neurons for future clinical applications in regenerative medicine.

T-2151
MIR-31/SDHA AXIS IS INVOLVED IN METABOLIC DIFFERENTIATED IN PARTIALLY VS FULLY REPROGRAMMED IPSCS

Lee, Man Ryul
Indiana University, Indianapolis, IN, USA

Induced pluripotent stem cells (iPSCs) cultures are a heterogeneous mixture composed of a few fully but predominantly partially reprogrammed cells. The metabolism of cells undergoing reprogramming is remodeled when somatic cells are reprogrammed into induced pluripotent stem cells (iPSC) and this metabolic shift is considered an essential component of the reprogramming process. iPSCs use less mitochondrial respiration but increased anaerobic glycolysis for their bioenergetic needs. In this study, we identified several micro RNAs (miRNAs) that are differentially induced in fully vs. stable partially reprogrammed iPSCs that were derived from human umbilical cord blood (CB) CD34+ cells. Among them, miR-31 suppressed expression of succinate dehydrogenase complex subunit A (SDHA), a protein essential for the mitochondrial Krebs cycle and a vital component of the mitochondrial electron transport chain (ETC) complex II. We found that the 3'UTR region of SDHA mRNA contains a binding target site for, and it's transcription is suppressed by, miR-31. MiR-31 overexpression in partially reprogrammed iPSCs lowered SDHA expression levels and lowered oxygen consumption rates to levels similar to fully reprogrammed iPSCs. However, miR-31

overexpression in partially reprogrammed cells did not increase the proportion of TRA1-60 positive cells in colonies but it did increase TRA1-60 positive cells when it was co-transduced with Yamanaka factors, resulting in nearly a 3 fold increase in reprogramming efficiency in cord blood CD34+ cells. These results suggest that miR-31 has an important regulatory/promotive role to drive complete reprogramming of partially reprogrammed iPSCs past the early, stable, partially reprogrammed stages of a multi-step reprogramming process induced by Yamanaka factors. These results also support the idea that a switch of mitochondrial respiratory metabolism to glycolytic metabolism is a critical step for lowering the threshold of iPSC reprogramming and also supports the notion of a multi-stage reprogramming process where metabolic remodeling might represent one fundamental aspect. This information not only illuminates valuable insights into potential designs to improve prototypic Yamanaka factor-driven iPSC production but also provides the most comprehensive analysis yet of one aspect of the molecular metabolic dynamics of multi-stage reprogramming processes.

T-2152
MECHANISMS OF ASCL1-MEDIATED REPROGRAMMING

Lee, Qian Yi, Wernig, Marius
Stanford University, Palo Alto, CA, USA

With an aging world population, neurodegenerative diseases are becoming some of the leading contributors to global disease burden, since age is a predominant risk factor for these disorders. Direct lineage reprogramming is a potentially powerful approach in disease modeling and drug discovery, since we can easily isolate fibroblasts from a patient and convert them directly into neurons to study disease progress. Our lab has previously shown that we can efficiently reprogram mouse embryonic fibroblasts (MEFs) into neurons with the transcription factors Brn2, Ascl1 and Myt1l. Human adult fibroblasts can also be reprogrammed, albeit much less efficiently, with the same three factors and the addition of NeuroD1. We have shown recently that Ascl1 acts as a pioneering factor in MEFs by immediately binding its cognate genomic sites and opening up the local chromatin to make it competent for the other transcription factors to bind. Now, we look into further into the mechanism of Ascl1 action in neuronal reprogramming. Our future plan is to find the differences of Ascl1 action between mice and humans that may account for the lowered efficiency of reprogramming in human fibroblasts.

T-2153
EPIDERMAL WNT/BETA-CATENIN ACTIVATION REPROGRAMS ADULT DERMIS TO A NEONATAL STATE

Lichtenberger, Beate M., Driskell, Ryan, Watt, Fiona M.
Centre for Stem Cells and Regenerative Medicine, King's College London, London, United Kingdom

In skin, the formation of hair follicles (HF) from developing epidermis is dependent upon a series of reciprocal interactions between cells of the epidermis and a condensate of mesenchymal cells of the underlying dermis which subsequently forms the dermal papilla (DP) of the HF, and occurs during late embryonic and early postnatal stages. Adult skin normally does not give rise to new HF suggesting that the properties of dermal fibroblasts change during postnatal development. However, activation of Wnt/β-catenin in adult epidermis expands the epidermal stem cell compartment and drives cells of the interfollicular epidermis and sebaceous gland into hair follicle lineages, thereby inducing HF neogenesis. The new ectopic follicles (EF) have associated dermal papillae implying cross-talk between Wnt-activated epidermis and dermis. Gene expression profiling of epidermal and dermal cells isolated from neonatal and adult skin and skin harbouring ectopic HF suggested that β-catenin-mediated reprogramming of the epidermis also involves reprogramming of the dermis to a neonatal state, which is reflected by massive remodelling of the extracellular matrix (ECM) in the dermis and increased proliferative potential of fibroblasts, which persists in vitro, and thus reveals a surprising ability of the epidermis to extensively remodel its local environment, even in an adult tissue. We have identified the signalling pathways involved in the expansion of different fibroblast lineages and ECM remodelling. Furthermore, we could show that the expansion of the papillary fibroblasts in the upper dermis upon epidermal Wnt activation affects wound healing and leads to formation of ectopic hair within the wound bed, which does not occur in wild-type skin.

T-2154
GENERATION OF AN EFFICIENT HUMAN SECONDARY REPROGRAMMING SYSTEM

Linderoth, Emma, Mileikovskaia, Maria, Hussein, Samer M., Nagy, Andras
Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

Much of what we currently know about the reprogramming process has been determined from studies on mouse cells. Translating and expanding those findings to human cells will be crucial in the development of iPSC technology for medicine. We are developing a human secondary reprogramming system for the efficient and reproducible generation of iPSCs, in order to study the reprogramming process in human cells in greater detail. With such a system, we can define cell state transitions that are achieved during the reprogramming process, allowing for a controlled time-window for the enhanced generation of specific cell types of particular therapeutic interest. We are currently using two different strategies to generate a highly efficient secondary reprogramming system. In the first approach, we use one of our well-characterized primary human iPSC (1°hiPSC) line, containing four separate, doxycycline (DOX)-inducible, piggyBac transposon-delivered expression vectors for Oct4, Klf4, c-Myc and Sox-2 (OKMS), as a starting point. The 1°hiPSC cells were grafted into immuno-compromised mice, leading to the formation of teratomas. Secondary fibroblast-like (2°FB) cells were derived from the resulting teratomas, and upon DOX-treatment, these 2°FB cells efficiently initiate the reprogramming process. Colonies start to appear after 8-10 days of DOX-treatment, and the efficiency of return reaches up to 10% based on limiting dilution assays. The second novel approach uses our CA1 human embryonic stem cells (hESCs) as a starting point. The hESCs were transfected with a polycistronic piggyBac vector expressing OKMS and mCherry reporter in a DOX-inducible manner. The established clones were then treated with DOX and high-transgene expressing clones were selected for further studies. These lines were differentiated in vitro by embryoid body (EB) formation or by spontaneous differentiation to produce fibroblast-like cells for further analysis. We expect that our human secondary reprogramming systems will serve as a powerful tool to analyse epigenetic regulation and cell states during human somatic cell reprogramming, and to study the role of novel modulators of pluripotency and differentiation.

T-2155

SINGLE TRANSFECTION OF A SYNTHETIC POLYCISTRONIC SELF-REPLICATIVE RNA WITH SMALL MOLECULES YIELDS HIGH NUMBERS OF HUMAN IPSCS

Lu, Min[1], Yoshioka, Naohisa[2], Dowdy, Steven[2], Chu, Vi[1]
[1]EMD Millipore, Temecula, CA, USA, [2]University of California, San Diego, La Jolla, CA, USA

Various methods utilizing DNA, RNA, miRNA and protein have been described to generate integration-free induced pluripotent stem cells (iPSCs). Some disadvantages to these methods include low reprogramming efficiency (i.e. DNA and protein), a requirement for negative selection and subsequent recloning steps to remove persistent traces of the virus (i.e. Sendai virus) or for daily transfections of four individual in vitro generated mRNAs over a 14 day period (i.e. mRNA based). Recently, an RNA species from non-infectious (non-packaging), self-replicating Venezuelan equine encephalitis (VEE) virus was engineered to encode all four reprogramming factors (RF; OCT4, KLF4, SOX2 and GLIS1) in a polycistronic RNA transcript that mimic cellular mRNA. Advantages to the VEE-RF system are that (1) the virus can self-replicate for a limited number of cell division hence obviating the requirement for multiple transfections, (2) the virus has capacity for incorporation of large polycistronic ORFs such as the 4 RF at sufficient high levels for reprogramming and (3) the virus only persists under positive selection and the ectopic RNA viruses can be quickly cleared when the positive selective pressure (i.e. puromyocin) is released. A single transfection of the VEE-RF replicon used in combination with a small molecule boost supplement that had previously been shown to enhance the kinetics and efficiency of lentivirus based reprogramming method efficiently reprogrammed two lines of human foreskin fibroblasts (HFFs). Interestingly the contribution of the small molecules to reprogramming efficiency was more significant in RNA-based reprogramming (~9 fold) than in lentiviral based methods (2-3 fold). RNA-based reprogramming efficiencies ranged from 0.3% in the slower proliferating BJ fibroblasts to 1.1% in the faster proliferating HFF. The resulting human iPSCs displayed normal ES cell-like morphology, stained positive for alkaline phosphatase and are moreover free of any residual VEE RNA. The fact that the same small molecule cocktail was able to boost iPSC colony generation in both viral and RNA approaches suggests that common signaling pathways critical for reprogramming have been activated and may be universally applied to enhance the reprogramming efficiency to other integration-free methods (i.e. DNA and protein based systems).

T-2156

SUCCESSIVE IPSC REPROGRAMMING INTERMEDIATES IDENTIFIED BY PROSPECTIVE ISOLATION AND SINGLE CELL MASS CYTOMETRY

Lujan, Ernesto
Genetics, Stanford University, Stanford, CA, USA

Reprogramming somatic cells to a pluripotent state by forced expression of transcription factors is a highly dynamic process. While extremely useful, this process is currently inefficient and the generation of heterogeneous populations over time impedes molecular analysis. In an effort to tease apart this heterogeneity we have utilized single cell mass cytometry. Like conventional fluorescent flow cytometry, this technique allows for the profiling of whole populations at the single cell level in a high-throughput manner; unlike conventional flow, we are able to detect protein levels for up to 30 intra and extra-cellular epitopes per cell with little spectral overlap due to distinct conjugated metal isotopes. With mass cytometry we have analyzed reprogramming factor stoichiometry, post-translational modifications of cell cycle and signaling proteins, pluripotency marker activation, and surface marker profiles for three different reprogramming lines, characterized partially reprogrammed lines, mouse ESCs and mouse iPSCs. From these analyses we find that reprogramming across different systems follows similar stepwise stages which can be clustered into 1) the repression of fibroblast specific markers 2) the emergence of an Oct4high Klf4high population 3) which gives rise to a population that resembles partially reprogrammed cells in cell cycle parameters and surface marker expression. These cells progress into fully reprogrammed Nanoghigh Sox2high CD54high and nonproductive Lin28high Nanoglow CD24high populations. Further, we have used these profiles in combination with novel surface markers to find productive routes to iPSC reprogramming. Our findings show that single cell analysis by mass cytometry can parse productive and nonproductive routes to reprogramming furthering our understanding of pluripotency induction.

IPS CELLS: DIRECTED DIFFERENTIATION

T-2157

MODELING HUMAN HETEROTOPIC OSSIFICATION USING DIRECTED DIFFERENTIATION OF HUMAN IPS CELLS

Barruet, Emilie C.[1], Kim, Hannah[1], Lwin, Wint[1], Morales, Marcela[1], Urrutia, Ashley[1], Schlieve, Christopher[1], White, Mark P.[2], Theodoris, Christina[2], Srivastava, Deepak[2], Hsiao, Edward[1]
[1]Institute for Human Genetics, University of California, San Francisco, San Francisco, CA, USA, [2]Gladstone Institutes, San Francisco, CA, USA

Musculoskeletal disorders affecting the bones and joints are major health problems for children and adults. Unfortunately, treatments for skeletal diseases are still rudimentary. The recent advent of human induced pluripotent stem cells (hiPSCs) provides an unparalleled opportunity to identify novel therapies for human skeletal diseases. One major regulatory pathway in bone formation involves bone morphogenetic proteins (BMPs). Patients with mutations in the Activin A Type I receptor (ACVR1), a BMP receptor, develop the debilitating disease fibrodysplasia ossificans progressiva (FOP). They show progressive ossification in muscle and tendon. The majority of ACVR1 mutations occur in a single amino acid (R206H) that may increase ACVR1 signaling activity. Recent data suggest that human endothelial cells carrying the ACVR1 R206H mutation may contribute to the formation of FOP lesions. Our overall hypothesis is that activated BMP signaling in endothelial cells increases heterotopic bone formation by increasing osteogenesis. In this study, we use a series of human iPSCs created from normal control and FOP donors. We previously showed that the FOP iPSCs not only demonstrate increased mineralization and enhanced chondrogenesis but also increased levels of genes expressed by endothelial cells (CD31) when cultured in mineralizing conditions. To determine if the ACVR1 R206H mutation leads to increased endothelial cell production, we used a recently-developed protocol to create human iPSC-derived endothelial cells. We successfully derived endothelial cells from our iPS lines. We found a yield of 25% phenotypic endothelial progenitors (CD31+/KDR+) from both control and FOP iPS lines. After sorting they yield more than 95% CD31+/CD144+ cells. iPS cell-derived CD31+/CD144+ cells cultured in mineralization conditions did not show mineralization. We are now using a new 3D protocol that recapitulates the endochondral bone formation through chondrogenic steps. This will allow us to test if the altered BMP signaling in FOP endothelial cells affects their osteogenic or chondrogenic properties. These studies use iPSCs created from patients with FOP, a rare and dramatic disease of massive heterotopic

ossification, to establish a robust in vitro model of human skeletal tissue formation that may be extended to iPSCs created from other skeletal conditions. The cellular mechanistic insights gained from these studies will establish a solid foundation for understanding the roles of non-bone tissues such as endothelial cells in skeletal formation and for identifying new therapies not only for FOP but also other diseases of abnormal skeletal formation.

T-2158
EFFICIENT AND SCALABLE GMP-GRADE CULTURE MEDIA SYSTEM FOR RAPID CARDIOMYOCYTE DIFFERENTIATION OF PLURIPOTENT STEM CELLS IN HUMAN DISEASE RESEARCH

Boucher, Shayne E., Kuninger, David, **Vemuri, Mohan C.**
Life Sciences Solutions, Thermo Fisher Scientific, Frederick, MD, USA

Introduction: Simple and robust derivation of spontaneously contracting cardiomyocytes derived from human pluripotent stem cells (hPSCs) would provide a valuable source of cells for basic research into cardiac biology and mechanisms of heart disease as well as applied studies in pharmacological drug discovery and toxicity screening. Currently a number of protocols exist for inducing embryoid bodies (EB) suspension or monolayer cultures of hPSC to differentiate into cardiomyocyte (Iglesias-Garcia et al 2013; Priori et al 2013). These cardiomyocyte differentiation protocols have led to varying results and differing purity levels of cardiomyocytes. To enable consistent differentiation of hPSCs, we developed a simplified cardiomyocyte differentiation media system, consisting of three ready-to-use components. This easy to use cardiac differentiation system is designed for monolayer hPSC affording flexible culture formats and a scalable workflow enabling generation of large numbers of consistent, spontaneously active cardiomyocytes. Methods: Briefly, hPSCs were maintained under feeder-free conditions using Essential 8 Medium. For cardiac induction EDTA-dissociated cells were seeded on Geltrex-coated surface as small clusters at ~1 - 2 x 104/cm2 by day 4 or 5 under serum-free condition. After reaching target confluence (Day 0), hPSC were incubated in an induction media for two days followed by addition of a second induction media and cultured for two additional days. In the final step, the induction media was replaced with maintenance media and cells were re-fed every other day for up to five weeks with beating cells usually first appearing on Day 7 and robust contracting syncytium by Day 10.The differentiated cells were analyzed for formation of contracting syncytium and for cardiomyocyte markers by flow cytometry and immunocytochemistry. Results: Multiple parameters were evaluated during differentiation media testing and our results demonstrate significant influences arise from the quality and confluence level of the PSC prior to induction and with the incubation period in different media. For example, several studies showed that hPSCs seeded at a density to reach 70 to 85% confluency by four days would yield large numbers of synchronously beating cardiomyocytes. When analyzed by quantitative flow cytometry, we observed troponin T type 2 (TNNT2)-immunoreactive cells strongly correlating to contracting cardiomyocytes. In cultures initiated with 90% or greater confluency, we typically observed lower yields of correlated TNNT2+ contracting cardiomyocytes. Subsequent immunocytochemistry studies verified expression of cardiac markers of ISL1, GATA4, MEF2C and MYH6 in Day 15 cardiomyocyte cultures. Conclusions: Our findings suggest the complete differentiation media system could serve as a standardized culture system for generating large numbers of consistent, spontaneously active cardiomyocytes in research studies. Further investigations will be performed to assess cardiomyocyte differentiation on different hPSC lines as well as verifying gene expression profiles, cardiac markers, and sensitivity to pharmacological agents.

T-2159
DEVELOPMENT OF SAFER STRATEGIES FOR THE AUTOLOGOUS CELL THERAPY OF DUCHENNE MUSCULAR DYSTROPHY BASED UPON MUSCLE PROGENITORS, IPS CELLS AND HUMAN ARTIFICIAL CHROMOSOMES

Casteels, Tamara[1], Benedetti, Sara[1], Hoshiya, Hidetoshi[1], Kazuki, Yasuhiro[2], Ragazzi, Martina[1], Gerli, Matti F.M.[1], Maffioletti, Sara M.[1], Messina, Graziella[3], Oshimura, Mitsuo[2], Cossu, Giulio[4], Tedesco, Francesco Saverio[1]
[1]Department of Cell and Developmental Biology, University College London, London, United Kingdom, [2]Department of Biomedical Science, Tottori University, Yonago, Japan, [3]Department of BioSciences, University of Milan, Milan, Italy, [4]Institute of Inflammation and Repair, University of Manchester, Manchester, United Kingdom

The limited availability of patient-specific, transplantable progenitors and the large size of the dystrophin gene represent significant obstacles in the development of therapies for incurable muscle disorders, such as Duchenne muscular dystrophy (DMD). The use of expandable, patient-specific progenitor cells and human artificial chromosomes (HACs) encoding the entire dystrophin locus (DYS-HAC), help in overcoming these two obstacles. Previous data from our laboratory showed amelioration in the dystrophic phenotype of mice transplanted with murine, DYS-HAC-corrected, vessel-associated mesoangioblast stem cells. Nevertheless, translation of this strategy to human myogenic progenitors requires the presence of cells with a remarkable proliferative capacity, able to withstand clonal expansion into clinically-relevant cell numbers (i.e. billions/patient) following HAC transfer. This could be particularly challenging when using autologous muscle-derived stem cells, whose pool is susceptible to exhaustion following the degeneration-regeneration cycles of a dystrophic muscle. Here we describe two complementary strategies for the generation of DYS-HAC-corrected mesoangioblasts from DMD patients: one directly from skeletal muscle biopsies and the other from autologous iPS cells. In the case of adult muscle derived-mesoangioblasts, reversible immortalization using lentivirally-delivered floxed human telomerase and Bmi1 cDNAs allowed bypassing of replicative senescence and permitted transfer of DYS-HAC. Four non-transformed and non-tumorigenic clones were generated, showing a stable karyotype and myogenic differentiation *in vitro* and *in vivo*. In the event that isolation of muscle-derived mesoangioblasts proves challenging, patient's fibroblasts or myoblasts can be reprogrammed to pluripotency and differentiated towards the perivascular/mesoangioblast lineage, as we have recently shown for Limb-Girdle 2D and Duchenne muscular dystrophies. Notably, cells were genetically corrected with lentivectors or HACs in both strategies. However, the above-mentioned reprogramming and differentiation steps still rely on the use of integrating viral vectors, which harbor a potential risk of insertional mutagenesis and cell transformation. Consequently, we have focused on and expanded a culture of DMD-iPSCs containing a deletion of exons 4 to 43 in the dystrophin gene, reprogrammed using non-integrating Sendai viruses and corrected with the DYS-HAC. We are now differentiating those cells towards the muscle lineage in order to then induce terminal differentiation with mRNAs for the myogenesis regulator MyoD. Alternatively, a novel DYS-HAC containing an inducible MyoD cDNA has been engineered and will be transferred into progenitors derived from the above DMD-iPSCs. If successful, this second strategy will not only be integration-free in its reprogramming, genetic-correction and differentiation steps, but it will equally generate cells ready for transplantation into pre-clinical mouse models of muscular dystrophy. Overall, this project may pave the way for the generation of a future autologous clinical-grade strategy for ex vivo gene and cell therapy of DMD based upon HACs.

T-2160
OVERCOMING INTRINSIC CELL LINE DIFFERENCES TO EFFICIENTLY DIRECT HUMAN PLURIPOTENT CELLS TO OLIGODENDROCYTES

Chen, Christine[1], Chu, Vi T.[2]
[1]Stem Cell Group, Bioscience Division, EMD Millipore, Temecula, CA, USA, [2]Millipore Corporation, Temecula, CA, USA

Protocols to derive oligodendrocyte precursor cells from human pluripotent cells vary widely in the reported efficiency of oligodendrocytes. Many of these protocols utilize a common set of extrinsic factors that are known to be important in oligodendrocyte development; these include sonic hedgehog, retinoic acid, PDGF and thyroid hormone. The role of intrinsic cell line differences has been less well understood and this may account for the wide variations in differentiation efficiency observed. To identify conditions that would override intrinsic cell line lineage propensity and specify cells toward an oligodendrocyte lineage, the effects on timing, duration, concentrations and combinations of extrinsic factors were determined in three pluripotent cell lines, two of which possessed known lineage propensity. H9 and H7 human ES cell lines are widely known to have neuroectodermal and mesodermal potential, respectively. A third human iPS cell line was also evaluated. To test the effects of extrinsic factors on OPC development, we modified a neural induction platform called NIM that was previously established to facilitate a pan-neuronal commitment of different pluripotent cells, irrespective of innate propensity. The commitment to neuroectodermal fate ($OCT4^{-}$ $PAX6^{+}SOX1^{+}$) occurs 72 hour post NIM induction. Exposure of cells to sonic hedgehog before the establishment of neuroectodermal patterning did not promote the expression of NKX2.2 and OLIG2, but rather directed cells towards a neuronal lineage. In addition, we found that addition of retinoic acid (RA) at specific timepoints, either earlier or later in the neural specification process exerted profound and opposing effects on oligodendrocyte lineage specification. Early application of RA gave rise to high PAX6 and low OLIG2 and NKX2.2 expressions, whereas later application of RA promoted high OLIG2 and NKX2.2 expressions. Insights into the effects of extrinsic factors at specific induction points is an important step towards establishing relevant cell models in patient specific iPS cells for diseases in which olidodendrocytes have been implicated.

T-2161
CHARACTERIZATION OF LONG NON-CODING RNAS INDUCED DURING CORTICAL NEURON DIFFERENTIATION FROM PRIMATE PLURIPOTENT STEM CELLS

Field, Andrew[1], Jacobs, Frank[2], Harte, Rachel[1], Ewing, Adam[1], Rosenkrantz, Jimi[1], Katzman, Sol[1], Salama, Sofie[3], Haussler, David H.[4]
[1]University of California, Santa Cruz, Santa Cruz, CA, USA, [2]UCSC Baskin School of Engineering, Santa Cruz, CA, USA, [3]University of California Santa Cruz, Santa Cruz, CA, USA, [4]HHMI - UC Santa Cruz, Santa Cruz, CA, USA

The cerebral cortex has undergone rapid changes in size and complexity in the primate lineage, yet the molecular processes underlying primate brain development are poorly understood. We have developed a common protocol for cortical neuron generation from human, chimpanzee and rhesus pluripotent stem cells that recapitulates early events in cortical development and enables us to do a comparative molecular analysis of this process. Here we focus on long non-coding RNAs (lncRNAs), which as a class have been implicated in gene regulation, differentiation of pluripotent cells into specific tissues, and, in some cases, are known to have a role in the fine-tuning of developmental processes. Despite their potential importance in driving the development of tissues, studies focusing on lncRNAs have been impeded by the low sequence conservation and extremely tissue-specific expression patterns of functionally relevant lncRNAs. For this reason, we developed a new approach focusing on both the sequence and expression conservation of lncRNAs in equivalent tissues among closely related primate species. Using our protocol for cortical neuron differentiation from human, chimpanzee, and rhesus pluripotent stem cells to model early brain development, we collected RNA for high throughput total transcriptome sequencing at regular timepoints during the differentiation protocol and identified over a thousand lncRNAs induced during cortical neuron generation in each species. Out of 2,684 putative lncRNA loci identified in our human samples, 2,357 (88%) share at least 50% sequence identity to loci in rhesus. 1,343 (57%) of these loci are expressed during rhesus neural differentiation. Based on preliminary datasets for only two of six neural differentiation time points, we find that chimpanzee neurospheres express 54% of the lncRNA loci detected in human. More are expected to be detected once the additional time points are added. In contrast, only 849 (32%) human loci share at least 50% sequence identity to regions in the mouse genome and, presumably, many less would be expressed in similar ES-derived mouse cortical neurospheres. Overall, both sequence and expression of cortical neuron associated lncRNA transcripts are more conserved over the primate lineage than over mammals as a whole, which may indicate that these lncRNAs contribute to primate-specific phenotypes. Identification of lncRNAs expressed during cortical development is a first step towards functional studies that will evaluate their importance during neurogenesis, provide insight to primate-specific and human-specific features of cortical development, and may also give insight to the role of many disparate genetic lesions that contribute to human neurological diseases.

T-2162
EFFICIENT ENDOTHELIAL DIFFERENTIATION OF URINE-HIPSCS GENERATED BY EPISOMAL VECTORS OR SENDAI VIRUS

Freund, Christian[1], Orlova, Valeria[1], van den Hil, Francijna[1], Maas, Saskia[1], Salvatori, Daniela[2], Mummery, Christine L.[1]
[1]Anatomy and Embryology, Leiden University Medical Centre, Leiden, Netherlands, [2]Central Animal Facility, Leiden University Medical Centre, Leiden, Netherlands

Human induced pluripotent stem cells (hiPSCs) are a valuable tool to study disease mechanisms in vitro and for the screening of compounds which could serve as novel drugs. A prerequisite is their derivation from easily accessible somatic tissue with non-integrating vectors and their efficient differentiation under defined conditions. Here we generated hiPSCs from two urine samples by episomal vectors (OCT3/4, SOX2, KLF4, L-MYC, LIN28, p53 suppression) or sendai virus (SeV; OCT3/4, SOX2, KLF4, c-MYC). hiPSCs were either generated in the presence of mouse embryonic fibroblasts (MEFs) or under defined conditions using E7 media. After picking hiPSC colonies were expanded and maintained on matrigel/mTESR1 or vitronectin/E8 media, respectively. hiPSCs expressed typical markers of pluripotent stem cells (OCT3/4, Nanog, TRA-1-81) and differentiated into derivatives of the three germ layers in the presence of fetal calf serum as assessed by immunfluorescent staining (ectoderm: βIII-tubulin, endoderm: α-fetoprotein, mesoderm: CD31 (PECAM)). For directed endothelial differentiation hiPSCs were cultured in BPEL media in the presence of BMP4, Activin A, VEGF and CHIR99021 for three days and in BPEL containing VEGF and SB431542 for another seven days as described previously by our group. The percentage of endothelial cells (ECs) expressing CD31 was determined by FACS: TESR1 and E8 hiPSCs cultures both gave rise to

~ 20±5% ECs. This is in line with our previous findings from hiPSCs generated from skin fibroblasts or blood outgrowth endothelial cells. ECs were enriched by magnetic bead purification using CD31-labeled beads. Purified ECs expressed VE-cadherin and VWF as assessed by immunofluorescent staining. Experiments to test purified ECs in vivo are ongoing. In conclusion we show here that urine cells can be reprogrammed with non-integrative vectors in the absence of MEFs and that the resulting hiPSCs efficiently differentiate into ECs under defined conditions.

T-2163
ROLE OF LYSOPHOSPHATIDIC ACID IN EMBRYONIC DEVELOPMENT OF ZEBRAFISH AND NEURAL STEM/ PROGENITOR CELLS

Frisca, Frisca[1], Kaslin, Jan[2], Davidson, Kathryn C[1], Goldshmit, Yona[2], Vandestadt, Celia[2], Pebay, Alice[1]
[1]Department of Ophthalmology, Centre for Eye Research Australia, University of Melbourne, Melbourne, Australia, [2]Australian Regenerative Medicine Institute, Monash University, Clayton, Melbourne, Australia

Lysophosphatidic acid (LPA) is a signaling phospholipid that induces pleiotropic effects in many cell types, mainly through binding its specific G-protein coupled receptors. Here, we assessed the role of LPA in embryonic development and neural stem /progenitor cell (NS/PC) maintenance/survival using *in vivo* and *in vitro* approaches. To study the role of LPA in embryonic development *in vivo*, we use zebrafish (ZF) as a model organism. Gain of function of LPA signaling induces significant embryonic phenotypes. We observed embryonic axis mesoderm defects in a dose-dependent manner following gain of function of LPA signaling which is accompanied by mis-expression of asymmetric nodal-related genes in the lateral plate mesoderm. Blocking $lpa_{1\text{-}3}$ receptors using the antagonist Ki16425 rescues the phenotype, indicating gain of function of LPA signaling induces this phenotype at least through $lpa_{1\text{-}3}$. This is further supported by the expression profiles of lpa receptor ($lpa_{1\text{-}3}$) observed in the early axis mesoderm formation during early embryonic development. Taken together, our data suggests that LPA signaling regulates axis mesoderm formation and establishment of left/right asymmetry in the early embryo. To further dissect and verify the signalling mechanism observed in the zebrafish following gain of function of lpa signalling, we use human pluripotent stem cell-derived NS/PCs, having previously established this cellular system as an accessible *in vitro* tool to study phospholipid signaling. We previously found that LPA functionally regulates the survival and maintenance of NS/PCs by inducing apoptosis and inhibiting neurosphere formation. Using this system, we stimulate LPA signaling in the maintenance of NS/PCs to examine the impact on various signalling pathways, such as Rho, Akt, and Erk pathways; considering the importance of these pathways in the embryogenesis of zebrafish as well as LPA signaling. We found that LPA on NS/PCs stimulates activation of Rho signalling but not activation (phosphorylation) of Akt and Erk. Inhibiting neurosphere formation by using the antagonist of the relevant pathway did not rescue the LPA effect in inhibiting neurosphere formation except Rho inhibitor. This data will be incorporated in the *in vivo* study to further dissect the possible mechanism underlying the gain of function of LPA signalling on the axis mesoderm formation of early zebrafish embryo.

T-2164
DNA-METHYLATION PATTERNS FOR TISSUE-TYPE AND AGING ARE ERASED IN MESENCHYMAL STROMAL CELLS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS.

Frobel, Joana[1], Hemeda, Hatim[1], Lenz, Michael[2], Denecke, Bernd[3], Saric, Tomo[4], Zenke, Martin[5], Wagner, Wolfgang[1]
[1]Helmholtz-Institute for Biomedical Engineering; Stem Cell Biology and Cellular Engineering, RWTH Aachen University Medical School, Aachen, Germany, [2]Aachen Institute for Advanced Study in Computational Engineering Science (AICES), RWTH Aachen University Medical School, Aachen, Germany, [3]Interdisciplinary Center for Clinical Research (IZKF), RWTH Aachen University Medical School, Aachen, Germany, [4]Institute for Neurophysiology, University of Cologne, Cologne, Germany, [5]Institute for Biomedical Engineering - Cell Biology, RWTH Aachen University Medical School, Aachen, Germany

Human mesenchymal stromal cells (MSCs) are heterogeneous cell preparations that lose differentiation potential during culture expansion. Standardization is hampered by variation in starting material and cell culture methods which renders comparison between studies difficult. In contrast, induced pluripotent stem cells (iPSCs) assimilate towards a ground-state. Therefore, generation of MSCs from iPSCs holds the perspective to provide more standardized and homogeneous MSC preparations. We have recently generated iPSCs from bone marrow MSCs. Here, we describe that these MSC-derived iPSCs can be re-differentiated into cells which closely resemble MSCs on functional level. The protocol involves the same culture conditions as used for initial MSC culture using human platelet lysate (hPL) as serum supplement. Within two weeks, iPSC-derived MSCs (iPS-MSCs) down-regulated pluripotency markers and displayed a typical fibroblast-like morphology. The immunophenotype of iPS-MSCs was the same as in primary MSCs ($CD14^-$, $CD29^+$, $CD31^-$, $CD34^-$, $CD45^-$, $CD73^+$, and $CD90^+$) - only CD105 was slightly less expressed in iPS-MSCs. Furthermore, iPS-MSCs were induced towards adipogenic, osteogenic, and chondrogenic lineages. Their differentiation potential was similar to primary MSCs, albeit adipogenic differentiation was less pronounced. Genome wide gene expression profiles supported the notion that iPS-MSCs closely resemble primary MSCs: marker genes for mesodermal lineage and MSCs were expressed at similar levels in iPS-MSCs and primary MSCs. However, DNA methylation (DNAm) profiles - analyzed by Infinium HumanMethylation450 BeadChip technology - revealed marked differences between iPS-MSCs and primary MSCs. Donor-specific differences in DNAm profiles were maintained throughout reprogramming and re-differentiation. However, characteristic DNAm patterns for bone marrow derived MSCs as compared to adipose tissue derived MSCs were not recapitulated in iPS-MSCs. Furthermore, age-related DNAm was reset in iPSCs and not reacquired in iPS-MSCs, indicating that the cells remain epigenetically rejuvenated. Senescence-associated DNAm changes - which accumulate during *in vitro* culture expansion of primary cells - were also reset by reprogramming into iPSCs but these changes were continuously adopted according to the number of population doublings upon loss of pluripotency. Overall, iPS-MSCs and MSCs are similar in function but iPS-MSCs reveal incomplete reacquisition of MSC-specific DNAm patterns - particularly of DNAm patterns associated with tissue type and aging. Our results indicate that iPS-MSCs provide an attractive and unlimited source of MSCs for standardized and rejuvenated preparations in regenerative medicine.

T-2165
CHARACTERIZATION OF DOPAMINERGIC NEURONS DIFFERENTIATED FROM PARKINSON'S DISEASE PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

Gill, Lisa Marie[1], Bratt-Leal, Andres[1], Slavin, Ileana[1], Gould, Sherrie[2], Peterson, Suzanne[1], Szücs, Attila[3], Sanna, Pietro[1], Houser, Meliisa[2], Loring, Jeanne F.[1]

[1]The Scripps Research Institute, La Jolla, CA, USA, [2]Scripps Clinic, La Jolla, CA, USA, [3]UCSD, La Jolla, CA, USA

Parkinson's disease (PD) is a degenerative disorder system resulting in severe motor deficits caused by the death of dopamine-generating cells in the region of the midbrain called the substantia nigra. Unfortunately, current pharmaceutical treatments do not reverse the loss of dopaminergic (DA) neurons and eventually become ineffective at treating the symptoms. With the development of induced pluripotent stem cell (iPSC) technology, it is now possible to generate pluripotent stem cells from the skin of a patient. Stem cell transplants for Parkinson's disease are currently being investigated in preclinical studies and may hold the key to treatment of this intractable disease. For our study, iPSCs were generated from the skin of nine Parkinson's disease patients under IRB approval with the goal of generating preclinical data in support of an autologous cell therapy trial. Pluripotency was verified through expression of pluripotent markers and through differentiation to the three germ layers in vitro. Patient iPSCs were used to generate DA neurons by a directed differentiation, floor plate precursor-based protocol and analyzed for expression of a panel of neural markers using qPCR. In differentiated neuronal cultures, we assessed expression of PITX3, TH, DAT, SERT, GAD1, PAX6, GIRK2, FOXA2, LMX1A and VMAT using qPCR. We were successful in developing DA neurons from multiple lines, but the efficiencies varied among patient-specific lines and we are modifying protocols to obtain the best results from each cell line. We also performed electrophysiological analysis on maturing neurons using a whole cell configuration patch-clamp technique. Synchronous firing activity in presynaptic neurons was evident by large magnitude excitatory postsynaptic currents (EPSCs),indicating that connectivity can be established within neuronal networks differentiated from iPSCs.

T-2166
DERIVATION OF PARATHYROID CELLS FROM HUMAN IPS CELLS

Esmaeili Shandiz, Alaleh[1], Suzuki, Shingo[1], Lee, Albert[1], Sargent, R Geoffrey[1], Yezzi, Michael J.[1], Orloff, Lisa A.[2], **Gruenert, Dieter C.**[2]

[1]Otolaryngology-Head and Neck Surgery, University of California, San Francisco, San Francisco, CA, USA, [2]Broad Center for Regenerative Medicine and Stem Cell Research, University of California, San Francisco, San Francisco, CA, USA

Parathyroid endocrine function can be compromised or lost as a result of disease, surgery or radiation and can lead a parathyroid hormone (PTH) imbalance that results in hypoparathyroidism (HPT) and leads to a breakdown in calcium homeostasis, seizures, bronchospasm, laryngospasm, bone mineralization, gastrointestinal dysfunction, cardiovascular disease, neurological dysfunction and/or cardiac rhythm disturbances. At present, HPT is only treatable through aggressive pharmacotherapy or through parathyroid gland (PG) rescue and transplantation. The prevalence of HPT in the US is about 105 individuals and therefore poses a significant societal health and economic burden. The development of induced pluripotent stem (iPS) cells has provided a new paradigm for repairing damaged tissues and organs with a patient's own cells, thereby circumventing the limitations of immune-rejection. The iPS cells used for this study were derived from functionally committed somatic cells. These iPS cells were exposed to culture conditions in vitro that promote endodermal differentiation and progression into cells that express features consistent with parathyroid progenitors and mature parathyroid. Cells were monitored throughout the differentiation protocol for the expression of genes characteristic of various embryonic endodermal developmental stages and of parathyroid origin and/or function. Several protocols were compared and for their ability to promote differentiation into cells that expressed parathyroid-specific glial cell missing2/chorion-specific transcription factorB (GCM2/GCMB) as well as PTH and the calcium sensing receptor (CaSR). One protocol that involved a late-stage exposure to Activin A in the presence of sonic hedgehog (SHH) was identified as the most effective at generating cells expressing properties of cells found in parathyroid glands. In this protocol, activin A was added after the progressive expression of pharyngeal pouch transcription factor marker genes (SIX1, HOXA3, EYA1, PAX1/PAX9 and GCM2/GCMB) and a decrease in airway and thymus progenitor markers, NKX2.1 and FOXN1, respectively. Primary parathyroid cell culture isolates were used as reference standards for cell and organ specific morphology and gene expression.

T-2167
INVOLVEMENT OF 5-HYDROXYTRYPTAMINE RECEPTORS OR CANNABINOID RECEPTORS ON DIFFERENTIATION OF MOUSE INDUCED PLURIPOTENT STEM CELLS INTO NEURAL PROGENITOR CELLS

Ishizuka, Toshiaki, Ozawa, Ayako, Arata, Munemitsu, Watanabe, Yasuhiro

National Defense Medical College, Tokorozawa, Japan

[Introduction] It was reported that 5-hydroxytryptamine (5-HT) promotes differentiation of neural progenitor cells (NPCs). On the other hand, it was shown that cannabinoid (CB) receptors may play a role in NPC proliferation. The present study examined whether stimulation with 5-HT receptors or CB receptors affects differentiation of mouse iPS cells into NPCs. [Materials and Methods] Mouse iPS cell differentiation was initiated by embryoid body (EB) formation. All-trans retinoic acid (ATRA; 1 μM), 5-HT, HU210 (a CB_1 receptor agonist), or HU308 (a CB_2 receptor) was added to the EB cultures for 4 days, and then EBs plated on gelatin-coated plates were cultured for 7 days. The differentiation potential from mouse iPS cells into NPCs was evaluated by Nestin expression using western blot analysis. [Results] Treatment with 5-HT (0.03 μM) significantly enhanced ATRA-induced Nestin expression and phosphorylation of cyclic AMP response element binding protein (CREB). Pretreatment with either GR113808 (a selective 5-HT_4 receptor antagonist) or H89 (a protein kinase A inhibitor significantly inhibited these effects of 5-HT. On the other hand, the treatment with either HU210 (3 nM) or HU308 (3 nM) inhibited ATRA-induced Nestin expression. But, neither HU210 nor HU308 affected the phosphorylation of CREB. Pretreatment with rimonabant (a CB_1 receptor antagonist) blocked the inhibitory effect of HU210. In addition, pretreatment with SR144528 (a CB_2 receptor antagonist) blocked the inhibitory effect of HU308. [Conclusion] While the stimulation with 5-HT_4 receptors may enhance the differentiation of mouse iPS cells into NPCs, the stimulation with CB_1 or CB_2 receptors may inhibit the neural differentiation.

T-2168

OPTIMIZING AND AUTOMATING DIRECTED DIFFERENTIATION AND ISOLATION OF BASAL FOREBRAIN CHOLINERGIC NEURONS FROM IPSCS

Jacob, Samson[1], Sproul, Andrew[1], Ortiz-Virumbrales, Maitane[2], Paull, Daniel J.[1], Ehrlich, Michelle[2], Gandy, Sam[2], Noggle, Scott[1]
[1]The New York Stem Cell Foundation, New York, NY, USA, [2]Departments of Neurology and Psychiatry and the Alzheimer's Disease Research Center, Icahn School of Medicine at Mount Sinai, New York, NY, USA

Basal forebrain cholinergic neurons (BFCNs) are a major class of vulnerable neurons susceptible to cell death in Alzheimer's disease (AD). A highly efficient protocol that can be adapted for large scale BFCN production would therefore be a valuable tool for AD mechanistic studies and therapeutic screening. Here, we describe an optimized method of differentiating iPSCs, using defined culture conditions and Magnetic Activated Cell Sorting (MACS), for isolation and propagation of BFCNs. We are currently adapting this protocol to an automated platform. Using distinct cell surface markers, our method helps separate neural crest cells of the peripheral nervous system that share similar marker expression from our targeted BFCN population, while positively selecting for cholinergic progenitors. By using this selection strategy we are able to enrich forebrain progenitor cells that can be maintained as 3D neuronal embryoid bodies (NEBs). Sorted NEBs can be propagated for long periods of time, at least 100 days in culture, while retaining their ventralized forebrain identity. NEBs can subsequently be dissociated into monolayer cultures and magnetically selected for mature neuronal markers for terminally-differentiated BFCNs. Ultimately our method will provide an automated pipeline for assays requiring large amount of specific neuronal cultures, such as therapeutic screening targeting acetylcholine producing cells.

IPS CELLS

T-2171

AN EFFICIENT FEEDER-FREE CULTURE CONDITION FOR THE GENERATION AND EXPANSION OF XENO-FREE HUMAN IPS CELLS

Hwang, Dong-Youn
CHA University School of Medicine, Seongnam, Republic of Korea

Clinical applications using induced pluripotent stem cells (iPSCs) are expected to become a realistic option for treating a variety of incurable diseases in the near future. Roadblocks to be removed before therapeutic applications of iPSCs are how to avoid the risk of xenopathogenic transmission and immune rejection caused by contaminated animal antigens. In addition, an efficient feeder-free culture condition would be of great value by reducing batch-to-batch variation, allowing standardization of culture conditions, facilitating scale-up, and saving time and effort. In this study, using signaling regulatory molecules that are potentially implicated in the maintenance of the stemness of hESCs, we established an efficient xeno-free and feeder-free condition for the long-term culture (>100 passages) of human pluripotent stem cells (hPSCs). Furthermore, we generated xeno-free iPSCs using the same culture condition. In summary, this xeno-free and feeder-free culture system would facilitate the safe clinical applications of both iPSC and ESC-based cell therapies in the near future. This work was supported by grants from the Stem Cell Research Program (2010-0020347) and 2012M3A9C7050130 from the MSIP, and A120254-1201-0000200 from the Ministry of Health and Welfare, Korea

T-2172

EFFECT OF HYDROGEL STIFFNESS ON GENERATION OF INDUCED PLURIPOTENT STEM CELLS

Park, Kwang-Sook, Kim, Jin-Su, Arai, Yoshie, Moon, Bo Kyung, Lee, Soo-Hong
CHA University, Seongnam-si, Republic of Korea

Cellular microenvironment is known to regulate their normal phenotypes through the physical and chemical interaction. When the microenvironment is physically or chemically changed due to external stimuli or diseases, cells in it happen to show the abnormal phenotype. Some researchers have tried to control the cell phenotype using physically or chemically modification of the microenvironment. They have modified the matrix protein composition, topography, and/or stiffness. In this work, we hypothesize that the surface stiffness may induce the abnormal phenotype which is able to increase the cell reprogramming. To control the surface stiffness, this work employed polyacrylamide hydrogels with various concentrations of acrylamide and bis-acrylamide. And then we observed the effect of surface stiffness on cell morphology and gene expression related to generation of induced pluripotent stem cells (iPSCs). Compared to stiff hydrogels, it was found that soft hydrogels induced the round shape while stiff hydrogels did the widely spread shape. In addition, soft hydrogels induce the higher gene expression of epithelial markers such as E-cadherin followed by increase in the generation of Oct4-GFP colonies.

T-2173

ROBUST GENERATION AND MAINTENANCE OF HUMAN INDUCED PLURIPOTENT STEM CELLS UNDER DEFINED AND XENO-FREE CONDITIONS

Ahmadian Baghbaderani, Behnam, Tian, Xinghui, Friedrich Ben-Nun, Inbar, Nie, Ying, Walsh, Patrick, Cadet, Jean, Burkall, Amy, Tran, Huan, Walde, Amy, Shah, Kevan, Menendez, Laura, Carpenito, Carmine, Neo, Boon Hwa, Keefe, Robert, Fellner, Thomas
Lonza Walkersville, Inc., 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) and by definition have the ability to indefinitely self-renew and become any cell type in the body. Because of these attributes, hiPSCs have become an important scientific tool and are spurring advancements in basic research, disease modeling, drug development, and regenerative medicine. However, the process to generate hiPSCs is inefficient and technically challenging, even when utilizing more efficient viral methods and mouse embryonic fibroblast feeder layers. Efficiencies fall even further when generating iPSCs using non-integrating technologies under defined conditions. These realities lead Lonza to focus on developing a robust system that could eventually be translated to GMP and clinical grade manufacturing. The resulting system, named L7 hPSC Reprogramming and Culture System, is comprised of a culture platform (medium, matrix, subculture reagent, and cryopreservation medium) that supports every-other-day feeding of human PSCs. In combination with an enhanced episomal reprogramming method, the resulting hiPSCs derived from blood cells 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 have lost the episomal plasmids. To further test the system, we expanded multiple hESC and hiPSC lines 40+ passages. In each case, the lines maintained high expression of pluripotency markers and a normal karyotype.

T-2174
CRYODEFENDING YOUR STEM CELLS

Aho, Joy L.[1], Herr, Greg[1], Tang, Yixin[2], Cross, Rosie[1], Andersen, Marnelle[1], Johnson, Wade[1], Owens, D. Jason[1], Tousey, Susan[1]
[1]*Stem Cells, R & D Systems, Minneapolis, MN, USA,* [2]*Antibody Development, R & D Systems, Minneapolis, MN, USA*

Efficient cryopreservation of stem cells is essential for the maintenance of consistent stem cell stocks. Many of the existing formulations of cryopreservation media rely on high percentages of poorly defined serum or albumin. Here, we demonstrate the use of CryoDefend™-Stem Cells media for defined, xeno-free, and protein-free cryopreservation of pluripotent, mesenchymal, and neural stem cells. Following cryopreservation in CryoDefend-Stem Cells media, viability was assessed post-thaw. Cells were then characterized by verification of stem cell marker expression via immunocytochemistry and flow cytometry as well as functional verification via *in vitro* directed differentiation. CryoDefend-Stem Cells media was found to have superior or equal performance to both traditional serum/albumin-containing cryopreservation media and existing commercially available defined media.

T-2175
LINEAGE CONVERSION STEP IN IPS CELL GENERATION INVOLVES HIGHLY MUTAGENIC PROCESS

Araki, Ryoko, Fujimori, Yuko, Sunayama, Misato, Kasama, Yasuji, Uda, Masahiro, Nakamura, Miki, Abe, Masumi
National Institute of Radiological Sciences, Chiba, Japan

The iPSC system holds great promise for regenerative medicine but its underlying molecular mechanisms have remained elusive. Major concern is its genome integrity and a large number of point mutations have been indeed identified in their genomes. Recently, we revealed that substantial numbers of point mutations arise during iPSC generation process, indicating that most SNVs identified in iPSC genome are iPSC generation-associated point mutations and not pre-existing SNVs. In our study, we performed allele frequency test on SNVs identified in iPSC genome and found that a significant portion of them exhibits less than 50% allele frequency, 25% and 12.5%. This means the presence of heterogeneity of point mutation profiles within an iPSC clone, indicating that point mutations occur after the onset of cell lineage conversion from somatic cell to pluripotent stem cell. We confirmed the fact using sub-lines that were established from single cells within an iPSC clone. Furthermore, the history of the emergence of each mutation was revealed through the comparison among sub-lines. Collectively, our observation implied that previous studies with whole genome sequencing (WGS) must underestimate the point mutation load in iPSCs. Here we performed WGS of the sub-lines to identify all point mutations including rare-frequent SNVs in iPSC genomes and to know when such mutations arise in the genome. In addition, to evaluate the method dependency, sub-lines were established from genome integration-free or retrovirus vector mediated iPSC clones. As a result, we identified point mutations in sub-lines nearly twice what observed in parent iPSC clones: ~500 for sub-lines and ~250 for their parent iPSC clones in integration-free iPSC system and ~ 1,000 for sub-lines and ~500 for their parent iPSC clones in retro virus vector mediated system. Thus conservative estimations on original iPSC have missed half of point mutations. More importantly, clear difference in total number and in ratio between 50%SNVs and les than 50%SNVs was revealed between integration-free and retro viral system. Furthermore, we also found a significant number of point mutations that were not observed in parent iPSC clone in WGS at all, indicating a presence of extremely low frequency SNVs caused in iPSC genomes. Therefore, in order to investigate the timing of the emergence of these novel point mutations, we performed amplicon sequencing for ~40 of SNV candidates and determined the allele frequency in parent iPSC clones precisely. Together, our results clearly shows that the lineage conversion in iPSC generation involves a highly mutagenic process and the process is sharply restricted to just after the onset of cell lineage conversion.

T-2176
THE ROLE OF MIR-199A-5P IN THE GENERATION OF INDUCED PLURIPOTENT STEM CELLS

Beh-Pajooh, Abbas, Cantz, Tobias, Eggenschwiler, Reto, Schambach, Axel
Hannover Medical School, Hannover, Germany

Introduction: Generating induced pluripotent stem cells (iPSCs) is a useful method to avoid destroying blastocysts to obtain their inner cell mass as an unlimited resource for pluripotent stem cells as a model tool. One of the promising methods to control reprogramming is to overexperss or inhibit certain micro RNAs. One promising candidate for investigating reprogramming is micro RNA-199a-5p, which was studied in the context of hepatic differentiation of pluripotent stem cells. It was previously shown that inhibition of microRNA-199a-5p enhances the differentiation of embryonic stem cell towards hepatic-like cells. We investigated the effect of miR-199a-5p during nuclear reprogramming for iPSC generation in mouse OG2 MEF. We demonstrated that inhibition of miR-199a-5p enhances the reprogramming efficiency by increasing the number of GFP positive OG2 iPS colonies. Methods and Results: We transfected OG2 MEFs with different micro RNAs including scramble, miR-199a-5p and its inhibitor and after 72 hours isolated micro RNAs and made cDNAs by primers designed specifically for miR-199a-5p and analyzed the expression of these micro RNAs with qPCR. The analysis showed the miR-199a-5p is properly transfected and has an effect on the expression of this micro RNA. Then we cultured OG2 MEFs and transduced with OKS dtomato reprogramming lentiviral construct and 24 h later transfected with different micro RNAs of Scramble, mimic and inhibitor by means of lipofectamine and let them grow in the presence of LIF. The anlaysis of the number of appearing GFP positive iPS colonies on day 14 showed lowered number of GFP positive colonies in the mimic and significantly elevated numbers in the cells treated with the inhibitor $P<0.05$. The same experiment was carried out but the OG2 MEF cells were transfected in the late phase of reprogramming, on day10 but no significant difference was observed in the number of GFP positive iPS colonies. Conclusion: Thus in our experiment we demonstrated for the first time that miR-199a-5p blocks the reprogramming process in the early phase of reprogramming but not late phase. Therefore inhibiting miR-199a-5p in OG2 MEFs during reprogramming increases the efficiency of reprogramming by increasing the number of GFP positive colonies.

T-2177
INDUCED PLURIPOTENT STEM CELLS DERIVED-NEURONAL CELLS FOR CELL REPLACEMENT THERAPY IN HUNTINGTON'S DISEASE

Choompoo, Narawadee[1], Vinh, Ngoc-Nga[1], Kelly, Claire M.[2], Rosser, Anne E.[1]
[1]*BRG, School of Biosciences, Cardiff University, Cardiff, United Kingdom,* [2]*Cardiff Metropolitan University, Cardiff, United Kingdom*

Huntington's disease (HD) is a neurodegenerative disease caused by a mutation in the huntingtin gene (HTT). The extended CAG repeat ultimately leads to loss of medium spiny neurons (MSNs) in the

striatum of the HD brain. Cell replacement therapy using primary human fetal tissue has shown 'proof of principle' as a strategy to treat this genetically inherited disease[1]. However, alternative cell sources need to be identified to overcome the ethical and logistical issues that are associated with using human fetuses. IPS cells were first introduced by Yamanaka in 2006 by direct reprogramming of fibroblasts to ES-like cells by using four defined factors (Oct4, Sox2, Klf4, c-Myc)[2]. Here we generated iPS cells by introducing reprogramming factors using the *piggyBac* Transposon[3] transduction system to human fetal fibroblasts and fetal neural stem cells which theoretically have the potential to be more readily reprogrammed and are genetically unrelated to the host. The iPS cell lines so established demonstrated many similarities to human embryonic stem (ES) cells in terms of their morphology, surface antigens expression, and proliferation rates. These iPS cells were then successfully manipulated to differentiate into neurons in culture by which expressed standard markers of premature and mature MSNs, Ctip2 and Darrp32; a proportion of these cells exhibited the general neuronal electrical properties of spontaneous and inducible action potentials. *In vivo* differentiation of these iPS cells when transplanted into quinolinic acid (QA) lesioned rats showed good graft survival and differentiation into several neuronal sub-types, as assaessed by hunu and nestin expression.

T-2178
EFFICIENT DERIVATION AND EXPANSION OF HUMAN IPS CELL FROM ADULT BLOOD CELLS UNDER FEEDER-FREE AND XENO-FREE CULTURE CONDITIONS: A CGMP COMPLIANT APPROACH

Chou, Bin-Kuan, Gao, Yongxing, Cheng, Linzhao
Hematology, Johns Hopkins University, Baltimore, MD, USA

The robust technology of generating human induced pluripotent stem (iPS) cells from postnatal somatic cells provides unprecedented opportunities to study human diseases and to use one's own cells for autologous transplantation. We previously published a method to reprogram human blood cells by 1-2 non-integrating plasmids expressing a set of reprogramming factors after one round of DNA nucleofection (Chou et al., 2011; Dowey et al., 2012). These plasmids contain the OriP/EBNA1 replicon that enables episomal replication in human somatic cells and prolonged expression of 5-6 reprogramming genes. After successful reprogramming to iPS cells, however, transgenes including ENBA1 are silenced, resulting in the loss of OriP/EBNA1 plasmids in dividing human iPS cells. We noticed that the reprogramming efficiency of adult peripheral blood mononuclear cells (MNCs) is 20-50 fold lower that that of umbilical MNCs even when two plasmids are used. In addition, we observed great variations of the efficiency of generating iPS cells from different persons. To achieve a consistently high efficiency of reprogramming for any person's blood cells, ideally under a cGMP-compliant condition that allows future clinical use, we made the following 3 major improvements. 1) We added the third OriP/ENBA1 plasmid that expresses the BCL2L1 encoding BCL-XL, based on our previous results with the BCL2 gene (Chou et al., 2011) and a recent paper (Su et al., 2013). We found that the addition of this plasmid increased iPS efficiency from adult MNCs by ≥10-20 fold in more than 50 consecutive blood samples. 2) With the improved efficiency, we next explored if we can eliminate mouse embryonic fibroblast (MEF) feeder that are previously critical to the reprogramming of human adult blood cells. With the chemically defined E8 medium devoid of any animal proteins, we achieved efficient reprogramming of human blood MNCs on the matrix of either Matrigel or a human recombinant protein expressed in and purified from E. Coli. In fact, the efficiency under the MEF-free and xeno-free condition is at least two fold higher in a dozen of human blood samples we tested. 3) We further determine if we can eliminate the tedious and time-consuming step of hand picking dozens of iPS cell clones. After reprogramming of blood MNCs with the E8 medium, the pool of sizable iPS cell clones together with partially reprogrammed cells were plated on the freshly coated matrix of recombinant human protein and cultured with E8 medium as we previously published for undifferentiated iPS cells (Wang et al., 2012). Once enough cells were obtained, the fully reprogrammed and undifferentiated iPS cells were selected by a MACS column based on the presence of TRA-1-60 surface antigen. The purified cells (≥95% positive) from dozens or hundreds of primary iPS cell clones can be quickly expanded. The pooled iPS cells have reduced genotypic variations as compared to the starting somatic cell population, and maintain stable normal genotypes and pluripotency after many subsequent passages in E8 medium over a recombinant matrix protein. Our recent improvements permit a consistent and reliable method to generate human iPS cells from a large and diverse population, and to generate iPS cells efficiently and under a cGMP-compliant condition for future therapeutic applications.

T-2179
SUBCORTICAL WHITE MATTER REPAIR BY TRANSPLANTATION OF STEM CELLS

Cruz, Angelica[1], Llorente, Irene L.[2], Cinkornpumin, Jessica[3], Lowry, William E.[2], Carmichael, Stanley T.[2]
[1]California State University, Northridge, Northridge, CA, USA, [2]University of California, Los Angeles, Los Angeles, CA, USA, [3]University of California, Los Angeles, Long Beach, CA, USA

The white matter regions of the brain contain the connections that link brain areas. Stroke in the brain's white matter accounts for 30% of all stroke subtypes and is the second leading cause of dementia. There is no therapy to promote recovery in this disease. If white matter damage remains untreated, cognitive and motor capabilities decline. Stem cell transplantation offers the possibility of providing a cellular approach to repairing injured white matter. We tested the effects of transplanting two different types of stem/progenitor cells in a mouse model of white matter stroke. Fifteen days after iPSC-NPCs transplantation double cortin positive cells were present , but white matter atrophy was not restored. Transplantation of iPSC-GRPCs generated astrocyte activation over time within the white matter, increasing the Glial scar around the infarct area. Growth and accumulation of oligo2/gfp+ cells around the infarct area increased overtime in the white matter and in the cerebral cortex after iPSC-GRPC transplantation. Ongoing studies concerning tissue variables of cell death and behavioral recovery are being established.

T-2180
GENETIC CORRECTION OF INDUCED PLURIPOTENT STEM CELLS FROM A WISKOTT-ALDRICH SYNDROME PATIENT NORMALIZES THE IMMUNE DEFECTS

Davis, Brian R.[1], Laskowski, Tamara J.[1], Van Caeneghem, Yasmine[2], Pourebrahim, Rasoul[1], Li, Xuan Shirley[1], Liao, Wei[1], Garate, Zita[3], Crane, Ana M.[1], Segovia, Jose Carlos[3], Holmes, Michael C.[4], Ni, Zhenya[5], Kaufman, Dan S.[5], Vandekerckhove, Bart[2]

[1]Center for Stem Cell and Regenerative Medicine, University of Texas Health Science Center, Houston, TX, USA, [2]Laboratory for Experimental Immunology, Ghent University, Ghent, Belgium, [3]Differentiation and Cytometry Unit, Hematopoiesis and Gene Therapy Division, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas, Madrid, Spain, [4]Sangamo BioSciences Inc, Richmond, CA, USA, [5]Department of Medicine and Stem Cell Institute, University of Minnesota, Minneapolis, MN, USA

Wiskott-Aldrich syndrome (WAS) is an X-linked primary immunodeficiency disease characterized by thrombocytopenia, recurrent infections and increased autoimmunity. This disease is caused by mutations in the WAS gene (WAS) which encodes for the WAS protein (WASp), exclusively expressed in hematopoietic cells and required for proper platelet production and lymphoid cell function. Patients with WAS are treated with allogeneic stem cell transplantation or lentiviral hematopoietic stem cell gene therapy. We investigated restoration of T- and NK-cell functionality following a virus-free zinc-finger nuclease (ZFN)- mediated genome editing strategy for correction of WAS. We generated induced pluripotent stem cells (iPSC) from skin fibroblasts of a WAS patient carrying an insertional frame-shift mutation. Subsequently, a WAS-2A-eGFP transgene was targeted at the endogenous chromosomal location by homology-directed repair using ZFN, thereby correcting the gene defect and creating a GFP reporter for WASp expression. Hematopoietic progenitor cells were generated from WAS iPSC and gene-corrected iPSC (cWAS) *in vitro* via spin embryoid bodies. Human embryonic stem cell lines WA01 and WA09 were used as controls. GFP expression was pronounced in all CD43+ hematopoietic lineages including myeloid, monocytic, lymphoid, erythroid and megakaryocytic lineages. Hematopoietic precursors were further cultured on OP9-DL1 to generate T and NK cells. NK cells were readily obtained from cWAS and WA01/WA09 progenitors, but to a far more limited extent from WAS progenitors. WAS-derived NK cells were unable to generate interferon-γ or tumor necrosis factor-α upon stimulation with K562. Cytokine production was restored in cWAS- derived NK cells. Interestingly, in T cell generation, although WAS, cWAS, and WA01 lines were able to generate CD5+CD7+ T cell precursors, only low numbers of CD3+ TCRαβ and TCRγδ cells were obtained with WAS in comparison with WA01. Significantly, T cell generation was restored in cWAS. Taken together these results indicate that targeted endogenous integration of the WAS gene in WAS-iPSC results in restoration of the lymphoid defect observed in WAS-iPSC. Transplantation of gene-corrected iPSC-derived hematopoietic precursors may offer an alternative to lentiviral gene therapy which carries an inherent risk for insertional oncogenesis.

T-2181
IMMUNOGENICITY OF HUMAN PLURIPOTENT STEM CELLS; ALLOGENIC VS. AUTOLOGOUS IN VITRO MODEL

de Rham, Casimir[1], Mutel, Cécile[1], Ivanyuk, Dina[2], Tieng-Caulet, Vannary[3], Krause, Karl-Heinz[3], Saric, Tomo[2], Villard, Jean[1]

[1]Transplant Immunology Unit, University Hospital, Geneva, Switzerland, [2]Institute for Neurophysiology, University of Cologne, Cologne, Germany, [3]Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland

Human pluripotent stem cells, such as human embryonic stem (hES) cells or human induced pluripotent stem (hiPS) cells possess huge potentials in regenerative medicine, especially in tissues transplantation. The main obstacle in transplantation is the origin of the tissue. An immune response mediated by NK and T-cells will give rise between genetically unrelated donor and recipient. NK cells are of special interest because human pluripotent stem cells do not express MHC class I, making them excellent targets for NK cells. In this study, we first characterized the expression of specific ligand, expressed by hES and hiPS treated with or without IFN-g, involved in the immune reaction. The expression level of MHC class I, MHC class II, HLA-E, HLA-G, MIC-A/-B, ULBP-1,-2,-3, CD274 and the co-activators CD40, CD80 and CD86 were analyzed by FACS. Then we analyzed the functionality of NK and CD8+T cells (cytotoxic assay and degranulation assay) against allogenic hES and hiPS cells. Finally, we set up an autologous in vitro model of NK and CD8+T cells against hiPS. Fibroblasts were obtained from two healthy donors and derived into hiPS cells. Later, blood samples were taken from these two specific donors, where NK and CD8+T cells were isolated. With these cells, we were able to perform simultaneously the allogenic and the autologous cells co-culture.Phenotyping expressions of ligands by hES and hiPS cells show a large heterogeneity. The killing activity of T cells is reduced due to the low expression of MHC class I at the cell surface of pluripotent stem cells. In contrast the NK cells activity is stronger with pluripotent stem cells and their derivatives. In fact, each cell line should be deeply analyzed if a clinical application is envisaged.

T-2182
COMPLEX DISEASE MODELING USING HIPSC-DERIVED HUMAN NEURONS TO IDENTIFY FDA-APPROVED DRUGS THAT MODIFY COMPONENTS OF THE GSK3-BETA SIGNALING PATHWAY

Dorsett, Laurel M., Tobe, Brian, Crain, Andrew, Winquist, Alicia, Pernia, Cameron, Snyder, Evan Y.

Sanford-Burnham Medical Research Institute, La Jolla, CA, USA

Until recently, our mechanistic understanding and development of drug treatments for neuropsychiatric disorders has been limited due to difficulties in observing live human neurons. With advancements in neuronal differentiation and cellular reprogramming technologies, we are now able to investigate drug action in human neurons derived from embryonic and induced pluripotent stem cells including those from clinically affected patients. GSK3β and its substrates have been associated with neurodevelopment and neuropsychiatric conditions and may be modulated by neuropsychiatric drugs. The putative functions of the GSK3β substrate CRMP2 in neurological disorders has expanded in recent years as it associates with neuronal cytosketelal components and calcium channels and mediates dendrite specification and axonal growth. We have utilized human stem cell derived neurons to test the effects of neuropsychopharmacologic compounds including anti-seizure and psychiatric medications on GSK3β and CRMP2 post-translational modification. Interestingly, a small number of drugs previously considered to operate via divergent mechanisms

appear to converge upon these proteins suggesting that some of these medications may have potential to be repurposed for use in new clinical applications. In addition, our studies provide further support for the possibility that CRMP2 may be an attractive therapeutic target for future drug development.

T-2183

A NOVEL REPROGRAMMABLE MOUSE LINE WITH DUAL FLUORESCENCE, REPORTING THE EXPRESSION OF THE YAMANAKA FACTORS AND THE ACTIVATION OF OCT4

Elbaz, Judith, Puri, Mira C., Nachmani, Bar, Nagy, Andras
Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

Background: Mouse embryonic fibroblasts (MEFs) can be reprogrammed to pluripotent cells by the forced expression of the four Yamanaka factors; Oct4, Klf4, cMyc and Sox2 (OKMS). When these factors are introduced into MEFs by stably integrated transgenes, their expression is variable from cell to cell, due to the random genomic insertion sites and the number of transgenes landing in the genome. This heterogeneity contributes to the low efficiency of the reprogramming. Objective: We aimed at developing an efficient 2º reprogramming system equipped with fluorescent visualization of both the Yamanaka factors (mCherry) and the Oct4 pluripotency hallmark gene (GFP) expressions, allowing the live monitoring of the reprogramming process in vivo and in vitro. Methods: The piggyBac transposon containing the doxycycline (dox)-inducible promoter driving OKMS linked to mCherry reporter and transposon with a ubiquitously expressed rtTA transgene were transfected into MEFs carrying an Oct4promoter-GFP transgene. Colonies were picked and dox-independent primary (1º) iPSCs were obtained. Four clones (carrying one or two OKMS-mCherry insertions) were selected for diploid embryo <-> iPSC aggregation. Secondary MEFs were derived from the resulting embryos at E12.5. Results: The 2º MEFs were incubated in dox and flow cytometry analysis confirmed that each clone activated OKMS-mCherry. Soon after dox induction, mCherry positive colonies formed and GFP fluorescence was observed as early as day 8 of reprogramming. To assess pluripotency, dox was removed from day 10 of reprogramming. About 82% of the 25 clones were dox-independent at day 18 of reprogramming. The efficiency of reprogramming, which was calculated using serially diluted 2ºMEFs, varied between 3.3 and 11.3%. To assess the dynamics between silencing of OKMS exogenous expression and the endogenous expression of OCT4, cells cultured for 30 days in dox were sorted for GFP and mCherry and subsequently cultured with or without dox. Flow cytometry revealed that a small population of the mCherry+ cells survive when cultured without dox and successfully activate the endogenous OCT4. Interestingly, some of the GFP+ cells cultured in dox after the sorting reactivate the previously silenced OKMS transgene. In addition, tail tip fibroblasts, bone marrow and peripheral blood reprogrammed successfully from adult chimeras derived from all 1o iPSC clones, and dox-independent 2º iPSCs were obtained within 20 days. Finally, germ line transmission was obtained for one of the 1º iPSC lines and this founder generated a new reprogrammable mouse, which is currently being characterized. Conclusion: We successfully established an efficient secondary reprogramming system and a new reprogrammable mouse line with double fluorescent reporter. This novel system will enable us to uncover specific properties and molecular events underlying the generation of pluripotent cells from the soma.

T-2184

AN ELITE MODEL FOR REPROGRAMMING - NEURAL CREST STEM CELLS AS AN OPTIMAL SUBSTRATE FOR REPROGRAMMING

Fahmy, Ahmed, Brokhman, Irina, Xu, Jie, van der Kooy, Derek J.
[1]*Molecular Genetics, University of Toronto, Toronto, ON, Canada*

The discoveries made by Takahashi and Yamanaka in reprogramming somatic cells either to pluripotent stem cells (called induced pluripotent stem cells, or iPSCs) or directly to other differentiated cells has opened up a new field in stem cell research. However these reprogramming techniques are plagued by problems relating to the efficiency of the process and the poor understanding of their cellular mechanisms. Recent studies were able to drastically improve the efficiency of reprogramming by inhibiting Mbd3, a core member of the nucleosome remodelling and deacetylation repressor complex. Despite this there still are no clear and non-ambiguous studies which show if all differentiated cells have the potential of being reprogrammed or whether there exists an elite subpopulation of cells that are more selectively being reprogrammed. We hypothesise that a primary source of iPSCs and directly reprogrammed cells are neural crest stem cells (NCSCs) found in culture. It is well established that multipotent NCSCs migrate to many parts of the developing embryo where they can produce a vast array of cell types, and indeed some of these NCSCs remain as undifferentiated stem cells throughout life. We traced the lineage of neural crest cells in mice embryos using a Wnt1-Cre and ROSA-YFP reporter system. We assayed head and trunk embryonic skin fibroblast samples and found that YFP positive cells (NC derived) were present in the primary cell culture and they increased in proportion with passage number. This shows that there exists neural crest derived cells in cultures identical to those used in reprogramming studies. We reprogrammed skin samples of mouse embryonic fibroblasts (MEFs) and found that over 90% of the iPSCs produced were YFP positive (from NC origin). Despite the fact that 10% of iPSC colonies were produced from YFP negative cells, this may not suggest that some non-NC cells also are reprogrammed, as the efficiency of the cre labelling may not be 100%. Furthermore YFP negative cells from skin of the head (non-NC derived) were incapable of being reprogrammed to iPSCs, while YFP positive cells normally produced iPSCs suggesting that the only cells in MEF samples capable of producing iPSCs are of NC origin. Further experiments will look at which subpopulation of the YFP cells (neural crest precursors or post mitotic cells) are responsible for the production of iPSCs. These studies can help better understand the cellular mechanism of reprogramming and will help devise more efficient techniques of reprogramming.

T-2185

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM HUMAN UMBILICAL CORD VEIN MESENCHYMAL STEM CELLS

Fathi, Fardin, Abdi, Mehdad, Zare, Sona, Anjamroz, Seyyed Hadi, Rezaei, Mohammad Jafar, Rostamzadeh, Jalal, Rostamzadeh, Jalal
Cellular and Molecular Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran

The aim of this study is to generate induced pluripotent stem cells from human umbilical cord vein mesenchymal stem cells with plasmid vector. Mesenchymal stem cells in this research were extracted from the human umbilical cord vein wall with thpe IV collagenase enzyme and then were cultured. These cells were transfected with plasmid vector which carried selfrenewal transcription factors OCT4 and SOX2 with electroporation. After 9 days the induced pluripotent stem cells like colonies were observed. The nature of obtained cells were evaluated with Immionocytochemistry and Alkalin phosphatase

assessments to determine expression of embryonic stem cells markers. Immionocytochemical analysis showed that these cells express the pluripotency markers OCT4, SSEA4, TRA1-60, TRA1-81, and were also possitive for Alkalin Phosphatase enzyme. This study revealed that transient expression of self renewal genes OCT4 and SOX2 can result in induced pluripotent stem cells like colonies from umbilical cord vein mesenchymal stem cells.

T-2186
LONG-TERM EXPANSION OF DOPAMINERGIC PROGENITORS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (IPSCS) AND DIFFERENTIATION INTO DOPAMINERGIC NEURONS IN-VITRO

Fedele, Stefania[1], Collo, Ginetta[2], Ehsaei, Zahra[1], Cavallieri, Laura[2], Kunath, Tilo[3], Christensen, Klaus[4], Graf, Martin[4], Jagasia, Ravi[4], Taylor, Verdon[1]

[1]Department of Biomedicine, University of Basel, Basel, Switzerland, [2]Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy, [3]MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, United Kingdom, [4]Pharma Research and Early Development, F. Hoffmann-La Roche, Basel, Switzerland

Mesencephalic dopaminergic neurons (mDA) are the main source of dopamine in the mammalian central nervous system. The degeneration of mDA cells and the consequent destruction of the nigrostriatal network cause Parkinson´s disease (PD). Despite numerous advances in our understanding having been made in the last few years, the molecular mechanisms leading to PD are not fully understood. Functional DA neurons derived from human induced pluripotent stem cells (iPSCs) are an important system to improve our knowledge of mDA development and differentiation, and potentially to identify new molecular mechanisms that underlie the disease. Dopaminergic neuron differentiation from human iPS cell lines has been described previously (Kriks et al. Nature, 2011). A combination of different signals, especially FGF8 and SHH, promotes the appearance of ventral mDA progenitors by day 11 in vitro. We have adopted and adapted this system and confirmed the co-expression of early DA markers including Lmx1A and FoxA2 by immunofluorescence and RT-PCR analysis. In order to expand these ventral mid/hindbrain progenitors as a potential source of enriched mDA neurons, we have developed and refined a new protocol. We describe that the Lmx1A and FoxA2 mDA progenitors can be maintained and passaged for more than one month in vitro as a homogeneous population while retaining their marker expression and differentiation potential. Moreover, we developed a protocol that allows us to cryopreserve and thaw these expanded DA progenitors many times. We have evaluated the long-tem cultures and whether the cryopreservation affects mDA differentiation capacity. Using differentiation conditions, expanded DA progenitors generate many TH+ by day 50 and day 80 in vitro which co-express mDA markers including Nurr1, DAT, AADC and VMAT2. Furthermore, we confirmed the generation of mature and functional human iPS-derived mDA neurons by electrophysiology and dopamine release studies. Taken together, our results describe a novel method for long-term expansion of dopaminergic progenitors that retain their differentiation potential to generate mature mDA neurons.

T-2187
UTILITY OF HPSC SCORECARDTM ASSAY IN ASSESSMENT OF FUNCTIONAL PLURIPOTENCY OF CELLS ACROSS THE IPSC WORKFLOW

Fergus, Jeffrey, Quintanilla, Rene, Lakshmipathy, Uma

Thermo Fisher, Carlsbad, CA, USA

Improvements in induced pluripotent stem cell (iPSC) reprogramming technologies have led to the generation of patient-derived stem cells from various sources and conditions, creating valuable tools in drug discovery and future cell therapies. The steep challenge of characterizing these resulting iPSCs is minimally addressed by current methods that rely on a combination of in vitro and in vivo cellular methods. Molecular analysis methods offer an appealing solution for rapid, quantitative, and comprehensive characterization. We had earlier reported the development of a hPSC ScorecardTM Panel comprising a 94-gene TaqMan® panel. The accompanying cloud-based analysis software computes the signature for self-renewal and lineage markers for test samples and compares the data against a pluripotent reference standard to generate scores. Over two hundred samples were analyzed using the ScorecardTM to determine pluripotency along several stages of the iPSC workflow. Established clones were subjected to spontaneous embryoid bodies to assess for trilineage differentiation potential. Established clones were further used for directed differentiation into specific lineages and the optimal combination of cytokines and length of differentiation was determined using ScorecardTM. To further simplify the process and minimize sample size, methods were developed for direct use of cell lysate in the assay without compromising the quality of the results. These results collectively demonstrate the simplicity, ease and consistency of this method to predict functionality, thus offering a much needed uniform standardization and qualification of pluripotent cell lines.

T-2188
DECIPHERING THE NAÏVE STATE OF PORCINE IPSC

Freude, Kristine Karla[1], Secher, Jan O.[2], Mashayekhi, Kaveh[3], Petkov, Stoyan G.[4], Nielsen, Troels[5], Ceylan, Ahmet[6], Luo, Yonglun[7], Muenthaisong, Suchitra[3], Dinnyes, Andras[3], Hyttel, Poul[1]

[1]Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark, [2]Department of Large Animal Sciences, Section for Veterinary Reproduction and Obstetrics, University of Copenhagen, Frederiksberg, Denmark, [3]BioTalentum Ltd., Gödöllö, Hungary, [4]Friedrich-Loeffler-Institut, Neustadt, Germany, [5]Neurogenetics Research Laboratory, Danish Dementia Research Centre, Department of Neurology, University of Copenhagen, Copenhagen, Denmark, [6]Department of Histology and Embryology, Ankara University, Faculty of Veterinary Medicine, Ankara, Turkey, [7]Department of Biomedicine - Department of Human Genetics, Novo Nordisk A/S and Aarhus University, Aahrhus, Denmark

Concerted efforts have been expended in deriving porcine induced pluripotent stem cells (piPSC) which are envisaged to more faithfully mimic human physiology than existing rodent-derived iPSC lines. While initial piPSC lines, first generated in 2009, exhibit the majority of hallmarks displayed by iPSCs derived from other mammalian species, this is not without some caveats. Firstly, all existing piPSC-like cells are afflicted by insufficient activation of endogenous pluripotency genes. Secondly and associated with this, lack of silencing of exogenous pluripotency genes is a general drawback: in contrast, human and murine episomal reprogramming approaches lead to integration of such transgenes. Thirdly, current culturing conditions fail to support the maintenance of either porcine embryonic stem cells (pESC) or piPSC. Lastly, piPSC are unable to reproducibly contribute to chimeric

embryos as demonstrated for mouse and rat iPSC. Acquiring insight into these caveats will greatly benefit the generation of *bona fide* piPSC lines. Here, we sought to examine these caveats through: 1) comprehensive characterization of the pluripotency state of piPSC-like cells and 2) comparative transcriptome analyses between the original neonatal fibroblasts, piPSC-like cells, and porcine embryos at embryonic day 6/7 and day 10/11. The inner cell mass (ICM) of day 6/7 porcine embryos represents the naïve state of pluripotency, whilst at day 10/11 the blastocysts have hatched and the ICM has given rise to the hypoblast and the epithelial epiblast constituting the embryonic disc. In the epiblast, we expected to detect a primed state of pluripotency. Togenerate piPSC, neonatal fibroblasts were transduced with a Tet-ON lentiviralconstruct expressing porcine Oct4, Sox2, c-Myc, and Klf4. A comparative study was then conducted on these piPSC-like cells using the 2i approach with either FGF or LIF to establish and maintain them in a näive (LIF) or primed (FGF) condition. Subsequently, the potency of piPSC-like cells was comprehensively assessed, including through chimeric embryo contribution assays. Finally, extensive RNAseq analysis was performed to compare iPSC-like cells with the original fibroblasts and cells derived from porcine embryos at days 6/7 and 10/11. In summary, we were able to show that piPSC-like cells derived and maintained on 2i LIF exhibit a more naïve state than those derived with 2i FGF. Moreover, we identify a unique gene expression profile indicating the missing links in regards to pluripotency factors and growth factor receptors. These will be incorporated in our future efforts to derive and maintain *bona fide* stable naïve pluripotent piPSC.

T-2189
ES CELLS VS. IPS CELLS: LINEAGE CONVERSION-ASSOCIATED POINT MUTATIONS

Fujimori, Yuko Hoki, Sugiura, Mayumi, Kasama, Yasuji, Sunayama, Misato, Uda, Masahiro, Nakamura, Miki, Ando, Shunsuke, Araki, Ryoko, Abe, Masumi
Stem Cell Biology and Bioinformatics Team, National Institute of Radiological Sciences, CHIBA, Japan

The iPSC system holds great promise for regenerative medicine but its underlying molecular mechanisms have remained elusive. In contrast, ES cells are golden standard of pluripotent stem cells and have long history in both basic and applied researches. Therefore, it is an interesting and important issue to know whether iPS cells and ES cells are identical type of pluripotent stem cells. Here we focus on the point mutations in their genomes. For achieving comprehensive point mutation analysis of ES cells, the genomes of both parents from which blastocysts were prepared are essential as controls. Here we established ES cell lines from an inbred mouse strain, C57BL/6, along with the genomes of both parents. Their fully developmental ability was confirmed by aggregation experiments; 100% chimerism and germline competence were verified. On the four ES lines and on their parent genomes we conducted whole genome sequencing (WGS): approximately 55% of the entire genomes with more than 10 redundancies. As a result, we identified 10 to 30 point mutations in their genomes, and then confirmed the mutations by Sanger sequencing. Interestingly, the frequencies were ~1/10 of those observed in iPSCs genomes, since over hundreds point mutations have been observed in various mouse and human iPSCs. Notably, we also found a difference in their profile between iPSCs and ES cells: transversion predominant for iPSCs and transition predominant for ESCs. Because, out findings strongly suggest that most point mutations observed in iPSCs genomes arose associated with their cell lineage conversion process from somatic cell to pluripotent stem cell, next we have focused on the issue. Using also C57BL/6 mouse, several integration-free iPSC lines were established with the four factors, Oct3/4, Sox2, Klf4 and c-Myc (4F) in plasmid vectors from MEF. The MEF was derived from single embryo, not litter mates, to remove the intra-strain SNPs, which could be false positives of point mutations. Then, WGS on the three such integration-free iPSC lines and their parent somatic cells was conducted and ~300 point mutations were identified in each genome. Sanger sequencing verified the results. In addition, no common SNV was observed between different iPSC lines, suggesting that these are not pre-existing SNVs in their parent cells. Indeed, ultra-deep sequencing did not detect any SNVs in the parent MEF fraction. Subsequently, we performed variant allele frequency test and observed a considerable number of SNVs at less than 50% allele frequency, strongly suggesting a presence of 25% and 12.5% allele frequency point mutations. Similar SNVs were also observed by a deep WGS; a clear peak was appeared around 25% allele frequency in addition to the 50% frequency. Together, our observation means important facts that 25% or less SNVs were not pre-existing ones and that substantial numbers of point mutations occur during the conversion process, especially in their initiation steps, of iPSC generation, since pre-existing SNVs must be observed as 50% allele frequency. This was finally concluded by the investigation of sub-lines derived from single cells in an iPSC colony. The heterogeneity of the point mutation patterns within an iPSC clone was confirmed and reflects the history of the emergence of each mutation. In the present study we also attempted to reduce point mutation frequency by the introduction of some genes.

T-2190
RESTORATION OF SPATIAL MEMORY DYSFUNCTION OF HUMAN APP TRANSGENIC MICE BY TRANSPLANTATION OF NEURONAL PRECURSORS DERIVED FROM HUMAN IPS CELLS

Fujiwara, Naruyoshi
St. Marianna University School of Medicine, Kawasaki-shi Kanagawaken, Japan

Purpose: Dementia is a memory loss disorder, which is caused by a variety of brain diseases and results from a gross disruption of neural cells, including cholinergic and GABAergic neurons. In this study, we tried to generate neurons with cholinergic neuron phenotype from hiPS cells mimicking embryonic development of neurons. We transplanted the cells into the bilateral hippocam- pus of PDAPP mice and found that their spatial memory function improved significantly. [Materials and methods] (Culture of hiPS cells) The hiPS cell lines, 201B7 and 253G1 (RIKEN, Tsukuba, Japan), were used in this study. Both cell lines gave essentially the same results in this study. The hiPS cell lines were maintained according to RIKEN cell preparation manual. We developed embryoid bodies (EB) from undifferentiated hiPS cells by 4-day floating culture (from day 0 to day 4). Then EB were cultured on fibronectin (FN)-coated dishes on day 4. From day 5, we cultured the cells in differentiation medium consisted of DMEM/F12 with N2 supplement. We added 1 mM retinoic acid (RA), 10mM noggin-Fc (NOG) and 10nM sonic hedgehog (SHH) in the dishes twice (on days 5 and 7) and cultured for up to 11 days (from day 8 to day 19). We performed immunocytochemistry to evaluate the differentiation of these cells. (Transplantation and Morris water maze test) We transplanted the neuronal precursors into the 10-week-old PDAPP mice at day 8. 2 μl/each side of cell suspension (2.0 × 105 cells, n=15) and vehicle (n=16) were injected into the bilateral hippocampus. We conducted the Morris water maze (MWM) test once before transplantation (1st trial started at day −6) and twice after transplantation (2nd and 3rd trials started at day 22 and 48, respectively) to assess mouse spatial memory function. Results: (Cell differentiation) We generated neuronal precursors with cholinergic neuron phenotype. Approximately 78% of cultured cells at day 8 were hNCAM positive by

flow cytometric analysis. The cells at day 8 expressed mRNA of ChAT, βIII-tubulin, nestin, NFM and VGAT by RT-PCR and cells expressed Nestin at day 8 (97.3 ± 1.2%), and its expression decreased on day 19 (5.9 ± 5.0%). They expressed βIII -tubulin (24.5 ± 9.1%), ChAT (34.7 ± 9.1%) and NFM (18.0 ± 7.5%) at day 8. (Transplantation and MWM test) Transplanted hiPS cell derived neural cells were localized in the bilateral hippocampal lesions and these cells expressed hNCAM. Mouse spatial memory function was assessed by MWM tests. Spatial memory function of PDAPP mice has already impaired at 2 months of age. After transplantation, escape latency of the cell transplanted PDAPP mice was significantly shorter compared to that of vehicle injected PDAPP mice ($p<0.01$). (Immunohistochemical analyses) We conducted histological analysis of serial brain sections of mice having neuronal precursors cell grafts at day 53 (45 days after transplantation). We found that hNuc positive cells simultaneously expressed ChAT and VGAT in the hippocampus. These results suggested that the neuronal precursors derived from hiPS cells kept alive as cholinergic and GABAergic neurons in the recipient mouse brain. Conclusions: Transplantation of neuronal precursors derived from hiPS cells kept alive as cholinergic and GABAergic neurons in the recipient mouse brain and contributed to the improvement of memory dysfunction of a dementia-mouse model.

T-2191
WHAT IS THE OPTIMAL DIFFERENTIATION STAGE OF IPSC-DERIVED CARDIOMYOCYTES FOR CARDIAC CELL THERAPY?

Funakoshi, Shunsuke[1], Kimura, Takeshi[1], Yamanaka, Shinya[2], Yoshida, Yoshinori[3]
[1]Kyoto University, Kyoto, Japan, [2]Center for IPS Cell Research and Application, Kyoto, Japan, [3]Center for IPS Cell Research and Application, Kyoto University, Kyoto, Japan

Human induced pluripotent stem cells-derived cardiomyocytes (iPSC-CMs) are one of the promising sources for cardiac cell therapy. Although several investigators reported the positive effect of the iPSC-CMs based cell therapy, the treatment efficiency was limited. One of the major reasons of limited efficiency should be a poor engraftment of injected cells. To overcome this problem, it is important to elucidate the optimal differentiation stage of iPSC-CMs for engraftment. Now we compared the engraftment efficiency of intramyocardially injected iPSC-CMs by the differentiation stages. Using cell lines continuously expressing luciferase, we traced the engraftment ratio(ER) of the injected purified iPSC-CMs over time by in vivo imaging system (IVIS). ER was calculated by IVIS signal at some time points divided by the signal just after injection. When we compared the ER of undifferentiatied iPSCs, day4 mesodermal cells, day8, day20, and day30 iPSC-CMs after the initial differentiation, the ER was significantly higher in day20-CMs (iPSCs; 0%, day4 mesodermal cells; 0%, day8-CMs; 41.2%, day20-CMs; 167.6%, day30-CMs; 67.4% at 2 month after the initial injection). To demonstrate their therapeutic potential, day20-CMs were transplanted into NOG mice with myocardial infarction. Compared to control, mice transplanted with day20-CMs showed significant functional improvement measured by echocardiography (ejection fraction at 3 months: 55.9% vs. 38.9%; $p<0.01$). These results suggested that iPSC-CMs 20 days after the initial differentiation were the optimal cells for iPSC-CMs based cardiac cell therapy.

T-2192
GENERATION OF TRANSGENE-FREE HUMAN NAÏVE-LIKE PLURIPOTENT STEM CELLS USING SENDAI VIRUS VECTORS.

Fusaki, Noemi[1], Ema, Masahiro[2]
[1]Ophthalmology, Keio University School of Medicine and JST, Shinjuku-ku, Tokyo, Japan, [2]Research Center for Animal Life Science, Shiga University of Medical Science, Seta, Tsukinowa-cho, Otsu City, Shiga, Japan

The pluripotent states of human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are considered to correspond to that of mouse-derived epiblast stem cells (EpiSCs). These cells are sensitive to trypsin treatment and maintained an undifferentiated state with bFGF. It was reported that ectopic induction of factors such as OCT4 combined with kinase inhibitors (2i) and forskolin resulted in converting EpiSCs to a naïve pluripotent state similar to that of mouse ESCs growing with LIF. However, naïve 2i/LIF conditions are not sufficient to elicit a self-renewal response in human ESC/iPSCs and result in their differentiation. Here we show that OCT4, SOX2 and KLF4 continuously expressing human iPSCs are resistant to trypsin treatment. Using temperature-sensitive Sendai virus vectors carrying four factors, OCT4, SOX2, KLF4 and c-MYC, we obtained transgene-free naïve-like human iPSCs under the culture condition with 2i/LIF. These naïve-like iPSCs are resistant to trypsin treatment, form dorm-shape colonies and express SSEA1 like mouse ESCs, grow over forty passages, and show pluripotency in vitro and in vivo. Expression of Xist is reduced in these cells. These transgene-free naïve-like human iPSCs will contribute to the characterization and standardization of iPSCs for use in a clinical setting.

T-2193
STEM CELL BIOBANKING AT THE CORIELL INSTITUTE FOR MEDICAL RESEARCH

Gandre-Babbe, Shilpa[1], Grandizio, Christine[1], George, Ken[1], Shterban, Shana[1], Clements, Victoria[1], Nguyen, Tuyen[1], Tang, Zhenya[1], Gerry, Norman[1], Tarn, Chi[1], Berlin, Dorit S.[1], Madore, Steven J.[2]
[1]Stem Cell Biobank, Coriell Institute for Medical Research, Camden, NJ, USA, [2]Coriell Institute for Medical Sciences, Camden, NJ, USA

Induced pluripotent stem cells (iPSCs) represent a powerful new tool for studying human disease at the cellular level. Many of the first iPSC lines described were derived from fibroblast cell lines originally part of the National Institute of General Medical Sciences (NIGMS) Human Genetic Cell Repository and the National Institute on Aging (NIA) Cell Repository collections located at Coriell. Subsequently many of these iPSC lines were original submissions to the newly established Coriell Stem Cell Biobank which was established to make large numbers of high quality iPSC lines available for research purposes through the Coriell Cell Repositories web catalog at ccr.coriell.org. Biobanking of iPSCs requires a systematic work flow with extensive molecular and cellular characterization and quality control testing. At Coriell expansion of the iPSCs to sufficient quantities for large scale distribution is performed in accordance with strict quality control criteria. Each cell line destined for distribution undergoes viability testing, microbial contamination testing (including PCR-based mycoplasma screening) and DNA fingerprint analysis. In addition, chromosomal integrity is assessed using G-banded karyotype analysis as well as genome-wide genotyping (Affymetrix 6.0 array). Pluripotency testing involves extensive molecular and cellular characterization. Surface expression of pluripotency markers is measured by flow cytometry. Cell lines are tested for pluripotency by embryoid body formation followed by qRT-PCR for expression of the tri-lineage specific genes. In some cases, the

Pluritest™ assay is performed to assess the pluripotency. Cell lines are available for research purposes to academic, non-profit and industry investigators upon completion of a material transfer agreement. Each cell line is supplied with a Certificate of Analysis, culturing protocols and troubleshooting tips. Currently there are a total of 82 human iPSC lines available through the Coriell catalog including lines derived from apparently healthy controls as well as various genetic disorders. The Coriell Stem Cell Biobank also includes high quality aliquots of the transgenic mES cell lines generated by Dr. Minoru Ko.

T-2194
FEMALE HUMAN INDUCED PLURIPOTENT STEM CELLS REACTIVATE THE SILENT X CHROMOSOME DURING REPROGRAMMING

Ghazvini, Mehrnaz
Reproduction and Development, The Erasmus Stem Cell Institute for Regenerative Medicine, Rotterdam, Netherlands

During embryonic development of placental mammals, gene dosage compensation between males and female in somatic cells is achieved by transcriptional silencing of one of the X chromosomes in a random manner. Initiation of X chromosome inactivation (XCI) is regulated by inhibitors and activators of XCI which coordinate expression and spreading of XIST RNA in cis. In comparison to mouse, initiation of XCI mechanism is less studied in human due to post-XCI state of most human stem cells. Human iPS cells could serve as a model for XCI initiation. Even though many recent publications addressed XCI in human iPS cells, many questions still remain open about this process during reprogramming. To obtain more insight in X inactivation state of human iPS cells, we reprogrammed primary fibroblast cells of a female donor heterozygous for large X-chromosomal deletions using single cell RT-PCR analysis. Our results reveal re-activation of inactive X-chromosome during reprogramming followed by randomly XCI process. This observation might indicate that XaXa state of human iPSCs is unstable in the current culture conditions.

T-2195
ROLE OF LEPTIN RECEPTOR (LEPR) IN PLURIPOTENCY AND LINEAGE DEVELOPMENT

Gupta, Manoj K.[1], Tata, Nageswararao R.[1], Kleinridders, Andre[1], DeJesus, Dario[1], Raeder, Helge[2], Hu, Jiang[1], Wagers, Amy J.[1], Kulkarni, Rohit N.[1]
[1]Joslin Diabetes Center, Boston, MA, USA, [2]KG Jebsen Center for Diabetes Research, Department of Clinical Medicine, Bergen, Norway

Leptin receptors (LepR) are expressed by various types of stem cells including mesenchymal stem cells, hematopoietic stem cells, embryonic stem cells and induced pluripotent stem cells. Leptin/LepR signaling is also a central regulator of metabolism. But, the role of leptin receptor in pluripotency, stem cell metabolism and lineage development is still controversial and poorly understood. In the present study, we explored LepR function in pluripotency, metabolism and lineage determination using induced pluripotent stem (iPS) cells as our model system. We successfully reprogrammed mouse embryonic fibroblasts into iPS cells from control B6 and db/db (LepR deficient) mice. Like B6-wt iPS cells, db/db iPS cells showed similar morphology and the presence of alkaline phosphatase enzyme which is characteristic of pluripotent stem cells. Electron microscopy analysis revealed the bigger size of db/db iPS cells than B6-wt cells. However, db/db iPS cells showed reduced Oct4 expression and decreased Stat3 signaling giving the note that LepR signaling involved in reducing the stemness of stem cells. The stimulation study described the absence of functional LepR signaling and the presence of reduced Leukemia inhibitory factor (LIF) signaling in db/db iPS cells. In vivo differentiation of these iPS cells revealed the presence of all three lineages including mesoderm, endoderm and ectoderm, and the formation of tumors by db/db iPS cells as compared to B6-wt iPS cells showing the lower chance as 50% in SCID mice and 37.5% in syngenic B6-mice. Furthermore, the metabolic characterization demonstrated the higher oxygen consumption rate (OCR) and the presence of slightly more number of mitochondria in db/db iPS cells. In conclusion, we have shown that LepR signaling is regulating the stemness and metabolic properties of pluripotent stem cells and impaired teratoma formation. Disruption of leptin receptor signaling has been shown to involve in the pathophysiology of various diseases including obesity and diabetes, this study and generated iPS cells will provide the tools to explore the role of LepR in the developmental defects of beta cells and adipocytes and to find the putative targets of leptin receptor signaling during the development of these diseases.

T-2196
USE OF EFFICIENT CONDITIONAL, INDUCIBLE AND RMCE TECHNOLOGIES IN THE CREATION OF THE ROSA26-IPSC MOUSE: A NOVEL RESOURCE FOR STUDYING CELLULAR (DE)DIFFERENTIATION AND COMPLEX DISEASE.

Haigh, Jody Jonathan[1], Haenebalcke, Lieven[2], Goossens, Steven[2], Pieters, Tim[2], Nguyen, Thao[1], Costa, Magdaline[1], Haigh, Katharina[1]
[1]Australian Centre for Blood Diseases, Monash University, Melbourne, Australia, [2]Inflammation Research Centre, VIB/Ghent University, Ghent, Belgium

The conditional Cre/loxP system and the doxycycline (Dox) inducible Tet-on/off system are widely used in the mouse to limit spatial/temporal transgene expression but often require time consuming, inefficient cloning/screening steps and extensive mouse breeding strategies. We have therefore developed a highly efficient Gateway and recombinase-mediated cassette exchange (RMCE)-compatible system to target conditional and/or inducible constructs to the ROSA26 locus of F1 hybrid Bl6/129 ESCs, called G4 ROSALUC ESCs. Control of cellular (de)differentiation in a temporal, cell-specific, and exchangeable manner is of paramount importance in the field of reprogramming. Here, we have used these technologies and generated a mouse strain that allows iPSC generation through the Cre/loxP conditional and doxycycline/rtTA-controlled inducible (COIN) expression of the OSKM (Oct-4, Sox2, Klf-4, c-Myc) reprogramming factors entirely from within the ROSA26 locus. After reprogramming, genes of interest can replace these factors_for example, to enhance lineage-directed differentiation with the use of a trap-coupled RMCE reaction. We have shown that, similar to ESCs, Dox-controlled expression of the cardiac transcriptional regulator Mesp1 together with Wnt inhibition enhances the generation of functional cardiomyocytes upon in vitro differentiation of such RMCE-retargeted iPSCs. This ROSA26-iPSC mouse model is therefore an excellent tool for studying both cellular reprogramming and lineage-directed differentiation factors from the same locus and will greatly facilitate the identification and ease of functional characterization of the genetic/epigenetic determinants involved in these complex processes. Along these lines we are presently using this model system to create iPS cells from complex mouse genetic and leukemic disease backgrounds for use in functional genetic screens. As well we have begun to use this model to examine synergy between OSKM expression and the use of stimulus-triggered acquisition of pluripotency (STAP technologies) in enhancing cellular reprogramming efficiencies. Initial results of these approaches will be presented.

T-2197
CCL2 ENHANCES PLURIPOTENCY OF HUMAN INDUCED PLURIPOTENT STEM CELLS BY ACTIVATING HYPOXIA RELATED GENES

Hasegawa, Yuki[1], Tang, Dave[1], Takahashi, Naoko[1], Hayashizaki, Yoshihide[2], Forrest, Alistair R.R.[1], Suzuki, Harukazu[1]
[1]DGT, RIKEN CLST, Yokohama, Japan, [2]RIKEN PMI, Wako, Japan

Standard culture of human induced pluripotent stem cells (hiPSCs) requires basic Fibroblast Growth Factor (bFGF) to maintain the pluripotent state, whereas hiPSC more closely resemble epiblast stem cells than true naïve state ES which requires LIF to maintain pluripotency. Here we show that chemokine (C-C motif) ligand 2 (CCL2) enhances the expression of pluripotent marker genes through the phosphorylation of the signal transducer and activator of transcription 3 (STAT3) protein. Moreover, comparison of transcriptomes between hiPSCs cultured with CCL2 versus with bFGF, we found that CCL2 activates hypoxia related genes, suggesting that CCL2 enhanced pluripotency by inducing a hypoxic-like response. Further, we show that hiPSCs cultured with CCL2 can differentiate at a higher efficiency than culturing with just bFGF and we show CCL2 can be used in feeder-free conditions in the absence of LIF. Taken together, our finding indicates the novel functions of CCL2 in enhancing its pluripotency in hiPSCs.

T-2198
CYTOGENETIC ABERRATION DIFFERENCES IN HUMAN ESC AND IPSC LINES: AN EIGHT YEAR COMPARISON OF CHROMOSOME CHANGES IN 2,607 ESC AND 5,203 IPSC LINES

Hazelbauer, Stephanie Ann[1], **Finger, Jared[1],** Balaban, Esra[1], Walling, Jason[1], Meisner, Lorraine[2]
[1]Cell Line Genetics, Inc., Madison, WI, USA, [2]Cell Line Genetics Inc., Madison, WI, USA

Cytogenetic analysis was performed on 2,607 human embryonic stem cell (ESC) lines and 5,203 human induced pluripotent stem cell (iPSC) lines during the years 2006 to 2013. Although both types of cell lines demonstrated a similar chromosome aberration rate, the types of aberrations were significantly different; suggesting that they arose by different mechanisms and this difference may be related to their respective methods of derivation. Under optimal conditions, ESC lines are derived from the inner cell mass of karyotypically normal embryos, while iPSC lines are derived from karyotypically normal adult cells that have been reprogrammed using integrating and non-integrating methods. In addition to inducing pluripotency, the reprogramming process can also induce random chromosome breakage and rearrangement. Although ESC lines initially have normal karyotypes, it has been shown that many develop numerical chromosome abnormalities in culture (predominantly trisomy 12p, 17q, 1q, and 20q). These specific trisomies typically demonstrate a proliferative advantage that enables trisomic clones to overtake a coexisting karyotypically normal cell line in relatively few passages. Our data will show that roughly half of the karyotypically abnormal ESC lines demonstrated numerical chromosome (whole chromosome) aberrations while the other half showed structural chromosome aberrations (the majority of which were unbalanced translocations). In contrast, iPSC lines demonstrated few numerical aberrations, while the majority of the aberrations consisted of structural rearrangements. Interestingly, the majority of balanced translocations were detected in iPSC lines at early passages, while balanced translocations in ESC lines were rare. We will present the different types and frequencies of chromosome aberrations found in ESC and iPSC lines.

T-2199
HUMAN INDUCED PLURIPOTENT STEM CELL SUB-FRACTIONS DEMONSTRATE PREFERENTIAL LINEAGE COMMITMENT AND PROPENSITY FOR DIFFERENTIATION INTO ENDOTHELIAL PROGENITOR CELLS

Ho, Mirabelle S.H.[1], Mei, Shirley H.J.[2], Schlosser, Kenny[2], Stewart, Duncan[1]
[1]Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Sprott Centre for Stem Cell Research, Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada, [2]Sprott Centre for Stem Cell Research, Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada

Acellular scaffolds hold tremendous promise for the creation of bioengineered organs for transplantation, and represent a promising approach to address the widespread shortage donor lungs. However, inadequate re-endotheliazation hampers current attempts to recellularize lung scaffolds, leading to organ construct failure due to thrombosis. The use of patient-specific induced pluripotent stem cells (iPSCs) circumvents the immune challenges and ethical dilemmas confounding human embryonic stem cells. Seeding decellularised lung scaffold with endothelial progenitor cells (EPCs) derived from iPSCs could potentially provide more complete re-endotheliliazation of biological scaffolds. We further hypothesized that different sub-fractions within a heterogeneous population of iPSCs possess inherent predisposition to differentiate down certain lineages. Specifically, we are interested in identifying the sub-fraction which demonstrates the greatest propensity to differentiate into mesodermal lineages such as EPCs. By utilising a double FACS strategy with pluripotency markers CD9 and EpCAM, four sub-fractions arbitrarily termed R3 ($CD9^{Hi}$-$EpCAM^{Hi}$; 24.3%), R4 ($CD9^{Mid}$-$EpCAM^{Mid}$; 18.3%), R5 ($CD9^{Low}$-$EpCAM^{Low}$; 40%) and R6 ($CD9^{Neg}$-$EpCAM^{Neg}$; 12.6%) were generated. Independent double-FACS performed with Nanog and Tra-1-60, as well as Q-PCR for pluripotency genes Nanog and GDF-3 confirmed that the R3 sub-fraction contains the highest proportion of pluripotent cells whilst R6 contain predominantly differentiated cell types. Preliminary Q-PCR performed on R3-R6 samples immediately post-FACS showed that expression of a mesodermal marker, MIXL1, strongly mirrored that of eNOS, a characteristic gene of endothelial. Relative to R3, the R6 sub-fraction showed 5.5-fold and 3.5-fold increases in expression of MIXL1 and eNOS respectively. R3-R6 cells formed stem cell colonies within 7 days of FACS as evidenced by positive immunostaining with Nanog and CD9. Q-PCR on cells 7 days post FACS demonstrate that relative to R3, the endodermal marker, GATA4, the mesodermal marker, MIXL1, and the ectodermal marker, PAX6, are preferentially expressed in R5 (8-fold increase), R4 (1.5 fold increase) and R6 (>10 fold increase), respectively. Sub-fractions demonstrate distinct preferential lineage commitment with the R4 sub-fraction showing most promise for differentiation to the mesodermal lineage and consequently into EPCs. Subsequent differentiation into EPCs to assess for differentiation efficiency across the four sub-fractions will employ a robust EC differentiation protocol modified from Tatsumi *et al*'s. Optimization of this protocol has found that MACS-selected $CD144^+$ cells displaying cobble-stone morphology typical of EPC cultures were enriched at an initial efficiency of 23.8%. Over a single passage, $CD144^+$ cells in the enriched sub-fraction increased to 76.8%. Moreover co-expression of EPC markers CD144 and CD31 was appreciably higher in the enriched sub-fraction (75.6%) compared to the depleted sub-fraction (1.9%). $CD144^+/CD31^+$ co-expressing cells underwent further expansion in successive passage to 87% in enriched sub-fraction. Identification of a specific iPSC sub-fraction predisposed to differentiate into EPCs could potentially reduce the experimental manipulations required for its directed differentiation whilst achieving therapeutically relevant

quantities of cells within a shorter time-frame.

T-2200
CHARACTERISATION OF AN IPS CARDIOMYOCYTE MODEL FOR INVESTIGATING COMPOUND RELATED STRUCTURAL CARDIOTOXICITY

Holder, Julie[1], Weekes, Claire J.[2], Schofield, Christopher A.[3], Walker, Tracy M.[4], Worby, Angela[3], Finnigan, Damon[3], Sridhar, Arun[5], Harris, Kate[2], Powell, Andrew J.[3], Nicholls, Andrew W.[2], Gandhi, Mitul[2]
[1]Regenerative Medicine DPU, GlaxoSmithKline, Stevenage, United Kingdom, [2]Safety Assessment, GlaxoSmithKline, Ware, United Kingdom, [3]Biological Sciences, GlaxoSmithKline, Stevenage, United Kingdom, [4]GlaxoSmithKline, Ware, United Kingdom, [5]Bioelectronics, GlaxoSmithKline, Stevenage, United Kingdom

Cardiovascular toxicology is a primary cause of compound attrition during preclinical and clinical development. The development of an *in vitro* model for early evaluation of drug efficacy and safety could assist in the identification of compounds which have a cardiac liability early in the drug discovery /development continuum. It is however critical that the cells used in the in vitro assay reflect the primary tissue and we have measured a number of parameters such as intracellular Cardiac Troponin, Creatinine kinase, LDH isoforms and beat frequency at different times after the cells were recovered from a frozen stock. Up to five different batches of cells were tested to evaluate batch to batch variability. Differences between batches were evident from the endpoints measured identifying the need to characterise each batch prior to use. As a baseline a set of measurable endpoints for characterisation of the cell batch have been developed as a result of this characterisation which closely describe a human iPS cardiomyocyte.

T-2201
REPROGRAMMING AND CHARACTERIZATION OF IPSCS FROM FAMILIES WITH EARLY ONSET FRONTOTEMPORAL DISEASE

Holst, Bjørn[1], Rasmussen, Mikkel[2], Schmid, Benjamin[1], Tubsuwan, Alisa[1], Hyttel, Poul[2], Nielsen, Jørgen[3], Clausen, Christian[1]
[1]Bioneer A/S, Hoersholm, Denmark, [2]University of Copenhagen, Faculty of Health and Medical Sciences, Frederiksberg C, Denmark, [3]Copenhagen University Hospital, København, Denmark

The recent ability to revert terminal differentiated cells (somatic cells) into so-called induced Pluripotent Stem Cells (iPSCs) has opened up new ways for investigating major human diseases such as Alzheimer's disease and diabetes, where proper models have thus far been lacking. The technology allows reprogramming of easily accessible somatic cells, like fibroblasts from a skin biopsy, into iPSCs, which can then be differentiated to mature cells, like neural subtypes, in which the disease-related pathomechanisms can then be investigated. Dominantly inherited mutations in MAPT, the gene that encodes tau, is known to cause frontotemporal dementia, proving that dysfunction of tau is sufficient to cause neurodegeneration and dementia. Common to diseases like frontotemporal dementia is the abnormally hyperphosphorylation of tau, resulting in accumulation of intra-neural tangles of paired helical filaments, twisted ribbons or straight filaments. The prevention of tau aggregation and propagation would be an approach to develop mechanism-based treatments for tauopathies. To obtain new and better disease models for frontotemporal dementia, we have isolated fibroblasts from skin biopsies of three familial early onset patients with mutations in the MAPT. Two of them are from the same family, a 58 year old symptomatic woman and her 29 year old asymptomatic daughter, both with an Arginine to a Tryptophane substitution at position 406 in the MAPT protein (R406W). The third patient is a 58 year old woman with a proline to a leucine change at position 301 of the MAPT protein (P301L). For each patient, three iPSC lines have been isolated together with age-matched controls, using an episomal based reprogramming system in an mTeSR1/matrigel-based setup. We present here a comprehensive characterization of the isolated iPSC lines at passage 10. Immunocytochemical staining for the pluripotency markers OCT4, NANOG, SSEA3, SSEA4, TRA-1-61 and TRA-1-81 and differentiation to embryoid bodies was performed, thus confirming their pluripotency. Moreover, qPCR analyses of OCT4, NANOG, KLF4, L-MYC and LIN28 showed that these markers were highly upregulated compared to fibroblasts. Genome editing approaches like transcription activator-like effector nucleases (TALENs) will be used to generate isogenic, gene-corrected controls allowing mutation-specific analyses.

T-2202
CELL DENSITY AND PROTOCOL OF CULTURE MEDIUM REPLACEMENT AFFECT THE PLURIPOTENCY OF HUMAN IPS CELL AGGREGATES IN SUSPENSION CULTURE

Horiguchi, Ikki, Urabe, Yusuke, Sakai, Yasuyuki
University of Tokyo, Tokyo, Japan

Application of induced plutipotent stem (iPS) cells to regenerative medicine requires development of scalable culture methods to obtain a large number of cells at reasonable costs. Suspension culture of iPS cell aggregates is one of the promising scalable culture methods. However, the behaviors of aggregates such as formation, growth and preservation of pluripotency are still not well studied and there are few reports on optimizing operations of scalable suspension culture such as inoculum cell density and protocol of changing culture medium from engineering point of view. We therefore investigated the effect of various operating condition such as initial cell densities and frequency of culture medium replacement on the behaviors in a small-scale suspension culture. The results showed that higher inoculum cell densities and changing of half volume of the culture medium at higher frequencies were effective in the maintenance of their pluripotency. Prior to initiation of suspension culture, cells were treated by Y27632 and dissociated by TrypLE select. After filtration through 40 μm cell strainer, iPS cells were suspended at concentrations of 1×105 ("high inoculum") and 5×104 ("low inoculum") cells/mL. After 7 days suspension culture, aggregates were aspherized in "low inoculum" group whereas aggregates kept spherical morphology in "high inoculum" group. Flow cytometry analyses showed that cells in "high inoculum" group maintained Nanog expression similar to those in the original iPS cells in adherent culture. However, more than half cells in "high inoculum" group became "de-undifferentiated" as evidenced by their loss of Nanog expression. We also compared different protocols of changing medium in terms of the volume and frequencies, that is, "whole volume/everyday", "whole volume/every two days" and "half volume/everyday". According to morphological observations, the cells in the "half volume/everyday" group maintained sphericity of aggregates even at "low inoculum" condition. In order to understand biological mechanism of de-undifferentiation, we performed ELISA and RT-qPCR analysis. These analyses suggested that cells secreted some growth factors such as bFGF, TGFb1, NODAL and BMP4. According to the ELISA analysis of bFGF, interestingly, the remaining concentration of bFGF in the "high inoculum" group was higher than that in "low inoculum" group. This result means that human iPS cells probably produce bFGF while consuming. These results indicated that iPS cells were able to condition culture medium to maintain their pluripotency and changing whole medium induced their de-undifferentiation because conditioned medium was replaced by non-conditioned flesh medium. In this case, it is expected that aggregates in "high inoculum" group were many

enough to re-condition flesh medium to maintain their pluripotency but aggregates in "low inoculum" group were affected by culture medium replacement to de-undifferentiate. These results are helpful in optimizing operating condition of suspension culture as scalable culture method. In addition, these results indicate the feasibility of reducing required amount of growth factors such as bFGF in order to reduce the running cost of iPS culture. We are now doing further experiments to understand which factors controls their de-undifferentiation. In addition, we are also designing culture system containing with dialysis to exchange substrates and wastes without removal of secreted factors.

T-2203
REGULATION OF DNA METHYLATION BY MIR-29 FAMILY DURING SOMATIC CELL REPROGRAMMING

Hysolli, Eriona[1], Tanaka, Yoshiaki[1], Lu, Jun[1], Weissman, Sherman[2], Park, In-Hyun[2]
[1]Yale University, New Haven, CT, USA, [2]Yale University School of Medicine, New Haven, CT, USA

Overexpression of Yamanaka factors OCT, SOX2, KLF4, and MYC reprograms somatic cells to generate induced pluripotent stem cells (iPSCs). Reprogramming to pluripotency is accompanied by global genomic and epigenomic changes. Histone modification and DNA methylation states in iPSCs are similar to those in embryonic stem cells (ESCs). However, epigenetic differences exist between iPSCs and ESCs. In particular, aberrant DNA methylation states found in iPSCs are a major concern for using iPSCs in a clinical setting. Thus, it is critical for future applications of iPSCs to find factors that regulate DNA methylation states in reprogramming. We asked whether non-coding RNAs that regulate both de novo methylation and demethylation would facilitate reprogramming. Through target prediction analysis and screening, we found that miR-29 family targets both de novo DNA methyltransferases DNMT3A, DNMT3B and demethylases TET1, TET3 and TDG. The suppression of miR-29 family improved, but overexpression of miR-29a decreased the efficiency of human somatic cell reprogramming. Through global DNA methylation and hydroxymethylation analysis, we found that DNA demethylation is a major event mediated by miR-29a in early reprogramming. Our findings would lead to developing a unique miRNA-based approach in generating iPSCs with proper epigenetic states.

T-2204
ANALYSIS OF LONG-RANGE CHROMATIN INTERACTION NETWORKS IN PLURIPOTENT STEM CELLS

Ikeda, Hiroki, Sone, Masamitsu, Yamanaka, Shinya, Yamamoto, Takuya
CiRA, Kyoto University., Kyoto, Japan

Three-dimensional (3D) organization of chromosomes has been studied both in pluripotent stem cells and differentiated cells. Recent works have demonstrated that chromatin interactions at pluripotency genes loci are reorganized prior to their reactivation in cellular reprogramming. However, little is known about rearrangements of chromatin interactions at other genes loci during somatic cell reprogramming. Here, we report the analysis of chromatin structures in pluripotent stem cells by using chromosome conformation capture combined with high-throughput sequencing (4C-seq), a method to detect genomic regions in close physical proximity to a chosen genomic site. We have investigated chromatin interactions at pluripotency genes loci, developmental genes loci and intergenic loci in human iPSCs and their original human dermal fibroblasts (HDFs). As a result, we identified different chromatin interactions at several gene loci between human iPSCs and HDFs. Interestingly, some interactions at developmental genes, which exhibit little expression in both cell types, are also changed during somatic cell reprogramming. In addition, we observed that polycomb proteins are enriched in tethered regions of developmental gene loci in hiPSCs. Our results indicate the importance of chromatin organization at developmental genes loci in the differentiation capacity of pluripotent stem cells.

IPS CELLS: DISEASE MODELING

T-2205
GENERATION OF A NOVEL HUMAN X-LINKED MYOTUBULAR MYOPATHY (XLMTM) MODEL VIA INDUCED PLURIPOTENT STEM CELLS

Bao, Yunhua, Huttner, Anita J.
Yale School of Medicine, New Haven, CT, USA

Introduction: X-linked myotubular myopathy (XLMTM) is a severe form of congenital myopathy that primarily affects skeletal muscles. Patients with XLMTM often present with severe perinatal weakness that can progress to respiratory failure, but with optimal medical support, some severely affected patients can survive for years. The pathogenesis of XLMTM is poorly understood but has been linked to MTM1 mutations. Recent progress in our understanding of human induced pluripotent stem cells (iPSCs) opened a new avenue for modeling human diseases. This dynamic recapitulation of disease process will help gain insight into disease pathogenesis and might potentially aid in finding specific therapeutic interventions such as stem cell and gene-based therapy. In this study we are aimed to generate the iPSCs from somatic cells of patients with XLMTM as well as to induce differentiation of these patient specific iPSCs into functional myocytes. Methods: Skin fibroblasts were collected during the autopsy of patients with XLMTM and age-matched individual who had no evidence of congenital diseases. Collected skin tissues were cultured in fibroblasts culture medium for three weeks. Generation of human iPSCs follow published protocols using a polycistronic lentiviral vector construct expressing human OCT4, SOX2, KLF4 and cMyc/mCherry. Formed colonies were characterized by immunostains and RT-PCR as well as karyotyping and microarray studies. Generated iPSCs are injected into the SCID mice to generate teratoma. In vitro differentiation and characterization of human iPSCs into cells of the muscle lineages were evaluated by FACS sorting using mesenchymal precursor and myogenic progenitor markers. Results: After two weeks of transduction, a large number of colonies had formed. The colonies show positive immunostaining of Oct4, Tri-1-60, and Nanog, as well as expression of human pluripotency genes hSox2, hNanog, hOct4, and hDNMT3B as revealed by RT-PCR. These results support the pluripotent nature of the cells. Teratomas composed of all three germ layers formed when iPSCs were injected into SCID mice. In addition, the iPSCs also demonstrate ability to differentiate into cells expressing muscle markers in vitro. In our preliminary studies with iPSCs derived from control subjects, we reached the 80% level of mesenchymal precursor (MP) marker CD-73 positive cells. Expended MP-positive cells with N-CAM through secondary FACS sorting, which results in 10% enrichment of induced skeletal myoblasts. Conclutions: Our study has demonstrated the successful generation of iPSCs from autopsy skin tissue of patients with XLMTM--a new tissue source for generating human iPSCs. These patient-specific iPSCs possess the ability to differentiate in vitro and in vivo, providing an effective model for myopathy studies.

T-2206

GENOME EDITING OF IPSCS FROM PARKINSON'S DISEASE AND MULTIPLE SYSTEM ATROPHY PATIENTS

Bi, Kun[1], Hermanson, Spencer[1], Thompson, David[1], Schuele, Birgitt[2], Langston, J. William[3], Vogel, Kurt[4]
[1]Cell Biology, Thermo Fisher Scientific, Madison, WI, USA, [2]Parkinson's Institute, Sunnyvale, CA, USA, [3]The Parkinson's Institute, Sunnyvale, CA, USA, [4]Thermo Fisher Scientific, Madison, WI, USA

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects 1-2% of the population over age 65. PD is marked by a loss of dopaminergic neurons in the midbrain substantia nigra pars compacta region. Because of lack of access to such tissue, or availability of good animal models of PD, iPSC-derived neurons hold promise in the development of model systems to study PD. We have generated iPSCs from patients harboring mutations in the PARKIN and LRRK2 genes, as well as a rare case with mutations in both the LRRK2 and GBA genes, and a patient with Multiple System Atrophy (MSA). In order to discern subtle, disease-relevant phenotypes in midbrain dopaminergic neurons derived from iPSCs, or to understand the impact a specific gene or mutation on those phenotypes, we generated a set of isogenic iPSC lines using the Transcription Activator-Like (TAL) effector nuclease technology. We deleted the alpha-synuclein (SNCA) gene (both heterozygous and homozygous alleles) in the MSA iPSC line via non-homologous end-joining repair mechanism, and reverted the LRRK2 and GBA mutations back to wild type in a PD iPSC line via homologous recombination to better understand any synergies between these mutations. These lines are currently being differentiated into midbrain dopaminergic neurons, and disease-relevant assays are being developed. In this presentation, we will describe the optimized iPSC TAL-editing workflow, the genomic analysis methods we have developed for colony screening to identify edited clones and the approach we took to screen for and analyze off-target effects. We hope to share our protocols and lessons learned to enable broader research community to successfully perform TAL-based genome editing of iPSCs.

T-2207

GENERATION OF INDUCED PLURIPOTENT STEM CELLS FROM URINE FOR THE STUDY OF BARTH SYNDROME

Blanner, Patrick[1], Neilson, Amber[1], Martinez, Rita[2], Cade, Todd[1]
[1]Washington University in St. Louis, St. Louis, MO, USA, [2]Washington University-St. Louis School of Medicine, St. Louis, MO, USA

Induced pluripotent stem cells (iPSCs) offer a tremendous opportunity for modeling and studying human disease, especially those diseases with known genetic origins. Barth Syndrome (BTHS) is a rare genetic disorder caused by a loss-of-function mutation in the tafazzin gene (TAZ). TAZ encodes a phospholipid acyltransferase that is located in the inner mitochondrial membrane and involved in the remodeling of cardiolipin. Clinical features of BTHS include cardiomyopathy, skeletal myopathy, growth delay, and neutropenia. This pronounced neutropenia in patients with BTHS makes it difficult to safely acquire somatic cells for reprogramming through commonly used methods such as skin punch biopsies or blood draws. To circumvent this issue, we have developed a completely non-invasive methodology to isolate renal epithelial cells from urine for the subsequent generation of iPSCs from BTHS patients. Employing this method, we were able to successfully isolate, expand and bank renal epithelial cells from 6 patients with BTHS, as well as 5 sibling controls. We were then able to create multiple iPSC lines from these patients in a completely feeder-free environment. Furthermore, we were able generate cardiomyocytes from these iPSCs for the subsequent study of BTHS in a more disease relevant context.

T-2208

THE AUTISM SPECTRUM DISORDERS STEM CELL RESOURCE AT CHOC CHILDREN'S: IMPLICATIONS FOR DISEASE MODELING AND DRUG DISCOVERY

Brick, David J.[1], Nethercott, Hubert E.[1], Montesano, Samantha[1], Banuelos, Maria G.[1], Stover, Alexander E.[1], Sun Schutte, Soleil[2], O'Dowd, Diane K.[2], Hagerman, Randi J.[3], Michele, Ono[3], Hessl, David R.[3], Tassone, Flora[4], Schwartz, Philip H.[1]
[1]Centers for Neuroscience and Translational Research, CHOC Children's Research Institute, Orange, CA, USA, [2]Developmental and Cell Biology, University of California, Irvine, Irvine, CA, USA, [3]Pediatrics, University of California, Davis, Sacramento, CA, USA, [4]Biochemistry and Molecular Medicine, University of California, Davis, Sacramento, CA, USA

The autism spectrum disorders (ASDs) comprise a set of neurodevelopmental disorders that are, at best, poorly understood but are the fastest growing developmental disorder in the United States. As animal models of polygenic disorders such as the ASDs are difficult to validate, the derivation of induced pluripotent stem cells (iPSCs) by somatic cell reprogramming offers an alternative strategy for identifying the cellular mechanisms contributing to ASDs and development of new treatment options. Access to statistically relevant numbers of ASD patient cell lines, however, is still a limiting factor for the field. Here, we describe a new resource with over 200 cell lines (fibroblasts, iPSC clones, NSCs, glia) from unaffected volunteers and patients with a wide range of clinical ASD diagnoses, including Fragile X syndrome (FXS). We have shown that both normal and ASD-specific iPSCs can be differentiated toward a neural stem cell phenotype and terminally differentiated into action-potential firing neurons as well as glia. The ability to evaluate and compare data from a number of different cell lines will facilitate greater insight into the cause(s) and biology of the ASDs and will be extremely useful for uncovering new therapeutic, as well as diagnostic, targets. Importantly, some drug treatments have already shown promise in reversing the neurobiological abnormalities in iPSC-based models of ASD-associated diseases. The ASD STEM Cell Resource at CHOC will continue expanding its collection and make all lines available upon request with the goal of advancing the use of ASD patient cells as disease models by the scientific community.

T-2209

GENOME-EDITED PLURIPOTENT STEM CELLS AS MODELS FOR DRUG RESPONSE AND PHARMACOGENOMICS

Singh, Sadananda[1], Maillet, Agnes[1], Tan, Kim[1], Mehta, Ashish[2], Shim, Winston[2], Pouladi, Mahmoud[1], **Brunham, Liam R.**[1]
[1]Translational Laboratory in Genetic Medicine, Singapore, [2]National Heart Centre Singapore, Singapore

Genetic association studies have recently identified a number of genetic variants that are associated with drug-response phenotypes. However, determining the functional significance of these pharmacogenomic variants remains a major challenge, in part because of the lack of appropriate model systems in which to assess their effects. The development of genome-editing tools such as TALENs and CRISPR-Cas has provided the opportunity to investigate the functional significance of specific genetic variants in human cell lines on an isogenic background. Here we describe an approach using genome-editing in pluripotent stem cells to model pharmacogenomic traits and interrogate the functional significance of specific genetic variants on cell-autonomous drug-response phenotypes. We will present an update on our results generating isogenic pluripotent stem cell-derived cardiomyocytes in which to model the functional significance of genetic

variants that have been associated with drug-induced cardiotoxicity. This represents a general approach for the functional assessment of genetic variants associated with drug-response phenotypes.

T-2210
DEVELOPMENT OF A STEM CELL-BASED CELLULAR SCREENING MODEL FOR BOTULINUM NEUROTOXIN INTOXICATION

Caglic, Dejan, Hrones, Morgan, Krutein, Michelle, Tobin, Dickerson J.
Chemistry, The Scripps Research Institute, La Jolla, CA, USA

Botulinum neurotoxin (BoNT) is the most toxic protein known to man with its LD50 ~1 ng/kg, where exposure to the toxin ultimately results in host paralysis and death. Despite the broad clinically utility of this toxin, it also can pose a safety threat if misused in the event of a bioterrorist attack. As a result, much effort has been put into the discovery and development of therapeutic strategies for the treatment of botulism. Traditionally, molecules with anti-BoNT activities have been identified through in vitro screening campaigns using fluorescent peptide substrates, or by using cellular models and low-throughput immunological detection of SNARE protein cleavage. Although these efforts have resulted in leads with good in vitro potency, all have failed when advanced to in vivo testing due to poor pharmacokinetic properties such as low aqueous solubility, high cytotoxicity and low cell permeability. In addition, cellular models that rely on secondary cell lines have been shown to have poor predictive value in lead advancement. These limitations, therefore, dictate the need for new cellular models that faithfully recapitulate the phenotype of BoNT intoxication, are better predictors of in vivo efficacy, and are compatible with existing automation and high-throughput screening procedures. We have taken advantage of human stem cell-derived motor neurons that have been demonstrated to exhibit high BoNT sensitivities and represent a feasible approach for expansion to cell numbers required for screening applications. We have proposed that a FRET-based reporter of BoNT activity allows for real time monitoring of the intoxication process. To achieve this goal, two different approaches have been used to engineer reporter expression in stem cells. In the first approach, cells are being prepared to constitutively express the BoNT reporter and allow for the direct comparison of BoNT sensitivity between differentiation stages. The second prong to our approach was to develop stem cell-derived motor neurons that express the BoNT reporter only in a specific differentiation stage. Stage-specific expression of the reporter protein allows for monitoring of intracellular BoNT activity in real time as well as enables the evaluation of the efficiency of the differentiation protocol and provides a means to purify cells of interest.

T-2211
MITOCHONDRIA PATHOLOGY IN STEM CELLS- DERIVED NEURONS FROM PATIENTS WITH PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS

Calafiore, Marco[1], Barca, Emmanuele[2], Douvaras, Panagiotis[1], Quinzii, Catarina M.[3], Fossati, Valentina[1]
[1]The New Stem Cell Foundation Research Institute, New York, NY, USA, [2]Department Of Neurology, H. Houston Merritt Clinical Research Center Columbia University, Medical Center, New York, NY, USA, [3]Department Of Neurology, H. Houston Merritt Clinical Research Center, Columbia University, Medical Center, New York, NY, USA

Autoimmunity and inflammation are typical hallmarks of multiple sclerosis (MS) from the early stage of the pathology, but what drives T lymphocyte to attack myelin is still unknown. During the past decade, gray matter atrophy has been increasingly recognized as a critical player in the progression of clinical and cognitive disabilities. Advances in MRI technology have allowed to establish the onset of neuronal loss prior to the loss of myelin and the formation of white matter lesions. However, whether axonal degeneration is caused by inflammation and oxidative stress or is intrinsic to neural cells is still a point of debate. If we could investigate the neuronal pathology alone, without the effects of the autoimmunity mechanisms, we could better understand the dynamics of MS. The success in generating any type of cells at will by reprogramming human skin fibroblasts to a pluripotent state opens the possibility to study the MS pathology in a controlled environment. For this study, we selected four individuals diagnosed with primary progressive multiple sclerosis (PP-MS) and four age and sex-matched controls. Skin biopsies were obtained from PP-MS patients and healthy controls recruited at the Tisch Multiple Sclerosis research center of New York (TMSRSNY), upon approval of specific IRB protocol. The controls were individuals not affected and without history of MS in the family, as well as not affected by autoimmune diseases or neurodegenerative diseases. iPSC lines were generated using the integration free, virus free mRNA/miRNA method. Cells were fully characterized and differentiated toward neurons. Stem cell-derived neurons from PP-MS patients show mitochondrial dysfunction and elevated production of reactive oxygen species in the absence of the immune system in culture, suggesting that neurodegeneration is a cell autonomous intrinsic process. Further investigation is required to understand how mitochondria dysfunction can lead to the development of MS.

T-2212
CHARACTERIZATION AND FUNCTIONAL APPLICATIONS OF HUMAN IPS CELL-DERIVED MIDBRAIN DOPAMINERGIC NEURONS

Carlson, Coby B.[1], Chase, Lucas[1], Mangan, Kile P.[1], Arnold, Brian[1], DeLaura, Susan[1], Thompson, Arne[1], Hesley, Jayne[2], Sirenko, Oksana[2], Cromwell, Evan F.[2], Ott, Vanessa[1]
[1]Cellular Dynamics International, Madison, WI, USA, [2]Molecular Devices, Inc, Sunnyvale, CA, USA

A major challenge in the field of neuroscience drug discovery has been the lack of robust and physiologically relevant systems for cellular analysis and disease modeling. Human induced pluripotent stem (iPS) cells have emerged as a promising source of material from which tissue-specific cell types can be derived. In particular, neuronal subtypes from specific regions of the brain can be generated and are proving to be more predictive of the human condition as compared to immortalized cell lines or primary rodent cultures. The controlled differentiation of human iPS cells into either forebrain neurons or midbrain dopaminergic (DA) neurons - and not into cardiomyocytes or hepatocytes, for example - is a complex process that is tightly regulated. In addition, the ability to repeat the method of differentiation consistently at a scale sufficient for drug discovery efforts has proven to be very difficult. We have previously presented on the derivation and characterization of human forebrain neurons. Here, we demonstrate how a midbrain DA neuron differentiation was adapted from published literature, and then further optimized, resulting in the ability to produce commercial quantities of cryopreserved midbrain DA neurons. Using methods of gene expression, flow cytometry, and high content imaging, we were able to monitor the development of these neurons during differentiation. The final product displays typical neuronal morphology, with long and branching processes, and expresses key markers for this midbrain DA neuron population, including Lmx1, FoxA2, tyrosine hydroxylase (TH), and Map2. Moreover, this differentiation method yields a highly pure population of cells (>80% for TH and FoxA2; negative when stained for Nestin). Importantly, these DA neurons were compared to forebrain neurons in various functional assay endpoints, including multi-electrode array (MEA), FLIPR calcium assays, and other

standard measurements of neurotoxicity. Our results demonstrate baseline differences in network level electrical activity, as well as robust and varied responses to pharmacological agents (including GABAergic and dopamine agonists). The data presented here underscore the value of using iPS cell-derived cell types and highlight the use of different neuronal subtypes in various applications.

T-2213
CURCUMIN LOADED NANOPARTICLE AMELIORATED RETINAL FUNCTION IN BEST VITELLIFORM MACULAR DYSTROPHY-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

Chiou, Shih-Hwa[1], Chang, Yun-Ching[1], Chou, Shih-Jie[1], Chen, Shih-Jen[2]
[1]Institute of Pharmacology, School of Medicine, National Yang-Ming University, Taipei, Taiwan, [2]Department of Ophthalmology, Taipei Veterans General Hospital, Taipei, Taiwan

Best vitelliform macular dystrophy, also known as Best disease (BD), is a form of inherited juvenile-onset macular degeneration. Although its pathogenesis has been linked to the mutation of retinal pigment epithelium (RPE) Bestrophin-1, effective therapeutics against BD have not been identified. In this study, we isolated BD patient-derived dental pulp-derived cells to generate patient-specific induced pluripotent stem cells (BD-iPSCs) and then differentiated BD-iPSCs into RPE-like cells (BD-RPEs) that were used as an expandable platform for in vitro drug screening. We employed several techniques such as flow cytometry, quantitative real-time reverse-transcriptase (RT)-PCR, miRNA/mRNA-microarray analysis in this study. Notably, Compared with unaffected sibling-derived iPSC-derived RPE cells (Centerl-RPEs), BD-RPEs exhibited normal RPE-specific markers but had a lower expression of the tight junction protein ZO-1 and Bestrophin-1 as well as reduced phagocytic ability. Among several candidate drugs that were screened, curcumin was the most effective for upregulating both the Bestrophin-1 and ZO-1 genes in BD-RPEs. To ensure the sustained release of curcumin, we encapsulated curcumin with PLGA nanoparticles. PLGA-coated curcumin nanoparticles (Cu-NPs) were efficiently internalized into BD-RPEs and restored both ZO-1 and Bestrophin-1 levels, as well as phagocytic ability and the function of voltage-dependent calcium channels. In conclusion, our findings demonstrated that nanomedicine-based therapy using curcumin may be an effective option for personalized BD therapy.

T-2214
INDUCED AGING IN IPSC-DERIVED CELL LINES FOR THE MODELING OF LATE-ONSET DISEASES

Cornacchia, Daniela[1], Miller, Justine D.[2], Zhang, Chao[3], Ganat, Yosif[1], Bowman, Robert L.[2], Milner, Teresa A.[3], Betel, Doron[3], Studer, Lorenz[1]
[1]Sloan-Kettering Institute for Cancer Research, New York, NY, USA, [2]Gerstner Sloan Kettering Graduate School of Biomedical Sciences, New York, NY, USA, [3]Weill Cornell Medical College, New York, NY, USA

Induced pluripotent stem cells (iPSC) represent a powerful technology for the study of human genetic disease. Our group has previously demonstrated the power of iPSC technology for modeling developmental disorders such as Familial Dysautonomia or Herpes Simplex Encephalitis. However, modeling the pathogenesis of late-onset disorders like Parkinson's (PD) or Alzheimer's disease (AD) has proven more difficult, as those disorders take decades to develop without signs of the disease early in life. In fact, current studies modeling PD using iPSC technology fail to recreate the severe degenerative pathology characteristic of the disease. These results raise a fundamental question as to how well iPSC-technology can reproduce age-dependent disorders. Recent work from our group provides compelling evidence that barriers in recreating age-related phenotypes in iPSC-derived cells can be attributed to a reversal of cellular age upon reprogramming. Transition through pluripotency brings cells back to a "younger" state, impeding the appearance of cellular phenotypes essential for disease modeling. We argue that most iPSC work done to date attempts to model late-onset disorders in iPSC-derived cells at an immature stage, when those cells are not yet expected to exhibit any degenerative disease phenotypes in the affected individual. Current approaches seek to trigger disease-phenotypes using strong stress paradigms, by challenging the system through damaging compounds such as reactive oxygen species or toxic chemicals. We reason that an acute exposure to toxic agents might not yield truly meaningful data, as it does not reflect the physiological progression of events that eventually give rise to disease. We propose that more faithful disease-modeling requires the implementation of the age component in iPSC-based systems. In line with this, our recent results demonstrate that cellular age-markers, which are erased during reprogramming, can be re-induced in iPSC-derived cells by ectopic expression of progerin, a mutant protein responsible for a form of premature aging known as Hutchinson-Gilford Progeria. This approach allowed for the first time to study late-onset phenotypes in iPSC-derived dopamine neurons from PD patients. The study also provided a first proof-of-principle that cellular rejuvenation through reprogramming inhibits the appearance of age-associated disease phenotypes, which can be triggered by accelerating cellular aging with specific factors. Here, we will present data on how to further refine our in-vitro aging paradigm with the goal of more faithfully reproducing phenotypes of late-onset diseases in iPSC-derived cells. To this end, we set out to identify global transcriptional and epigenetic "aging-signatures" associated with normal aging via comparative profiling of primary cells from young and old donors and their iPSC-derived progeny. Aligning these results to profiles of progerin-induced aging will determine to which extent premature aging resembles normal aging. Differential factors identified by our studies are employed to yield an improved "aging-cocktail", aimed at testing our primary hypothesis that induced in-vitro aging allows the development of more faithful models of late-onset degenerative disorders including PD. Our study should yield novel insights into the molecular basis of normal and premature aging and further facilitate iPSC based modeling of age-dependent disorders.

T-2216
ALLELE-PREFERRED TARGETED CORRECTION OF CFTR GENE IN CYSTIC FIBROSIS HUMAN INDUCED PLURIPOTENT STEM CELLS

Crane, Ana M.[1], Kramer, Philipp[1], Bui, Jacquelin H.[1], Li, Xuan Shirley[1], Gonzalez-Garay, Manuel L.[2], Hawkins, Finn[3], Liao, Wei[1], Mora, Daniela[1], Wang, Jianbin[4], Sun, Helena C.[4], Paschon, David E.[4], Guschin, Dmitry Y.[4], Gregory, Philip[4], Kotton, Darrell[3], Holmes, Michael C.[4], Davis, Brian R.[1]
[1]Center for Stem Cell and Regenerative Medicine, Brown Institute of Molecular Medicine, Houston, TX, USA, [2]Center for Molecular Imaging, Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center, Houston, TX, USA, [3]Boston University Pulmonary Center, Boston, MA, USA, [4]Sangamo BioSciences, Inc., Richmond, CA, USA

Recently developed reprogrammingand genome editing technologies make possible the derivation of corrected patient-specific pluripotent stem cell sources - potentially useful for the development of new therapeutic approaches. Starting with skin fibroblasts from patients diagnosed with Cystic Fibrosis, we have derived and characterized induced pluripotent stem cell (iPSC) lines. We then utilized zinc finger

nucleases (ZFNs), designed to target the endogenous *CFTR* gene, to mediate correction of the inherited genetic mutation in these patient-derived lines via homology directed repair (HDR). The corrected Cystic Fibrosis iPSCs, when induced to differentiate *in vitro*, express the corrected *CFTR* gene; importantly,*CFTR* correction resulted in restored expression of the mature CFTR glycoprotein. We observed an exquisitely sensitive, homology-dependent preference for targeting one *CFTR* allele vs. the other. Such allele-specific action offers the potential for preferential targeting of uclease-mediated correction to specific mutant alleles.

T-2217
EFFECTS OF HPRT DEFICIENCY IN HUMAN INDUCED PLURIPOTENT STEM CELLS AND NEURAL PROGENITOR CELLS

Crapper, Liam[1], Maussion, Gilles[1], Gigek, Carolina[2], Diallo, Alpha[1], Turecki, Gustavo[1], Ernst, Carl[1]
[1]McGill University, Montreal, QC, Canada, [2]Universidade Federal de São Paulo, São Paulo, Brazil

In humans HPRT deficiency causes Lesch-Nyhan disease (LND), a rare developmental disorder with a variety of metabolic and neurological symptoms including chronic self injury, gout, dystonia, and intellectual disability. While the metabolic symptoms of LND can be easily treated this has no impact on the neurological symptoms, which remain poorly understood. This is in part due to the difficulty of studying LND in animal models, which do not show any behavioural phenotypes resulting from HPRT deficiency. We have created novel models of LND by deriving induced pluripotent stem cell lines (iPSCs) and iPSC derived neural progenitors from patients with LND, and by knocking down HPRT1 expression in human ventral midbrain derived neural precursor cells. Using transcriptome sequencing and western blotting, we have identified substantial alterations in the expression of genes related to metabolism and protein translation.

T-2218
HUMAN UTERINE POLYP STEM CELLS DEDIFFERENTIATION INTO EARLY EMBRYO WITH HATCHING ACTIVITY THROUGH THE IPS GENERATING PROCESS

Dah-Ching, Ding, Chu, Tang-Yuan
Department of Obstetrics and Gynecology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan

By introduction of Oct4, Sox2, Klf4 and cMyc, human adult somatic cells can be reprogramed into embryonic stem cell capable of pluripotent differentiation. We show here in multiple lines of human endometrial polyp and cervical polyp mesenchymal stem cells (proved by morphology, surface markers and differentiation ability), introduction of these four transcription factors could induce a dedifferentiation of these cells into early embryo in three days, ranging from one-cell, two-cell, four-cell embryos, morula to blastocyst. These early embryos resembled human early embryo derived from IVF in morphology, hatching activity and expression of early embryonic genes and proteins, including Oct4, Sox2, Nanog and ZP2. In contrast, mesenchymal cells derived from normal endometrium could not be induced to dedifferentiated to such early embryos. After 30 days of culture, iPS can be formed (proved by pluripotency gene expression, in vitro and in vivo differentiation). We conclude that mesenchymal stem cells derived from tumorous stroma of the uterine mucosa could be dedifferentiated to early embryo by the iPS cocktail. Polyps of the Mullerian duct derived organ may harbor some epigenetic marks making them vulnerable to reprograming to the earliest developmental stage. This study provides a simple model to derive early human embryo by in vitro.

T-2219
CREATING A FAMILY-BASED MODEL SYSTEM FOR STUDYING LONG QT SYNDROME

Dementyeva, Elena[1], Grigor'eva, Elena[1], Vyalkova, Anna[1], Medvedev, Sergey[1], Shevchenko, Alexander[1], Elisaphenko, Eugeny[1], Bairamova, Sevda[2], Pokushalov, Evgeny[2], Ivanova, Ludmila[1], Zakian, Suren[1]
[1]Institute of Cytology and Genetics, Novosibirsk, Russian Federation, [2]State Research Institute of Circulation Pathology, Novosibirsk, Russian Federation

Long QT syndrome (LQTS) is a disease detected as a prolongation of the QT interval on electrocardiogram and leads to an increased risk of ventricular tachycardia, which may cause ventricular fibrillation and sudden death. To date the most perspective approach to model the disease is generating patient-specific cardiomyocytes carrying LQTS causing mutations using induced pluripotent stem cell technology. A number of studies have shown that such cardiomyocytes can reproduce main features of the syndrome and are a promising tool for studying LQTS mechanisms and searching for methods of effective therapy. However, LQTS is characterized by a significant variety in disease severity even among people carrying the same mutation, which requires some additional approaches to the disease modelling. In this work, several patients with documented LQTS symptoms were searched for disease causing mutations. A fibroblast culture was derived from a patient carrying V254M mutation in the KCNQ1 gene and was used for reprogramming and generating induced pluripotent cells. The cells obtained demonstrated the same pattern of gene expression and methylation as human pluripotent stem cells and differentiated into derivatives of three germ layers. The patient-specific induced pluripotent stem cells can be further differentiated into cardiomyocytes to model LQTS in vitro. Family analysis revealed several carriers of the V254M mutation that have not demonstrated any symptoms of the syndrome before. Some single nucleotide polymorphisms that have been shown to be associated with prolongation of the QT interval and LQTS severity were analyzed in symptomatic and asymptomatic carriers. These findings allow us to create a family-based model system consisting of symptomatic and asymptomatic carriers of the same mutation and healthy controls for a deeper study of the disease.

T-2220
MODELING A METABOLIC LIVER DISORDER USING INDUCED PLURIPOTENT STEM CELLS: FAMILIAL HYPERCHOLESTEROLEMIA TYPE IIA

Dianat, Noushin[1], Awan-toor, Sarah[1], Fourrier, Angélique[2], Poiron, Claire[2], Steichen, Clara[1], Goulinet Mainot, Sylvie[1], Caron, Jérôme[1], Saheb, Samir[3], Bruckert, Eric[3], Weber, Anne[1], Nguyen, Tuan Huy[2], **Dubart-Kupperschmitt, Anne[1]**
[1]Inserm U972, Villejuif, France, [2]Inserm UMR-S 1064, Nantes, France, [3]Pitié-Salpêtrière hospital- Endocrinology and Metabolism service, 75651 Paris, France

Human hepatocytes differentiated from patient's induced pluripotent stem cells (iPSCs) allow to assess the feasibility to transplant genetically modified autologous hepatocytes as an alternative to allogenic hepatocyte or liver transplantation for the treatment of genetic disorders. Familial hypercholesterolemia type IIa (FH) is an autosomic dominant genetic disease due to mutations in the gene encoding the receptor of Low Density Lipoproteins (LDLR). In heterozygous FH patients (1/500), hypercholesterolemia is already present at birth and early atherosclerotic lesions can be detected. In

these patients pharmacological treatment in particular statins are efficacious at reducing plasmatic cholesterol level, but cardiovascular complications occur from the age of 50 years. Homozygous FH patients (1/106) have very high plasmatic cholesterol level (>10 g/l) and other specialized cholesterol-lowering treatment are required such as LDL apheresis to avoid heart failure and death occurring at an early age in absence of treatment. Liver transplantation is otherwise the only curative treatment since hepatocytes are the only cells in the organism able to eliminate cholesterol via bile. The aim of our study is to generate iPSCs specific of homozygous FH patients, to differentiate them into hepatocytes, to correct the genetic defect in vitro and to evaluate the correction in FH-iPSC-derived hepatocytes compared to uncorrected in terms of molecular regulation of cholesterol pathways. We have generated several lines of FH-iPSCs from a skin biopsy from one patient homozygous for the A519T mutation in the LDLR, using a polycystronic lentivector encoding OCT3/4, SOX2, C-MYC and KLF4. Five iPSC lines were characterized for expression of stem cell markers (OCT4, NANOG, SSEA-4, TRA-1-60) by RT-PCR, immunocytochemistry and flow cytometry. Expression of the reprogramming transgenes was extinguished after approximately 10 weeks of culture depending on the cell line. DNA sequencing confirmed the presence of the original fibroblast mutation in the FH-iPSC lines. Their ability to internalize the LDL fluorescent ligand was decreased compared to normal fibroblasts. FH-iPSC lines were differentiated into bipotent hepatic progenitor cells expressing alpha fetoprotein, HNF4α and cytokeratin 19. They were then further differentiated in more mature hepatocyte-like cells characterized by the expression of specific hepatocytic markers such as albumin, alpha-1 antitrypsin and transcription factors such as HNF1β, HNF4α, FoxA2. As a first gene therapy approach, hepatic cells were transduced with a lentiviral vector expressing the human LDLR cDNA under the control of a hepatic specific promoter (Apolipoprotein A-II). Transgene expression was detected by western blot analysis. In another approach, insertion of the correcting transgene cassette (ApoA-II-LDLR) was targeted to the AAVS1 safe harbor genomic site using transcription activator-like effector nucleases (TALENs). Corrected FH-iPSCs were selected by puromycin resistance and were characterized at the molecular level for accurate targeted integration. Differentiation of these corrected clones is underway to quantify fluorescent LDL internalization and to study cholesterol pathway regulation. Our approach paves the way for preclinical studies in animal models using patient-iPSCs corrected for the genetic defect.

T-2221
CELLULAR MODELING OF FABRY DISEASE USING HUMAN INDUCED PLURIPOTENT STEM CELLS

Do, Hyo-Sang[1], Park, Sang-Wook[1], Lee, Beom-Hee[2], Yoo, Han-Wook[2], Han, Yong-Mahn[1]
[1]*Department of Biological Sciences and Center for Stem Cell Differentiation, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Republic of Korea,* [2]*Department of Pediatrics, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea*

Fabry disease (FD) is a recessive X-linked lysosomal storage disorder which is caused by α-galactosidase (*GLA*) deficiency. Dissipation of GLA activity results in excessive globotriaosylceramide (Gb3) accumulation in the most cell types, thereby leading to progressive complications. In particular, accumulation of Gb3 in vascular cells causes life-threatening complications such as ischemic stroke, hypertrophic cardiomyopathy, and renal failure at the terminal stage of Fabry disease. Here, induced pluripotent stem cells were generated from dermal fibroblasts derived from a Fabry patient (FD-iPSCs) by ectopic expression of Yamanaka's factors. The FD-hiPSCs exhibited low galactosidase activity and excessive Gb3 accumulation in undifferentiated state. Also, FD-iPSCs could differentiate vascular cells such as endothelial cells (ECs) and smooth muscle cells (SMCs) with functionality of tubule-like structure formation, although FD-ECs and SMCs represented Gb3 accumulation. Accumulated Gb3 was cleared by treatment with alpha-galactosidase recombinant protein (Fabrazyme®) during vascular differentiation of FD-iPSCs. These results indicate that the FD-iPSCs will be valuable cell sources for studying pathophysiology of Fabry disease at the cellular level.

T-2222
CRISPR/CAS9 BASED GENETIC MODIFICATION OF A1AT DEFICIENCY IPSC

Eggenschwiler, Reto, Beh-Pajooh, Abbas, Cantz, Tobias
Translational Hepatology and Stem Cell Biology, REBIRTH-Cluster of Excellence, Hannover Medical School, Hannover, Germany

Alpha 1-antitrypsin (A1AT) inhibits a wide variety of proteases in the serum by covalent binding. A congenital point mutation (E342K) leads to the expression of the PiZ isoform of A1AT, which polymerizes and gets retained in the endoplasmic reticulum of A1AT-producing hepatocytes. Homozygosity for PiZ (PiZZ) causes a 90% reduction of A1AT serum concentration and thus, severe alpha 1-antitrypsin deficiency. In this case A1AT levels are not sufficient to inhibit neutrophil elastase and the increased breakdown of elastin in the lung leads to chronic obstructive pulmonary disease (COPD). Additionally, in some patients, the accumulation of misfolded protein in hepatocytes can cause liver cirrhosis. Here, we investigate the use of CRISPR/Cas9 based genomic precision engineering in A1AT deficiency PiZZ induced pluripotent stem cells (iPSC). For this purpose, we evaluated the homologous directed recombination (HDR) gene targeting potency of four A1AT-specific sgRNAs together with Cas9_D10A nickase in an in vitro reporter assay. By using the two most potent opposite strand sgRNAs for introduction of a double nick and variation of donor plasmid amount we were able to reach up to 1% gene targeting efficiency. Finally, we compared the gene targeting accuracy of Cas9 nuclease and Cas9 nickase in patient-specific PiZZ iPSC by co-transfection of a piggyBac-flanked puromycinΔtk-selectable donor. Using a multiplex PCR based analysis we found that most of the Cas9 nuclease targeted clones had off-target integrations, while all of the nickase targeted clones showed correct monoallelic integration in the A1AT locus. Moreover, when we applied the double nick-technique we found biallelic donor integration in 40% of all clones. In selected biallelic targeted clones we then transfected piggyBac transposase and subjected the cells to FIAU counter-selection. Resistant clones were further analyzed for successful transgene excision. Taken together, we have successfully established CRISPR/Cas9 based precision genome engineering for high efficiency and high accuracy use in human cells. Using a double-nick strategy and an appropriate donor we were able to genetically correct disease-specific PiZZ iPSC.

T-2223
SYSTEMS BIOLOGY APPROACHES TO MODELING HUNTINGTON'S DISEASE

Ellerby, Lisa M., An, Mahru C., Zhang, Ningzhe, O'Brien, Robert
The Buck Institute for Research on Aging, Novato, CA, USA

Huntington's disease (HD) is an autosomal dominant inherited disease caused by a CAG expansion in the huntingtin *HTT* gene. HD patients have a movement disorder, psychological symptoms and progression impairment of cognition. We have previously published the use of isogenic genetically corrected induced pluripotent stem

cells from HD patients to model the disease. To extend this work we have generated an allelic isogenic HD stem cell model using traditional homologous recombination and CRISPR/Cas9 technology. We will report the analysis of this newly generated model using systems biology approaches geared at integrating proteomics, genomonic and metabolomics. From this analysis we have found a number of key signaling pathways that are critical to HD biology and disease.

T-2224
DIFFERENTIAL ROLE FOR DNA LIGASE 4, ARTEMIS AND DNA-PKCS IN THE GENERATION, DNA DAMAGE RESPONSE AND MYELOID DIFFERENTIATION OF HUMAN IPS CELLS

Felgentreff, Kerstin[1], Likun, Du[2], Weinacht, Katja[3], Dobbs, Kerry[1], Bartish, Margarita[2], Giliani, Silvia[4], Schlaeger, Thorsten[3], DeVine, Alexander[3], Schambach, Axel[5], Woodbine, Lisa[6], Davies, E. Graham[7], Baxi, Sachin[1], van der Burg, Mirjam[8], Gennery, Andrew[9], Manis, John[10], Pan-Hammarström, Qiang[2], Notarangelo, Luigi[1]

[1]Division of Immunology, Boston Children's Hospital, Boston, MA, USA, [2]Clinical Immunology and Transfusion Medicine, Karolinska Institute, Stockholm, Sweden, [3]Division of Hematology/Oncology, Boston Children's Hospital, Boston, MA, USA, [4]A. Nocivelli Institute for Molecular Medicine, Brescia, Italy, [5]Hannover Medical School, Hannover, Germany, [6]University of Sussex, Brighton, United Kingdom, [7]Great Ormond Street Hospital, London, United Kingdom, [8]Department of Immunology, Erasmus MC, Rotterdam, Netherlands, [9]University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, United Kingdom, [10]Department of Transfusion Medicine, Boston Children's Hospital, Boston, MA, USA

Non-homologous end joining (NHEJ) is required for efficient repair of DNA double strand breaks (DSBs) and V(D)J recombination. NHEJ defects in humans cause severe combined immunodeficiency (SCID), increased cellular sensitivity to ionizing irradiation (IR) and can be associated with growth retardation, microcephaly and neuro-developmental delay. Efficient repair of DNA DSBs is needed for reprogramming of somatic cells into induced pluripotent stem cells (iPSCs). To compare the specific contribution of DNA ligase 4 (LIG4), Artemis, and DNA-PKcs in this process, we reprogrammed patient-derived fibroblast cell lines. LIG4 and catalytic DNA-PK, but not Artemis deficiency, were associated with markedly reduced reprogramming efficiency, which could be partially restored in the LIG4-deficient fibroblasts by genetic complementation. Furthermore, we identified an increased rate of genomic instability in LIG4-deficient iPSCs. Whereas no deficient response to IR could be observed in non-synchronized iPSCs, synchronization of cell cycles revealed severe DNA repair defects in all NHEJ-deficient iPSC lines. Impaired targeted myeloid differentiation was observed in LIG4-, but not Artemis- or DNA-PKcs-mutated iPSCs. These results indicate a critical importance of the NHEJ pathway for somatic cell reprogramming, with a major role for LIG4 and DNA-PKcs, and a minor, if any for Artemis.

T-2225
PARKIN MUTATIONS REDUCE THE COMPLEXITY OF NEURONAL PROCESSES IN IPSC-DERIVED HUMAN NEURONS

Feng, Jian[1], Ren, Yong[1], Jiang, Houbo[1], Fan, Kevin[1], Wang, Jun[1], Janoschka, Stephen[2], Wang, Xiaomin[3], Ge, Shaoyu[2]

[1]Department of Physiology and Biophysics, State University of New York at Buffalo, Buffalo, NY, USA, [2]Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, NY, USA, [3]Department of Neurobiology, Capital Medical University, Beijing, China

Parkinson's disease (PD) is characterized by the degeneration of nigral dopaminergic (DA) neurons and non-DA neurons in many parts of the brain. Mutations of parkin, an E3 ubiquitin ligase that strongly binds to microtubules, are the most frequent cause of recessively inherited Parkinson's disease. The lack of robust PD phenotype in parkin knockout mice suggests a unique vulnerability of human neurons to parkin mutations. Here, we show that the complexity of neuronal processes as measured by total neurite length, number of terminals, number of branch points and Sholl analysis, was greatly reduced in induced pluripotent stem cell (iPSC)-derived TH^+ or TH^- neurons from PD patients with parkin mutations. Consistent with these, microtubule stability was significantly decreased by parkin mutations in iPSC-derived neurons. Overexpression of parkin, but not its PD-linked mutant nor GFP, restored the complexity of neuronal processes and the stability of microtubules. The results suggest that parkin maintains the morphological complexity of human neurons by stabilizing microtubules.

T-2226
IMPLEMENTATION OF CELLULAR REPROGRAMMING TO STUDY NEURODEVELOPMENTAL DISORDERS

Galat, Yekaterina[1], Perepitchka, Mariana[1], Burton, Barbara[2], Charrow, Joel[2], Iannaccone, Philip[1], **Galat, Vasil**[2]

[1]Pediatrics, Ann and Robert H. Lurie Children's Research Center, Northwestern University Feinberg School of Medicine, Chicago, IL, USA, [2]Pathology, Ann and Robert H. Lurie Children's Research Center, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

In vitro studies of monogenic neurological disorders with early onset symptoms greatly contribute to the overall understanding of these syndromes. Thus far, the work exploring cellular mechanisms of neural dysfunction as a consequence of Smith-Magenis Syndrome SMS or GRIN2B mutations has been very limited. Smith-Magenis Syndrome (SMS) is a rare neurobehavioral disorder, caused by a chromosomal deletion or mutation in the *RAI1* gene, which is characterized by multiple congenital defects including intellectual disabilities, sleep disturbances, craniofacial and skeletal disorders. *RAI1* is a transcription factor and overexpression of *RAI1*, i.e. duplication of 17p11.2 causes another neurobehavioral disorder, the Potocki-Lupski syndrome (PTLS). A recent study showed that *RAI1* regulates the transcription of the circadian genes, as well as the *BDNF* gene, previously implicated in autism. A remarkable hallmark of the phenotype is an inverted pattern of plasma melatonin levels that contributes to sleep disorders. GRIN2B encodes the N-methyl-D-aspartate (NMDA) receptor subunit NR2B. The NMDA receptor channel has been shown to be involved in long-term potentiation, an activity-dependent increase in the efficiency of synaptic transmission thought to underlie certain kinds of memory and learning. The NR2 subunit acts as the agonist-binding site for glutamate. This receptor is the predominant excitatory neurotransmitter receptor in the mammalian brain. Several recent studies have identified association of mutations in the *GRIN2B* gene with autism, schizophrenia and epilepsy. *NR2B*, the protein encoded by *GRIN2B*, is potentially targetable and modifiable through already existing compounds e.g. NMDA receptor antagonist memantine, which represents an interesting option for patients carrying activating mutations in this gene. Here, we present the successful generation and characterization of a novel patient specific iPSC model for SMS and GRIN2B mutations both of which manifest in a complex phenotype, including intellectual disability. This model provides a unique opportunity to induce neuronal differentiation in stem cells from siblings that differ in single dominant mutations critical to normal neuronal differentiation. The model was established using a non-integrating Sendai virus reprogramming system. The generated iPSCs display embryonic stem cell-like morphology, express stem

cell markers, and are capable of differentiation to three germ lineages. We further differentiated the iPSC lines to neural progenitors. We believe that by comparing gene expression data from mutant iPSCs, to "control" iPSCs and their corresponding neuronal derivatives it will be possible to discover mutation related alterations that can then be linked to impaired human neuronal development and provide a basis for discovery of novel therapeutic targets. These models present a unique tool for neurophysiology studies and provide useful insight into the complex nature of Autism spectrum disorders (ASD)

T-2227
USING CELLS FROM PATIENTS WITH MULTIPLE SYSTEMS ATROPHY OR PARKINSON'S DISEASE (PARK2, LRRK2, AND/OR GBA MUTATIONS) TO BUILD STEM CELL DERIVED DISEASE MODEL SYSTEMS

Hammer, Bonnie[1], Bi, Kun[1], Hermanson, Spencer[1], Thompson, David[1], Lebakken, Connie[1], Reichling, Laurie[1], Piekarczyk, Marian S.[1], Sampsell-Barron, Tori[1], Huang, Y. Anne[2], Vangipuram, Malini[2], Westfall, Jessica[2], Hancock, Michael[1], Revankar, Chetana[1], Langston, J. William[2], Vogel, Kurt[1], Schuele, Birgitt[2]
[1]Thermo Fisher Scientific, Madison, WI, USA, [2]The Parkinson's Institute, Sunnyvale, CA, USA

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects 1% of people over age 60 and more than five million people worldwide. PD research has been hindered by a lack of access to diseased tissue to study. However, with recent advances in stem cell biology, it is now possible to derive induced pluripotent stem cells (iPSCs) from control or PD patient fibroblasts and differentiate them into neurons. Concomitantly, there have been advances in gene editing technologies which now allow select mutations to be corrected or genes to be knocked out within iPSCs, resulting in pairs of cell lines with the same genetic background that differ by the presence or absence of specific disease-linked mutations or genes. Recently, Life Technologies has collaborated with the Parkinson's Institute to develop PD model systems using donor fibroblasts collected at the Institute. We created a set of six iPSC lines from three PD patients (harboring PARK2, LRRK2 and/or GBA mutations), one MSA patient, and two age-matched controls. TALEN gene editing was utilized to correct mutations/knock out genes within two of the patient derived iPSC lines. All of the iPSC lines were differentiated into neural stem cells (NSCs). These NSCs were directly utilized in functional assays to compare their response to a number of different cellular stressors and were further differentiated into dopaminergic neurons. We will complete our disease modeling by using the pairs of isogenic lines generated in this study to assess functional differences between edited and unedited cells in fully differentiated dopaminergic neurons.

IPS CELLS: EPIGENETICS

T-2229
GENOME-WIDE ANALYSIS REVEALS MULTIPLE CHROMATIN AND TRANSCRIPTIONAL STATES DURING REPROGRAMMING TO IPS CELLS

Hussein, Samer M., Puri, Mira C., Tonge, Peter, Consortium, Project Grandiose, Nagy, Andras
Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

Recent studies indicate that transcription factor-driven somatic cell reprogramming to induced pluripotent stem cells (iPSCs) reshapes the global genetic, epigenetic, and transcriptional landscapes of cells to achieve pluripotency. To study these changes, together with international collaborators, we conducted a massive parallel screen using next generation sequencing (NGS) to profile the transcriptome (microRNA, lncRNA (long non-coding RNA) and mRNA), CpG methylation, ChIP-sequencing (for chromatin marks: H3K4me3, H3K27me3 and H3K36me3), in addition to quantitative mass spectrometry profiling of the global and cell surface proteome of reprogramming cells. Samples were collected at specific time points during reprogramming of an inducible secondary mouse embryonic fibroblasts towards iPS cells. To reach an iPSC state we show that cells transition through multiple phases accompanied by a widespread change in chromatin state with specific transcriptional signatures, defined by both coding and non-coding gene sets. Interestingly, the dynamics of H3K27me3 drive early events during reprogramming and promote a transient open/primed chromatin state near key developmental regulators, with continued net up-regulation of gene expression. When high transgene expression is maintained, H3K27me3 is re-acquired and cells undergoing reprogramming stabilize but completely deviate from the iPSC fate, although they maintain an alternate pluripotent state. Lowering transgene expression during this transient state drives the cells closer to iPSCs. We have thus characterized a permissive cell state during reprogramming that may be highly amenable to specific environmental cues. We also show that acquisition of iPSC-like chromatin marks and DNA methylation is achieved late in reprogramming, including gain of bivalent chromatin. Our data provide panoramic insight into the mechanisms of transgene driven reprogramming, highlighting in particular the role of specific gene expression networks and their genomic and epigenetic underpinnings.

T-2230
COORDINATED REGULATION OF RE-METHYLATION BY DNMT1 AND DNMT3A/3B AFTER DEMETHYLATION THROUGH 5-HYDROXYMETHYLATION IN MOUSE EMBRYONIC STEM CELLS

Kubiura, Musashi[1], Okano, Masaki[2], Kimura, Hironobu[3], Tajima, Shoji[3], Kimura, Hiroshi[3], Tada, Masako[4]
[1]Tottori University, Yonago, Tottori, Japan, [2]RIKEN, Kobe, Japan, [3]Osaka University, Suita, Osaka, Japan, [4]Chromosome Engineering Research Center, Tottori University, Yonago, Tottori, Japan

DNA cytosine methylation (5mC) and histone modifications regulate gene expression and development in mammals. 5mC is preferentially accumulated in the heterochromatic regions and plays important roles in gene silencing and genome stability. Mouse embryonic stem cells (ESCs) possess their own 5mC profiles with the balance of DNA methyltransferase (Dnmt) activity and tet methylcytosine dioxygenase (Tet) activity. We have found that 5mC to 5-hydroxymethylcytosine (5hmC) conversion is cyclically achieved in the euchromatic regions but not in the heterochromatic regions of mouse ESCs and human induced pluripotential stem cells. This finding shows that changes in chromatin structure also extensively influence the levels of 5hmC, leading to passive control of 5mC levels. It has been reported that 5hmC conversion initiates DNA demethylation in a dependent and/or independent manner for cell cycles. However, the re-methylation mechanisms after 5mC to 5hmC conversion have not been fully discussed. The major aim of this study was to examine which Dnmt enzymes are responsible for the re-methylation event following 5hmC conversion. We visualized the intra-chromosomal distribution of 5hmC using an immunostaining technique in mouse ESCs deficient for maintenance DNA methyltransferase (Dnmt1 KO ESCs) or deficient for de novo DNA methyltransferases Dnmt3a/3b (DKO ESCs). Half of the Dnmt1 KO ESCs showed a similar 5mC distribution pattern as wild-type ESCs but the other half of the cells showed cell

cycle-mediated dilution of 5hmC on all chromosomes, in which 5hmC signals were exclusively enriched on one of the two sister chromatids of all chromosomes. Thus, the 50/50 5hmC distribution on chromatids in Dnmt1 KO ESCs may suggest that both Dnmt3a/3b and Dnmt1 play important roles for re-methylation in the echromatic regions in ESCs after Tet-mediated demethylation.Moreover, Dnmt1 may be solely involved in re-methylation, because 5hmC-positive chromosomal regions were still detected in DKO ESCs after long-term culturing. Furthermore, a quantitative increase in total 5hmC volume and 5hmC-positive cells were detected in the differentiated DKO ESCs after epiblast cell induction culturing. The 5mC to 5hmC conversion was extended to the pericentric regions in both differentiated Dnmt1 KO ESCs and DKO ESCs. This shows that both Dnmt1 and Dnmt3a/3b independently achieve maintenance of 5mC in the pericentric heterochromatin regions. Our chromosome-wide observation of 5hmC localization shows that Dnmt1, 3a, and 3b coordinately act on a genome-wide re-methylation in the euchromatin of mouse ESCs.

T-2231
WNT5A AND SFRP1 ORCHESTRATE EFFICIENCY OF DOPAMINERGIC NEURON DIFFERENTIATION FROM PROTEIN-BASED IPS CELLS

Kwon, Yoo-Wook, Cho, Hyun-Jai, Yang, Han-Mo, Lee, Sae-Won, Park, Young Bae, Lee, Eun Ju, Hur, Jin, Kim, Hyo-Soo
[1]*Seoul National University Hospital, Seoul, Republic of Korea*

In patients with Parkinson's disease (PD), stem cells can serve as therapeutic agents to restore or regenerate injured nervous system. Here, we differentiated two types of stem cells; mouse embryonic stem cells (mESCs) and protein-based iPS cells (P-iPSCs) generated by non-viral methods, into midbrain dopaminergic (mDA) neurons, and then compared the efficiency of DA neuron differentiation from these two cell types. In the undifferentiated stage, P-iPSCs expressed pluripotency markers as ES cells did, indicating that protein-based reprogramming was stable and authentic. While both stem cell types were differentiated to the terminally-matured mDA neurons, P-iPSCs showed higher DA neuron-specific markers' expression than ES cells. To investigate the mechanism of the superior induction capacity of DA neurons observed in P-iPSCs compared to ES cells, we analyzed histone modifications by genome-wide ChIP sequencing analysis and their corresponding microarray results between two cell types. We found that Wnt signaling was up-regulated, while SFRP1, a counter-acting molecule of Wnt, was more suppressed in P-iPSCs than in mESCs. In PD rat model,transplantation of neural precursor cells derived from both cell types showed improved function. The present study demonstrates that P-iPSCs could be a suitable cell source to provide patient-specific therapy for PD without ethical problems or rejection issues.

T-2232
GENETIC, EPIGENETIC AND TRANSCRIPTIONAL DIFFERENCES BETWEEN HUMAN IPSCS AND SCNT-ESCS DERIVED FROM THE SAME SOMATIC CELLS

Ma, Hong[1], Morey, Robert[2], Daughtry, Brittany L.[1], Sabatini, Karen[2], Thiagarajan, Rathi D.[3], Kang, Eunju[1], Tippner-Hedges, Rebecca[1], Ahmed, Riffat[1], Marti-Gutierrez, Nuria[1], Amato, Paula[1], Wolf, Don P.[1], Laurent, Louise[2], Mitalipov, Shoukhrat M.[1]
[1]*Oregon Health and Science University, Beaverton, OR, USA,* [2]*University of California, San Diego, La Jolla, CA, USA,* [3]*Sanford Consortium for Regenerative Medicine/UCSD, La Jolla, CA, USA*

Human iPSCs have been shown to differ from native ESCs derived from embryos with respect to their epigenetic and transcriptional signatures. It has also been observed that iPSCs tend to carry more genetic abnormalities than ESCs, though rigorous comparisons have not been carried out. To determine if the iPSC-specific signatures are secondary to reprogramming technique (i.e. overexpression of a small number of pluripotency-associated transcription factors) or to reprogramming per se, we compared iPSC lines to genetically matched pluripotent stem cell lines derived by somatic cell nuclear transfer (SCNT-ESCs). High-resolution single nucleotide polymorphism genotyping revealed that the iPSC lines contained an average of 3-fold more de novo copy number variants compared to SCNT-ESCs. Moreover, genome-wide DNA methylation analysis indicated that the iPSC lines contain 8-fold more CpG sites that retain the DNA methylation state of the parent somatic cell than their SCNT counterparts, consistent with incomplete reprogramming. Both genome-wide DNA methylation and transcriptome analyses showed that SCNT-ESCs shared features with both IVF-ESCs and iPSCs, but clustered most closely with IVF-ESCs. We conclude that transcription factor-based reprogramming is associated with higher frequency of genetic and epigenetic aberrations and residual somatic epigenetic memory indicative of incomplete reprogramming and reprogramming errors. In contrast, SCNT provides more faithful reprogramming and superior maintenance of genetic and epigenetic stability.

CHROMATIN IN STEM CELLS

T-2233
SIRT1-DEFICIENT HEMATOPOIETIC STEM AND PROGENITOR CELLS RECAPITULATE AN AGING-LIKE PHENOTYPE

Ghaffari, Saghi[1], Bigarella, Carolina[1], Izac, Brigitte[1], Donovan, Michael[1], Dieguez-Gonzalez, Rebeca[1], Brugnara, Carlo[2], Sinclair, David[2], Rimmele, Pauline[1]
[1]*Icahn School of Medicine at Mount Sinai, New York, NY, USA,* [2]*Harvard Medical School, Boston, MA, USA*

The decline of stem cell function with age is implicated in the loss of tissue homeostasis and diseases of aging. Aging of hematopoietic stem cells (HSC) is associated with an increase in the stem cell pool and a decline in stem cell function associated with their myeloid skewed differentiation with age. Although defects in the DNA damage repair program, increased tumor suppressor function and epigenetic dysregulation have all been implicated in HSC aging, the mechanisms underpinning the age-associated alterations of HSC lineage specification remain unknown. Here we show that the loss of NAD-dependent Sirtuin (SIRT)1 deacetylase that is a critical evolutionary conserved regulator of organismal longevity, results in an aging-like phenotype of HSC. We show using an adult-tamoxifen inducible SIRT1 knockout mouse model to circumvent the potential developmental adaptation of germ-line deleted SIRT1 mice (only 10% of mice reach adulthood), that loss of SIRT1 compromises severely the CD48-CD150+LSK long-term repopulating HSC frequency and function. Importantly, differentiation of SIRT1-deleted HSC is skewed towards myeloid lineage as generation of granulocyte-monocyte progenitors (GMPs) is specifically enhanced while common lymphoid progenitors (CLPs) are reduced. These abnormalities in SIRT1-deleted mice combined with anemia and significant increase of neutrophils and decrease of lymphocytes in the peripheral blood and altered expression of associated genes are strikingly reminiscent of aged HSC and their myeloid-biased differentiation. In agreement with this, like in the old bone marrow, the frequency of CD150+ Lin-Sca1+cKit+ (LSK) cells expressing CD41 that depicts myeloid-biased HSC was significantly increased in young SIRT1-deleted bone marrow. In addition, SIRT1-deleted HSC in young adult bone marrow like old HSC, expressed

significantly increased levels of P-Selectin, Fos and PML and decreased levels of Sox4. We further show using double SIRT1-Foxo3 mutant mice that SIRT1 controls HSC homeostasis via the transcription factor Foxo3. These combined findings suggest that SIRT1 may be implicated in the control of HSC aging and their lineage specification.

T-2234
TRANSCRIPTIONAL REPRESSION BY BRG1-SWI/SNF COMPLEX AFFECTS PLURIPOTENCY OF HUMAN EMBRYONIC STEM CELLS

Wang, Yuan
East China Normal University, Shanghai, China

Previous reports demonstrated that the SWI/SNF complex plays an important role in mouse embryonic stem cells (mESCs), but it remains to be determined if this complex is required for the pluripotency of human embryonic stem cells (hESCs). Using RNA interference, we demonstrated that depletion of BRG1, the catalytic subunit of SWI/SNF complex, led to impaired self-renewing ability and dysregulated lineage specification of hESCs. A unique composition of BRG1-SWI/SNF complex in hESCs was further defined by the presence of BRG1, BAF250a, BAF170, BAF155, BAF53a, and BAF47. Genome-wide expression analyses revealed that BRG1 participated in a broad range of biological processes in hESCs through pathways different from those in mESCs. In addition, ChIP-seq demonstrated that BRG1 played a repressive role in transcriptional regulation by modulating acetylation levels of histone H3 at lysine 27 at the enhancers of lineage specific genes. Our data thus provide valuable insights into novel mechanisms by which transcriptional repression affects the self-renewal and differentiation of human embryonic stem cells.

T-2235
ROLE OF THE DE NOVO DNA-METHYLTRANSFERASES DNMT3A AND DNMT3B IN EPIDERMAL STEM CELL FUNCTION: IMPACT ON TISSUE HOMEOSTASIS AND AGEING

Aznar-Benitah, Salvador
Oncology, Institute for Research in Biomedicine (IRB Barcelona), Barcelona, 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 ageing, 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 the de novo DNA methyltransferases Dnmt3a and Dnmt3b as candidates relevant for the transition from dormancy to activation, lineage specification, and 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 (ChIP-seq). Our results show that the activity of Dnmt3a and Dnmt3b is highly dynamic during epidermal stem cell differentiation with little functional overlap between them. They also uncover unexpected and previously unknown functions of both proteins that are independent of their DNA-methyltransferase activity. I will discuss the relevance of these pathways in adult tissue homeostasis and ageing.

T-2237
STABLE NON-RANDOM X CHROMOSOME INACTIVATION IN HUMAN EMBRYONIC STEM CELLS: CULTURE ARTEFACT RATHER THAN A PARENT-OF-ORIGIN SPECIFIC EVENT

Geens, Mieke, Barbé, Lise, Spits, Claudia, Dée, Kimberly, Van Haute, Lindsey, Sermon, Karen D.
Research Group Reproduction and Genetics, Vrije Universiteit Brussel, Brussels, Belgium

Female human embryonic stem cell (hESC) cultures have previously been shown to display variable X chromosome inactivation (XCI) patterns. Unlike mouse embryonic stem cells, most hESC already display XCI at the undifferentiated state. Moreover, a predominant occurrence of non-random XCI patterns has been reported; but the origin of this non-random pattern in hESC remained unresolved. In this study we found that all fifteen female hESC lines we investigated display stage III XCI, with major loss of XCI marks such as *XIST* expression and repressive histone modifications and, in some instances, erosion of methylation. Moreover, all lines which were informative for the microsatellite markers on the X chromosome we studied (11/15) show a non-random XCI pattern after methylation-sensitive DNA restriction. This pattern is maintained during long-term culture and after differentiation both to somatic cells and towards the trophoblast lineage. When comparing the hESC lines to the DNA of the donors, we found for the first time that there is no predominant inactivation of either the X chromosome inherited from the female or male donor, strongly suggesting that there is no imprinting event directing the choice of XCI and pointing to a culture-induced occurrence of non-random XCI.

T-2238
HIERARCHICAL ANNOTATION OF CHROMATIN STATES IN STEM CELLS

Marco Rubio, Eugenio[1], Meuleman, Wouter[2], Pinello, Luca[1], Wang, Jianrong[2], Kellis, Manolis[2], **Yuan, Guo-Cheng**[1]
[1]Dana-Farber Cancer Institute, Boston, MA, USA, [2]MIT, Boston, MA, USA

Epigenetic mechanisms play an important role in stem cell processes, but our understanding of epigenetic regulation is still incomplete. One major difficulty is that chromatin forms complex three-dimensional structures, leading to multiple layers of organization of the genome. Recently, consortiums like ENCODE have generated high-resolution, genome-wide distributions of the first-order chromatin structure, such as histone modifications, but methods that use them to identify multi-layer chromatin structures are still lacking. To fill this gap, we have developed a Hierarchical Hidden Markov Model (HHMM) with two-layers of chromatin states, which we call domain- and nucleosome-level states, respectively. Using this method, we analyzed a ChIPseq dataset of 9 histone marks in human embryonic stem cells, and identified a number of chromatin domains that can be validated by independent studies. At the same time, nucleosome-level states detected variations in histone modification patterns at high resolution. We found that the same nucleosome-level states may be associated with different biological functions depending on the domain-level contexts. Our approach has uncovered higher order chromatin states and provides novel insights into epigenetic regulation in normal development and diseases.

EDUCATION AND OUTREACH, ETHICS AND PUBLIC POLICY, HISTORY AND SOCIAL ISSUES

T-2239
HOW SHOULD THE NATIONAL FOOTBALL LEAGUE TACKLE UNPROVEN STEM CELL TREATMENTS?

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

From Olympians to weekend marathoners, many athletes are receiving unproven treatments in an effort to heal injuries non-surgically or to speed up recovery times after surgery. This group includes high-profile U.S. National Football League (NFL) players, several of whom have publicly advocated for these types of treatments and cite them as major reasons they are able to continue their careers. Some treatments, such as the use of anabolic steroids and human growth hormone, were studied and then banned by the league. But others, including stem cell treatments, are relatively new and have not been thoroughly reviewed in a similar manner. Consequently, stem cell treatments are not perceived as harmful as long as a banned substance is not included in the treatment. However, it is unclear whether this should or will remain the case if the types of therapies and numbers of players receiving them increase and if negative consequences arise from the use of therapies which have not been clinically researched. Each year, more than 700 stem cell clinics around the world open their doors to "stem cell tourists." Patients travel mostly from industrialized countries to developing ones in an effort to treat many ailments_ranging from autism to multiple sclerosis to paralysis_for which there is no cure and treatment options are limited. Despite the fact that scientists have not empirically demonstrated any clinical benefit, the use of stem cells as orthopedic therapies is becoming more commonplace and has drawn the attention of elite athletes, including several NFL players. In this presentation, we describe the types of stem cell treatments obtained by players in the NFL and locations of the clinics in the United States and abroad. We also review the intended and unintended consequences of high-profile players receiving and advocating for these types of therapies. Our findings suggest that American football players are increasingly seeking out new and unproven treatments such as stem cell therapies to help accelerate recoveries from injuries. Most seem to come from cities which have clinics promoting stem cell therapies locally, although they ultimately obtain the treatment abroad since it is not permitted in the United States. Overall, we determined that with the rise of new and unproven stem cell treatments, the NFL faces a daunting task of trying to better understand and oversee the use of these therapies in order to protect the health of its players. Based on data obtained from internet searches of clinic press releases, journal and news articles, as well as blogs on NFL players, it seems that players may not be aware of the risks they are taking. Moreover, because players have an outsized influence as celebrities and role models, their use of unproven stem cell treatments may lead others to view the treatments as acceptable medicine. We conclude that it is vital that the NFL and other sport leagues review these procedures to determine how best to support, evaluate, and possibly regulate stem cell therapies to ensure the safety of players and the people they influence. If the NFL stays abreast of the development of these therapies, it will protect its players and the league itself by enabling new and beneficial treatments while curbing illegitimate usage.

T-2240
CANCER STEM CELLS: ONTOLOGY AND THERAPIES

Laplane, Lucie
Gustave Roussy, Villejuif, France

What is stemness? This question entangles biological and metaphysical issues. I will use both disciplines to defend two claims (they are the result of a large bibliographical analysis on a corpus containing 1412 articles on stem cells or cancer stem cells): (i)The current biological data can be used to defend four conceptions of stem cells, which correspond to four ontologies of stemness (categorical, dispositional, relational, and systemic). More precisely, current data are insufficient to determine whether stemness is a categorical property, a disposition, a relational property, or a system property (each conception will be described during the presentation). (ii)The ontological status of stemness matters for biology because each conception of stem cells has diverse consequences for anti-cancers therapies. More precisely, the effectiveness of therapeutic strategies will depend on the ontology of stem cells. I will focus my talk on the consequences of the four ontologies of stemness regarding the cancer stem cell theory. Can we cure cancers by targeting the cancer stem cells? Most partisans of the cancer stem cell theory claim, with Reya, Morrison, Clarke and Weissman (2001), that "'in order to cure cancer, it is necessary and sufficient to kill cancer stem cells". I will show that this is true if and only if stemness is a categorical property or a disposition. Another therapeutic strategy under development is the niche-targeting. I will show that such therapies can cure cancers if and only if stemness is a disposition or a relational property. Neither cancer stem cell targeting nor niche targeting can cure cancers if stemness is a system property. I will suggest that efforts should be made to develop more effective differentiating therapies. On a theoretical level, this is the only strategy that can cure cancers in every case (categorical, dispositional, relational, and systemic). The results of my research show (i) how important it is to determine what is stemness, and (ii) how crucial it is that philosophers and biologists work together. Lastly, I would like to end by opening some questions: can we deduce the nature of one kind of stem cells from another kind of stem cells? For example, if stemness is a disposition in hematopoietic stem cells, is it also a disposition for muscle stem cells? Can we deduce the nature of one kind of stem cells in a species from the same kind of stem cells in another species? For example, if stemness is a disposition for mice hematopoietic stem cells, is it the same for human hematopoietic stem cells, or drosophila hematopoietic stem cells? And, is the ontology of stemness robust in cancers? For example, can stemness be a categorical property of cancer stem cells at the beginning of the development of a cancer and then become a systemic property?

T-2241
ETHICAL CONTENTION AND THE DEVELOPMENT OF CAREERS IN STEM CELL SCIENCE

Levine, Aaron D.[1], Pjecha, Matthew[2], Karmali, Ruchir[2]
[1]Georgia Tech, Atlanta, GA, USA, [2]School of Public Policy, Georgia Tech, Atlanta, GA, USA

Stem cell research remains a field both full of potential and surrounded by ethical contention. The implications of these ethical questions weigh heavily on policy makers and the public, but may also affect researchers themselves. This study investigated the diverse range of challenges encountered by stem cell researchers both in their graduate training and their early careers in faculty positions and assessed the extent to which these challenges were related to ethical controversy surrounding the field. Data collection consisted of a series of qualitative interviews with early career stem cell scientists. In these interviews, early career

stem cell scientists were asked to reflect on their experiences as graduate students, post-docs and junior faculty and discuss how they confronted the challenges associated with their field. While ethical concerns relating to the research itself were reported by many interviewees, most of the challenges reported were related to securing funding and materials amid a complex regulatory bureaucracy, both in graduate school and as early career faculty. These challenges were often perceived as being greater than those experienced by peers in less ethically contentious fields. Participants regularly reported frustration over the large amount of time spent meeting oversight requirements. Furthermore, the shifting policy environment led some scientists to change research topics or avoid certain topics entirely, due to uncertainty about future regulations and restrictions. In the transition from graduate student to post-doc to faculty, participants highlighted the importance of networking, particularly having colleagues and advisors willing to put students in contact with departments in other institutions. Maintaining productivity with extremely limited time and managing a laboratory also emerged as consistent challenges associated with navigating the first few years in a faculty position. This study concludes by considering the implications of these findings for policymakers aiming to improve science education and facilitate the development of scientific careers in stem cell science and other ethically contentious fields.

T-2242
THE UBC STEM CELL CLUB: IMPROVING THE QUALITY AND QUANTITY OF MEMBERSHIP ON CANADA'S STEM CELL DONOR DATABASE

Fingrut, Warren, Charman, Erin, Sokalski, Kristen, McIntyre, McKyla, Boudreau, Kalun, Hicks, Riley, Li, Calvin, Subedi, Manisha
University of British Columbia, Vancouver, BC, Canada

Many patients with blood diseases require a hematopoietic stem cell transplant as part of their treatment. These patients need to find a donor that is a genetic match, and they frequently rely on unrelated donors. Canada's stem cell donor database is used to match potential donors to patients in need. Individuals age 17-35 can register to join this database online or at stem cell drives, where they provide consent and swab their cheeks to provide a tissue sample. It is challenging to secure a match for a stem cell transplant; currently, over 1000 Canadians cannot find a match. Patients are more likely to match to a donor in their own ethnic group. Additionally, younger donor age improves outcomes and survival of transplant recipients, and males are the preferred donors, as female donors increase the risk of the recipients developing chronic graft-versus-host disease. However, males under age 35 only represent 12% of the current Canadian donor-database (5% non-Caucasian males). The UBC Stem Cell Club was founded two years ago, aiming to increase membership on the stem cell donor-database and address the need for young, ethnically-diverse, and male registrants. We operate at all four distributed sites of the UBC medical program. We aim to recruit over 1500 donors each year and to target the most-needed demographics: ethnically-diverse males under age 35. The UBC Stem Cell Club has established a community partnership with OneMatch Stem Cell and Marrow Network: we are accredited to run drives independently. We run stem cell drives targeting ethnically diverse and male university students. We also piloted a rural stem cell drive in Inuvik, NWT, Canada, targeting young, Aboriginal males. Prior to all drives, volunteers complete training emphasizing our target demographics and achievement of informed consent. Results were compiled from post-event reports. We have coordinated 21 stem cell drives (17 at university campuses), and recruited 2309 potential stem cell donors. Our campus drives in Metro Vancouver, Victoria, Prince George, and Kelowna have signed up 1191, 434, 95, and 87 registrants respectively. Our community drives have recruited 457 registrants. From November 2012 - February 2014, 73.5% of the 1212 registrants recruited at our university drives were male, and 100% were under 35. From October 2013-February 2014, of the 118 males recruited at our university campus drives, 34.7% self-reported as non-Caucasian and 86.4% were age 25 and under. Our Inuvik and Prince George stem cell drives collectively recruited 28 Aboriginal males under age 35, increasing the representation of this demographic group on Canada's stem cell donor registry by up to 5.5%. The UBC Stem Cell Club recruits students as well as the general public in BC to become stem cell donors. Our drives improve the quantity and quality of membership on Canada's stem cell donor database.

T-2243
HUMAN PLURIPOTENT STEM CELL RESEARCH AND DATA SHARING AGREEMENTS: DEVELOPING PROPORTIONAL THRESHOLDS FOR INTERNATIONAL CONSORTIA, BIOBANKS AND REGISTRIES

Isasi, Rosario[1], Andrews, Peter W.[2], Baltz, Jay M.[3], Bredenoord, Annelien L.[4], Burton, Paul[5], Chiu, Ing-Ming[6], Hull, Sara Chandros[7], Jung, Ji-Won[8], Kurtz, Andreas[9], Lomax, Geoffrey[10], Ludwig, Tenneille E.[11], McDonald, Michael[12], Morris, Clive[13], Ng, Huck Hui[14], Rooke, Heather[15], Sharma, Alka[16], Stacey, Glyn N.[17], Williams, Clare[18], Knoppers, Bartha Maria[19]
[1]Centre of Genomics and Policy, McGill University, Montreal, QC, Canada, [2]Department of Biomedical Science, University of Sheffield, Sheffield, United Kingdom, [3]Ottawa Hospital Research Institute, University of Ottawa, Ottawa, ON, Canada, [4]Julius Center, Department of Medical Ethics, University Medical Center Utrech, Utrech, Netherlands, [5]School of Social and Community Medicine, University of Bristol, Bristol, United Kingdom, [6]Natl Health Research Institute, Jhunan, Taiwan, [7]NHGRI Bioethics Core, National Institutes of Health, Bethesda, MD, USA, [8]Division of Intractable Diseases, National Institute of Health, Chungcheongbuk-do, Republic of Korea, [9]Berlin Center for Regenerative Therapies, Berlin, Germany, [10]CIRM, San Francisco, CA, USA, [11]WiCell Research Institute, Madison, WI, USA, [12]W. Maurice Young Centre for Applied Ethics, University of British Columbia, Vancouver, BC, Canada, [13]NHMRC, Canberra ACT, Australia, [14]Genome Institute Singapore, Singapore, Singapore, [15]ISSCR, [16]Ministry of Science and Technology, New Delhi, India, [17]National Institute for Biological Standards and Control, Hertfordshire, United Kingdom, [18]Department of Sociology and Communications, Brunel University, London, United Kingdom, [19]Centre of Genomics and Policy, McGill University, Montreal, QC, Canada

Biobanks serve as the primary resource for access to authenticated, quality-controlled and ethically sourced human pluripotent stem cell (hPSC) lines. Their scientific value is predicated on the quality of the data associated with their curated cell lines. Proposals to integrate existing biobanks with electronic medical records and other health administrative databases to create converging genomic platforms will be an important step in building robust core infrastructures that can support the generation of data and stem cells at a population scale. Collections of hPSC lines including well-annotated genomic, epigenomic, and donors'- research participants' phenotypic and demographic data are essential, given that genetic and epigenetic variations contained in the lines could have an impact on the value of their associated data. Comprehensive data curation facilitates disease modeling, drug development, and further contributes to the understanding of genetic variation and its role in normal cell behavior. Moreover, next generation sequencing technologies - from microarrays to whole genome analysis - provide data on a wide range of healthy and sick participants with the potential to contribute to the clinical translation of cell based-therapies and personalized medicine. Several scientific and social developments are prompting reconsideration of

the ways in which the scientific and ethical imperative of data sharing and security are not only conceptualized but also implemented. Data sharing is envisaged as a tripartite responsibility of data producers, users, and funders. These factors, together with reports of the ease of re-identification in the scientific literature and popular press, contribute to changing public attitudes regarding the meaning of individual privacy and attendant expectations about the fiduciary duties of data stewards. In the context of hPSC research specifically, this presentation will propose guidance for research consortia, stem cell banks, and registries, in the drafting of data sharing agreements that are proportional to the nature of cell line derivation (e.g. hESC vs. iPSCs). We offer policy recommendations for determining thresholds for the release of individual and summary data (e.g. genomic, epigenomic, phenotypic, and demographic) as well as identity profiles (e.g. STR, SNP, etc.) associated with an hPSC line to *bona fide* researchers. It is our hope that the policy framework we propose will further stimulate foresight debate and policy action. We trust that our recommendations will also address the concerns of a wide range of stakeholders including researchers, policy-makers, funders, patients and participants alike.

T-2244
OUTLOOK OF PROJECTS TO SUPPORT STEM CELL R AND D POLICY IN KOREA

Kim, Moo Woong[1], Kim, Min Jung[1], Kim, Janghwan[1], Choi, Byung Hyune[2], Park, So Ra[2], Han, Yong-Mahn[3], Hyun, Byung Hwan[1]
[1]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea, [2]Global Stem Cell and Regenerative Medicine Acceleration Center, Incheon, Republic of Korea, [3]Korea Advanced Institute of Science and Technology, Daejeon, Republic of Korea

As future medical paradigm is gradually switched from disease customized treatment to person customized treatment, interests and expectations in stem cell research are increasing. In addition, therapeutic agents for incurable diseases which were impossible to be cured previously and chronic diseases are expected to be released to the global market on the basis of the rapid development of stem cell technology and achievement of excellent research. Every country is actively promoting the support policy to develop related technology and preoccupy the market due to increase in the value of stem cell research and outlook of industry vitalization. Korea is taking the lead in the commercialization of global stem cell therapeutic agents as acquiring item licenses of three stem cell therapeutic agents*. Moreover, Korea makes efforts to establish various policies and institutions of science and technology to vitalize stem cell research at national level. Korea has established and implemented 'stem cell research comprehensive promotion plan ('07~'15) for the mid-/long-term, and finds plans for stem cell R and D efficient investment. In particular, as rapidly changing R and D environmental change and future outlook analysis are becoming important, Korea is operating a professional organization to support strategic planning and practical facilitation through issue analysis in the field of stem cells. Thus, this report would like to introduce the current situation of stem cell R and D policy support in Korea with KoNSCRT (Korea Network for Stem Cell Research and Tech-Development) project in Ministry of Science, ICT and Future planning and GSRAC (Global Stem Cell and Regenerative Medicine Acceleration Center) in Ministry of Health and Welfare which are domestic major stem cell policy support projects.

T-2245
A STUDY ON BUILDING PATIENT-RESEARCHER PARTNERSHIP FOR CLINICAL TRIALS USING IPSCS

Muto, Kaori[1], Kusunose, Mayumi[2], Arimatsu, Yasuyoshi[3], Doi, Kentaro[3], Higashijima, Jin[4], Takahashi, Masayo[5]
[1]The Institute of Tokyo, The University of Tokyo, Tokyo, Japan, [2]Institute of Medical Science, University of Tokyo, Tokyo, Japan, [3]Japanese Retinitis Pigmentosa Society, Tokyo, Japan, [4]Shinshu University, Matsumoto, Japan, [5]RIKEN Center for Developmental Biology Kobe Institute, Chuo-ku, Kobe, Japan

Patient involvement to clinical research was highly recommended. "Collaborative partnership" was added as eighth principle for ethical clinical research (Emanuel et al, 2000) and Japanese stem cell research guidelines assign principle investigators to consider opinions from advocacy groups seriously (2-1- (5)). Since the world's first clinical trials using iPSCs for patients with exudative Age-Related Macular Degeneration (ARMD) was approved in Japan in 2013, clinical trials with iPSCs have attracted attention of Japanese patients with incurable diseases who expect better medical treatments. Some advocacy groups have begun to request active involvement as prospective research subjects. However, we have few successful examples. Retinitis Pigmentosa (RP) is one of the targeted diseases for clinical trials using iPSCs. To prepare successful patient involvement and to establish better protocols based on patients' opinions, we conducted a dialogue session with a principle investigator (PI) of clinical trials using iPSCs. Leaders (Arimatsu and Doi) of Japanese Retinitis Pigmentosa Society (JRPS) called for a dialogue session. A research ethicist (Muto), a clinical ethicist (Kusunose) and a science communicator (Higashijima) have prepared the session with them and invited a PI (Takahashi). The session's purpose was to provide prospective research participants with information on participation and build a patient-researcher partnership. Session goals were to provide participants an opportunity to (1) learn about risks, benefits, rights, and duties of human research subjects; (2) understand the concept of autonomous participants; (3) identify information patients see as vital in considering research participation; (4) begin to build a trusting relationship; (5) confirm workshop design efficacy. A verbatim transcript of the sessions was analyzed. We held a three-hour-and-a-half workshop with 40 members of JRPS on October 20, 2013. The processes were: (1) explanation of the protocol by the PI, (2) Q and A to the PI, (3) lectures on ethical points of views, (4) small group discussions (8 groups, consisting of 6 people), (5) presentation by all small groups, and (6) commentary by the PI. Throughout, participants showed a positive attitude about the protocol, though most had concerns about safety, mental health care, and additional limitation of daily life during the trial period. Participants asked for advice on avoiding adverse events by their efforts to reduce dropout and information on recommended medical examination for clearing inclusion criteria. The PI replied all questions by her commentary. We concluded that the workshop achieved initial goals and such opportunities should be continued until the trial would start. Both patients and the PI gained new knowledge and perspectives, realizing issues they had never considered. The whole processes of the session were transcribed and would be distributed as textbooks for patients. We also learned that an independent ethical intervention would be meaningful. For example, ethical advice for securing consent withdraws by a research team and also by an advocacy group was new to both, who shared prompt completion of trials as a common goal.

T-2246

STEM CELL TOURISM, PHYSICIAN INVOLVEMENT AND THE ROLE OF PROFESSIONAL REGULATION

Zarzeczny, Amy

Johnson-Shoyama Graduate School of Public Policy, University of Regina, Regina, SK, Canada

The growth of an international market for stem-cell based interventions, commonly referred to as stem cell tourism, is a complex and multi-faceted phenomenon. Operating primarily on an online, direct-to-consumer basis, stem cell tourism involves the pursuit of stem-cell based interventions that fall outside the approved standard of care for a given condition and in the absence of peer-review or ethics oversight for experimental treatment. Patients bear the expense of these interventions directly, and the cost typically appears to be substantial. Stem cell tourism has been associated with a number of concerns including physical risk to the patient as a result of the intervention, financial hardship for the patient and his/her supporters, reputational vulnerability for the field of legitimate stem cell science, and adverse implications for the medical system in the patient's home jurisdiction, among others. Over the last number of years, a wide range of responses has developed in an attempt to address these concerns. Governments have tightened national regulations, patient advocacy organizations have issued advice to their members, and improved public information and resources on topic have been made available, among other efforts. Nonetheless, research suggests the market continues to thrive, increasing calls for further action. Physicians play a variety of roles in the stem cell tourism market, ranging from being sources of information, advice and referrals to directly providing the stem-cell based interventions. In many jurisdictions around the world, physicians are subject to some form of professional regulation. For example, in Canada, physicians are members of a self-regulating profession and are held to certain professional standards. In this research, we examine the role of professional regulation in responding to physician involvement in stem cell tourism. We have produced a series of case studies from various jurisdictions around the world looking at instances where physicians have been subject to professional discipline for their involvement in stem cell tourism. We conducted a field survey to gather data on physician participation in this market and on the relevant regulatory regimes in associated jurisdictions. We also conducted interviews with representatives from provincial Colleges of Physicians and Surgeons (the professional regulatory bodies for physicians in Canada) to inform an assessment of how this conduct would be treated in Canada. The results from this research highlight the potential professional regulation has for addressing concerning conduct on the part of physicians in the stem cell tourism market. Professional regulation is a relatively direct and efficient mechanism and may prove a valuable tool for policy efforts in this realm.

NEURAL CELLS

T-3001

HUMAN ES CELL-BASED MODELING OF PEDIATRIC GLIOMAS BY K27M MUTATION IN THE H3.3 HISTONE VARIANT

Funato, Kosuke[1], Major, Tamara[1], Lewis, Peter W.[2], Allis, C David[3], Tabar, Viviane[1]

[1]*Memorial Sloan-Kettering Cancer Center, New York, NY, USA,* [2]*University of Wisconsin, Madison, WI, USA,* [3]*Rockefeller University, New York, NY, USA*

Human pluripotent stem cells (hPSCs) have been the subject of intense study in the past decade, raising hopes for translational applications, disease modeling and greater understanding of human development. Here we explore a novel aspect of hPSCs, namely the ability to model human tumors *in vitro* and *in vivo*. Diffuse intrinsic pontine gliomas (DIPG) are an aggressive and highly fatal form of pediatric brain tumors that arise in the brainstem of young children. Recent data suggest that a single amino acid substitution mutation in the H3.3 histone variant, known as K27M, occurs at high frequency in DIPGs, along with mutations in the *PDGFRA* and *TP53* genes. Here we differentiate human ES cells (H9) into neural precursors (NPC) using the dual Smad inhibition protocol. Lentiviral constructs encoding for a constitutively active form of PDGFRA, shRNA for p53 and K27M were introduced into the NPCs singly or in combinations, and maintained under selection. Our data show that expression of K27M was sufficient to induce some increased proliferation, compared to mock or WT H3.3 expression. The oncogene combination of PDGFRA and sh-p53 also resulted in increased proliferation, but the combination of all 3 factors resulted in a substantial increase in the Ki-67 index (32.4 ± 1.2 % *vs* 15.2 ± 3.6 % in control NPCs, $p<0.001$). Under low density culture conditions, cells in all groups had poor survival and expansion, but cells expressing the 3-factor combination exhibited robust growth (>5-fold, $p<0.04$). Additional *in vitro* testing demonstrates an increase in migration and matrigel invasion by the PDGFRA/sh-p53/K27M group, compared to the rest (>1.9-fold, $p<0.007$). These data provided convincing evidence of transformation *in vitro*. *In vivo* evidence was obtained by injecting the different cell groups into the brainstem of NOD-SCID mice. This led to the emergence of infiltrative tumors of human origin in the pons and the surrounding subarachnoid cisterns that resemble human DIPGs (in 58% of animals receiving cells with all 3 factors *vs* 11% for the combination of PDGFRA, sh-p53 and WT H3.3; mock infected cells did not lead to any tumors). Genomewide analysis indicates global changes in the chromatin landscape, and enrichment of the H3K27me3 marks at several master regulator gene sites. Furthermore, comparison with the reported data from DIPG patients shows a shared expression signature between our transformed cells and patient tumors with K27M mutation. Overall, these data support a transformative role for the K27M mutation on neural precursors. We also demonstrate that human ES cells represent an excellent platform for the modeling of human tumors *in vitro* and *in vivo*, which could potentially lead to the elucidation of the molecular mechanisms underlying neoplastic transformation and the identification of novel therapeutic targets.

T-3002

SPONTANEOUS FIRING PATTERNS AND PHARMACOLOGICAL RESPONSE IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONS

Odawara, Aoi[1], Alhebshi, Amani[1], **Trujillo, Michael**[2], Gotoh, Masao[3], Suzuki, Ikuro[1]

[1]*Graduate School of Bionics, Tokyo University of Technology, Tokyo, Japan,* [2]*Alpha MED Scientific, Berkeley, CA, USA,* [3]*Tokyo University of Technology, Tokyo, Japan*

Neuronal cells can be generated from Human induced pluripotent stem cells (hiPSCs), providing a very important alternatives to studies in humans and model organisms. The use of hiPSC-derived neurons affords insight into the mechanisms of neurological diseases and the identification of novel therapeutics. Long-term electrophysiological viability of cultured human iPSC-derived neurons has been sparsely investigated. Here, we used a multi-electrode array system (MED64) to investigate the functional characteristics of hiPSC-derived neurons with respect to their long-term spontaneous activity and drug responsiveness. We demonstrated that hiPSC-derived neurons are electrophysiologically active for >120 days in culture and demonstrated

long-term spontaneous activity. Three spontaneous firing patterns were observed: 1) tonic firing (single spike firing at 0.03-4.2 Hz, 2) burst firing (numerous spikes over a period of time) and 3) a combination of burst and tonic firing. After >3 months in culture, we observed synchronous burst firing activity due to synaptic transmission within neuronal networks. Compared with rat neurons, hiPSC-derived neurons required a longer time to reach functional maturity. Furthermore, addition of the GABA receptor antagonist bicuculline and AMPA/kainite receptor antagonist CNQX induced significant changes in the firing rate. These results suggest that long-term electrophysiological measurements in hiPSC-derived neurons using the MED64 system may be beneficial for clarifying the functional characteristics of human neuronal circuits and for drug screening applications.

T-3003
REGULATION OF MIDBRAIN DOPAMINERGIC NEUROGENESIS BY LXR LIGANDS

Arenas, Ernest[1], Toledo, Enrique M.[1], Kitambi, Satish S.[1], van Wijk, Kim[1], Sinha, Indranil[1], Islam, Saiful[1], Ravindran, Geeta[1], Theofilopoulos, Spyridon[1], Jakobsson, Tomas[2], Gyllborg, Daniel[1], Saltó, Carmen[1], Ernfors, Patrik[1], Steffensen, Knut[2], Linnarsson, Sten[1]
[1]MBB, Karolinska Institute, Stockholm, Sweden, [2]Laboratory Medicine, Karolinska Institute, Stockholm, Sweden

Liver X receptors (LXRs) alpha (NR1H3) and beta (NR1H2) are ligand dependent nuclear receptors that are required for midbrain dopaminergic and motor neuron development and maintenance. We have recently identified endogenous brain LXR ligands that constitute an entirely new class of highly selective and cell type-specific regulators of neurogenesis and survival. However, the molecular mechanisms by which some LXR ligands regulate midbrain dopamine (mDA) neurogenesis, while others regulate red nucleus neurogenesis is unknown. In order to investigate this mechanism we first performed RNA-sequencing, on midbrain neurospheres treated with Cholic acid (CA) or 24,25-epoxy-cholesterol (EC), to induce red nucleus or dopaminergic neurogenesis, respectively. We found that the transcriptional profiles of midbrain cultures treated with CA or EC were very different and that Wnt/β-catenin signaling was significantly enriched (p-value=3.77E-9, KEGG pathways) in the regulatory network of EC, but absent in that of CA. Moreover, we found that LXRs regulated Wnt/β-catenin signaling in a ligand dependent manner. This finding provided a link to neurogenesis because Wnt/β-catenin controls multiple aspects of mDA neuron development, including mDA neurogenesis in the midbrain floor plate. We next performed LXR Chromatin-IP combined with sequencing (ChIP-Seq) and found that most direct target genes are known for their involvement in lipid metabolism. Provided the multiple links between lipid metabolism and neural development, we are currently performing loss and gain of function studies of direct LXR target genes in order to identify whether they regulate mDA neurogenesis. Finally, we previously found that EC, but not CA, promoted mDA neurogenesis in mES cell cultures. We recently examined the potential application of EC to promote mDA differentiation in human pluripotent stem cells and found that that EC increases the numbers of human mDA neurons, suggesting that LXRs ligands may contribute to the development of cell replacement and regenerative therapies for Parkinson's disease.

T-3004
P53 INHIBITION PROVOKES THE DIRECT CONVERSION OF HUMAN FIBROBLASTS INTO MULTIPLE NEURONAL SUBTYPES

Babos, Kimberley Nicole[1], Kisler, Kassandra[2], Li, Yichen[1], Shi, Yingxiao[1], Zlokovic, Berislav V.[2], Ichida, Justin[3]
[1]Department of Stem Cell Biology and Regenerative Medicine, University of Southern California, Los Angeles, CA, USA, [2]Department of Physiology and Biophysics, University of Southern California, Los Angeles, CA, USA, [3]Department of Stem Cell Biology and Regenerative Medicine, USC, Los Angeles, CA, USA

Direct lineage conversion provides the fastest route to the production of patient-specific neurons for translational studies. We previously identified a collection of 7 transcription factors that convert mouse and human fibroblasts directly into spinal motor neurons, the nerve cells that actuate muscle movement and are selectively lost in patients with amyotrophic lateral sclerosis. However, the inefficiency of reprogramming and the difficulty in generating bona fide neurons directly from adult patient samples currently limits the utility of this approach. Here, we show that upon expression of a dominant-negative form of p53, we see upwards of a 20-fold increase in the production of induced motor neurons (iMNs) from adult human fibroblasts upon p53 inhibition, leading to approximately half of the starting fibroblasts becoming motor neurons. We have validated these iMNs to possess gene expression patterns and electrophysiology signatures typical of those seen in bona fide motor neurons. Furthermore, by introduction of an optimized, light-activated channelrhodopsin protein into our reprogramming system, we demonstrate that iMNs generated under p53 inhibition form functional neuromuscular junctions. Strikingly, we also find that p53 inhibition permits mouse and human somatic cells to reach more complete conversion, as p53-inhibited iMNs possess more mature, complex morphological components and electrophysiology. Additionally, we find that p53 inhibition improves conversion into other neuronal subtypes, as evidenced by its ability to promote the generation of GABAergic neurons from somatic fibroblasts when using an alternate transcription factor cocktail. Treatment with a small molecule p53 inhibitor also increases the number of iMNs generated, demonstrating the translational potential of p53 inhibition in lineage reprogramming. Notably, we find that p53 acts through an apoptosis-independent mechanism during the early stages of this reprogramming system to increase conversion. We show that following p53 inhibition, cell division is markedly increased and this is accompanied by a sharp upregulation of neurogenic transcription factors. Conversely, limiting cell division by irradiation or mitomycin C treatment at levels that are non-toxic to neurons potently reduces induced neuron formation. Thus, our results show that p53's inhibitory role in reprogramming extends beyond iPSC generation and indicate that cell division in early stages of reprogramming also drives conversion into post-mitotic cell types. Transient inhibition of this pathway allows for the highly efficient production of bona fide, subtype-specific neurons directly from adult patient fibroblasts.

T-3005
DIRECT CONVERSION OF ADULT SPINAL CORD-DERIVED OLIGODENDROCYTE PROGENITOR CELLS TO A NEURONAL FATE

Bazarek, Stanley[1], Marr, Robert A.[1], Peterson, Daniel A.[2]
[1]Neuroscience, Rosalind Franklin University, North Chicago, IL, USA, [2]Center for Regenerative Medicine, North Chicago, IL, USA

The lack of cell replacement following neurological injury, limits the regenerative response of the CNS. Progress in understanding the

biology of neural stem cells has raised interest in using stem cells for replacing neurons lost to injury or to disease. As existing committed and uncommitted cells in the CNS do not naturally progress to a neuronal fate, it will be necessary to engineer a conversion to a neuronal fate. Advances in cellular reprogramming provide new tools for re-specification of cell fate and provide a potential alternative to cell transplantation, namely the direct in vivo conversion of resident CNS cell populations for neuronal replacement. Success in this approach will require the generation of relevant neuronal subtypes. The aim of this study was to evaluate the effect of various neurogenic transcription factors, including sox2, mash1, olig2, pax6, and neurogenin2, that are related to cell specification during development on fate induction and subtype specificity on resident glia in the spinal cord. We have used cultures of adult- spinal cord derived oligodendrocyte progenitor cells (OPCs) to evaluate the potential for their engineered conversion to neurons. OPCs are the most abundant cycling population in the adult CNS and their isolation provides an ideal in vitro assay for screening neuronal determinants. Our results show that retroviral delivery of neurogenin2 or the combination of sox2 and mash1 to adult spinal cord OPCs in vitro can directly convert these cells into neurons through transcription factor mediated reprogramming and provide an alternative therapeutic strategy for neuronal replacement in the adult spinal cord.

T-3006
DECIPHERING THE SPATIAL AND DYNAMIC GENETIC REGULATION OF THE MOUSE OLFACTORY BULB NEUROGENESIS

Beclin, Christophe[1], Wild, Stefan[2], Mahnoun, Yann[1], Bosio, Andreas[3], Cremer, Harold[1]
[1]CNRS-IBDM, Marseille, France, [2]Miltenyi Biotec, Bergisch Gladbach, Germany, [3]Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

In the postnatal and adult mouse brain neuronal stem cells (NSC) boarding the lateral ventricles generate permanently neuronal progenitors that migrate into the olfactory bulb (OB) where they differentiate into inter-neurons. These new neurons are highly heterogeneous with respect to neurotransmitter phenotype, position in their target layers and innervation pattern. This neuronal heterogeneity is based on regionalized neural stem cells occupying defined positions along the wall of the lateral ventricles. In this project we aimed at identifying molecular mechanisms that control (1) the regionalization and determination of the stem cell in the SVZ and (2) the differentiation sequence that drives a stem cell into a mature neuron. To specifically characterize the diversity of neuronal precursors at the gene expression level we used in vivo electroporation technics that allows the targeted labeling of defined pools of NSC in the SVZ. After electroporation, labeled neuronal precursors progress in cohort along the differentiation sequence to integrate in the OB. FACS sorting of GFP electroporated NSC allowed us to isolate neuronal precursors issued from defined regions of the SVZ at specific successive differentiation stage, from a very immature to a differentiated state. RNA amplification of sorted cells combined with micro-array and bioinformatic analysis allowed high-resolution analysis of gene expression in space and time. At a global level we demonstrated a striking difference in the control of cell-cycling between progenitors in the lateral and dorsal SVZ. We validated this difference by BrdU immuno-staining and functional analysis. We propose that this differential control of cell division of neural progenitors in the SVZ participates in the control of the repartition between the different neuronal subtypes generated in the OB. At the level of individual genes we identified a set of candidate factors for defined functions in stem cell determination and differentiation. Using the same in vivo electroporation technics that allowed us to label the sorted cells we are currently analyzing the functional involvement of these factors in the neurogenic process.

T-3007
TNF ALPHA SIGNALING IN NEURAL STEM CELLS

Belenguer-Sánchez, Germán[1], Morante-Redolat, Jose Manuel[2], Porlan, Eva[1], Martí-Prado, Beatriz[1], Delgado, Ana[1], Farinas, Isabel[1]
[1]Biología Celular y Parasitología, Universidad de Valencia, Valencia, Spain, [2]Biología Celular y Parasitología, Universidad de Valencia/ CIBERNED, Valencia, Spain

Neurogenesis persists in specific niches of the adult mammalian brain and is supported by long-lived stem cells present in these locations. The subependymal zone (SEZ) of the adult murine brain is a very active neurogenic niche in which a relatively quiescent population of radial glia/astrocyte-like GFAP+ neural stem cells (NSC) continually produce new neurons and oligodendrocytes, via a population of rapidly-diving transit-amplifying progenitor cells. Although some intrinsic determinants are known to regulate stem cell division, the observation that stem cells can respond to excessive cellular demand in pathological situations or after traumatic injury suggests that signals present in their microenvironment or niche contribute to the regulation. Tumor necrosis factor (TNF) has been characterized as a pro-inflammatory cytokine prototypically involved in the innate immune response, but it is a multifunctional protein with a broad range of activities in different systems. Although TNFα is characteristically produced by immune cells, it is also expressed in many other cell types. In the adult brain, TNFα is produced by microglia and infiltrating macrophages, but can also be produced by astrocytes and neurons upon injury. Increasing evidence indicates that immune cells and immunological mediators modulate neurogenesis. In this context, effects of pro-inflammatory cytokines that are produced under non-physiological conditions, such as irradiation, inflammation, status epilepticus or stroke, on neurogenesis have been described. However, their effects appeared sometimes contradictory, suggesting potentially distinct effects depending on the cell or receptor type involved. TNFα interaction with the TNF-R1 generally triggers apoptosis and cytotoxicity whereas engagement of the TNF-R2 is usually associated with cell protection and proliferation. Neurogenesis of mice carrying deletions in each or both TNFα receptors exhibit alterations in the generation of new neurons in the dentate gyrus under basal conditions, an indication that a physiological source of TNFα regulates adult neurogenesis in this neurogenic niche. In the present work, we have evaluated the involvement of TNFα signaling in the SEZ by analyzing the role of the two TNFα receptors, both *in vivo* and *in vitro* neurosphere cultures using specific TNFR1 and TNFR2 agonist and TNFR *knock-out* mice for each receptor. We find that each receptor mediates a different biological response under physiological conditions and upon inflammation modulating proliferation, self-renewal and the balance of symmetrical/asymmetrical divisions of both neural stem cells and the related cancer stem cells present in glioma. Biochemical data shows that the observed TNFR1 related responses depend on transcriptional activation of Nf-κB signaling while TNFR2 seems to be acting trough an independent pathway.

T-3008
CXXC5 INTERACTS WITH TET3 DIOXYGENASE - IMPLICATIONS FOR THE REGULATION OF WNT SIGNALING IN NEURAL STEM CELLS

Biswas, Moumita, Hermanson, Ola
Karolinska Institute Neuroscience, Stockholm, Sweden

We initially characterized CXXC5 and its role in the cytoplasm as a BMP4-regulated modulator of Wnt signaling through its interaction

with the signaling intermediate Dishevelled. BMP4 and Wnt3a are crucial for proper development of the forebrain, and treatment with BMP4 and Wnt3a of cortical neural stem cells (NSCs) results in increased differentiation into AMPA-responsive neurons in vitro. CXXC5 mRNA is localized to a domain of the dorsal developing cortex, close to and overlapping with BMP4 and Wnt3a mRNA, and recent reports have strengthened the coupling between BMP-Wnt signaling and CXXC5 function. Already in the initial report we noted that CXXC5 was localized both in the cytoplasm and in the nucleus in NSCs (J Biol Chem, 2009). CXXC-domains have been shown to bind to DNA in different manners, and may play an essential role for the recruitment of TET3 dioxygenase to DNA and thereby the control of 5 mC hydroxylation. TET dioxygenases have been implicated in the regulation of stem cell characteristics. We have recently unveiled an unexpected role for TET3 in NSCs being required for proper glucocorticoid regulation of Wnt-signaling modulators and DNA methylation. There are several isoforms of TET3 with or without a CXXC domain. We hypothesized that there was an interaction, physical and/or functional, between TET3 and CXXC5 in NSCs controlling expression of Wnt signaling modulators and thereby fundamental characteristics of NSCs and forebrain development. Indeed, we have found that nuclear CXXC5 and TET3 exist in several isoforms in NSCs and co-immunoprecipitation experiments revealed a direct interaction between CXXC5 and TET3. As both CXXC5 and TET3 are regulating Wnt signaling in neural stem cells, current experiments are aiming at dissecting the specific roles for the genes, isoforms, and interactions of these protein products in regards to BMP and Wnt signaling, and thus NSC characteristics.

T-3009
MODELING DISEASE CAUSING DEFECTS IN LIPID METABOLISM IN MOUSE AND HUMAN NEURAL STEM CELLS

Bowers, Megan[1], Vidmar, Mojca[1], Jessberger, Sebastian[2]
[1]Brain Research Institute, University of Zurich, Zurich, Switzerland, [2]University of Zurich, Zurich, Switzerland

In the hippocampus, new neurons are generated throughout life from neural stem progenitor cells (NSPC) residing in the dentate gyrus in a process known as adult neurogenesis. Several studies in which these adult born neurons were ablated have established a requirement for hippocampal adult neurogenesis in specific types of learning and memory in rodents. Although it is now clear that adult neurogenesis persists throughout life in the hippocampus of humans as well, it remains controversial whether adult neurogenesis plays a similarly important role in human cognitive processes. Recent work has identified fatty acid synthase (FASN), a key enzyme for *de novo* lipogenesis, as a critical regulator of NSPC proliferation, and consequently adult neurogenesis, in the mouse. Furthermore, FASN function is required for hippocampus-dependent spatial learning and memory in a mouse model of running-induced stimulation of adult neurogenesis. Underscoring the relevance of these findings to humans, a point mutation in *FASN* was identified in individuals with non-syndromic cognitive impairment. Taken together, these findings suggest an intriguing hypothesis that the cognitive deficits observed in humans with *FASN* mutations may be explained, at least in part, by impaired adult neurogenesis. To address this hypothesis, and to determine the neurobiological phenotype caused by the FASN point mutation, zinc finger nucleases (ZFNs) were engineered to generate mouse knock-in and human embryonic stem cell (hESC) lines with the FASN point mutation linked to cognitive impairment in humans. Two founder mice transheterozygous for the point mutation and a deletion were successfully generated from two rounds of pronuclear injections. Similar to mouse NSPCs, pharmacologic inhibition of FASN function inhibits proliferation of neural progenitor cells derived from hESCs *in vitro*, suggesting a conserved requirement for FASN function in NSPC proliferation. Future analyses will focus on the effect of the FASN point mutation on neural stem cell properties, mature neuronal function, and lipid metabolism in NSPCs and neurons derived from targeted hESCs. In combination, these *in vivo* and *in vitro* models will potentially elucidate the relationship between defects in *de novo* lipogenesis and neurobiological dysfunction in humans.

T-3010
3RD VENTRICLE NEURAL STEM CELL NICHE DYNAMICS FOLLOWING CEREBRAL ISCHEMIA

Bronstein, Robert, Tsirka, Stella
Pharmacological Sciences, Stony Brook University, Stony Brook, NY, USA

It is now well established that the adult mammalian brain maintains neural stem cell reserves in two discrete regions, the dentate gyrus (DG) of the hippocampal formation, and in the sub-ventricular zone (SVZ) lying along the lateral ventricle wall. In recent years the 3rd ventricle lying proximal to the hypothalamus has also been shown to house limited-potential progenitors, most likely either α or β tanycytes (a radial glia-like cell population), which slowly differentiate in response to leptin and insulin-growth factor (IGF) signalling - finally adding neuronal and glial cells (NG2+) to the arcuate and paraventricular nuclei. The proliferative zone of the 3rd ventricle also extends into thalamic territory, though whether adult neurogenesis occurs in these dorsal reaches of the 3rd ventricle remains an open question. We studied the response of 3rd ventricle neural stem cells (NSCs) to focal thalamic lacunar ischemia in wild-type (WT) C57Bl6/J mice as well a mouse model with a global knock-out of the high mobility group B2 (HMGB2) gene - loss of which leads to heightened neurogenesis in the adult SVZ NSC niche. We have previously established a role for this chromatin protein (HMGB2) in modulation of embryonic neurogenesis via shotgun proteomics, and in the adult HMGB2 knock-out mouse SVZ. It is critically important to characterize and potentially "boost" endogenous cell replacement by NSCs following ischemic injury in thalamic and hypothalamic territories, potentially through signalling of chromatin/transcription factors such as HMGB2, which could lead to insights into conditions such as Dejerine-Roussy syndrome (thalamic pain syndrome) as well as heat shock/exhaustion (hypothalamic in origin) - as both are caused by ischemic damage to those particular brain regions.

T-3011
A UNIQUE PALISADE PATTERNED EXTRACELLULAR MATRIX SUSTAINS THE TERMINAL SCHWANN CELL STRUCTURE IN THE HAIR FOLLICLE STEM CELL NICHE

Cheng, Chun-Chun[1], Ban-Sanzen, Noriko[1], Watt, Fiona M.[2], Fujiwara, Hironobu[1]
[1]RIKEN Center for Developmental Biology, Kobe, Japan, [2]Centre for Stem Cells and Regenerative Medicine, London, United Kingdom

Extracellular matrix (ECM) is important in establishing tissue organization and function. By virtue of its remarkable heterogeneity in composition, the ECM contributes to the spatial organization of cell-specific environments and integrates different types of cells into exquisite organs, such as hair follicles. The upper bulge of the hair follicle is surrounded by the piloneural complex which is composed of sensory afferents and terminal Schwann cells (tSCs). Although the neural targeting to the upper bulge seems essential for proper sensory functions and hair follicle stem cell regulation, the underlying mechanisms are unknown. Here, we found that an ECM protein EGFL6

was specifically localized around the upper bulge and showed highly organized palisade pattern. EGFL6 was tightly associated with tSCs by interdigitating with their protrusions. The palisade pattern of EGFL6 was developed just after the formation of the parallel protrusions of tSCs during the hair follicle morphogenesis. In Egfl6 null mice, the morphology and position of tSCs were disrupted: the protrusions of tSCs were shorter and frequently collapsed and stacked. The position of tSCs was slightly lower along the hair follicle, overlapping with the K15-popsitive bulge area. Taken together, we identified a unique palisade patterned ECM in the upper bulge and demonstrated that it is essential for the morphogenesis and positioning of tSCs.

T-3012
NEURAL STEM CELL ACTIVITY IN HUMAN NEUROGENIC ZONES IN ALZHEIMER'S DISEASE

Chou, Athena Vai Lam[1], Mackenzie, Ian R.[2], Roskams, Jane[3]
[1]Department of Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada, [2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada, [3]Zoology, University of British Columbia, Vancouver, BC, Canada

Adult neural stem cells (NSCs) in the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus (DG) give rise to olfactory interneurons and hippocampal granule cells, a process that declines during normal aging. However, adult neurogenesis does not appear to alleviate the progressive neuronal loss in Alzheimer's disease (AD). Early-onset familial AD contains mutations in presenilin 1 and 2 (PS1 and PS2), both of which encode the catalytic subunits of the γ-secretase, which can cleave Notch receptors upon Notch activation. This, in turn, regulates NSC maintenance and the expression of a downstream target, Brain Lipid Binding Protein (BLBP, FABP7), in adult neural stem/progenitor cells (NSPCs). So the combination of increased progenitor demand due to neuronal loss, coupled with NSC dysregulation by abnormal proteolysis of Notch receptors by γ-secretase could contribute to ongoing AD pathogenesis. We have tested if the neurogenic zones of post-mortem human brain contain surviving putative NSPCs. In AD post-mortem brain, we have identified potential NSPCs along the lateral ventricle in the SVZ expressing Sox2 and BLBP. The cytoarchitecture of the neurogenic niches is highly variable among AD patients, demonstrating significant structural and cellular heterogeneity between individuals. Also, the proliferative activity of BLBP+ cells is dependent on the cell density in surrounding enviornment. These data strongly suggest that some NSPCs are active in the neurogenic niche in AD. In addition, we have identified a population of BLBP+ putative progenitors in the SGZ showing radial glia morphology with mitotic activity, whereas the remaining BLBP+ cells are located in the molecular layer of the hippocampus with a protoplasmic morphology. In the hippocampus of AD patients, there are fewer number of BLBP+ cells and these cells do not have radial glia-like morphology. This is potentially due to the presence of amyloid plaques and neurofibrillary tangles in the hippocampus.

T-3013
EFFECT OF ADIPOSE DERIVED MESENCHYMAL STEM CELLS ADMINISTRATION AND THERAPEUTIC INDUCTION OF HYPOTHERMIA ON DELAYED NEURONAL DEATH AFTER TRANSIENT GLOBAL CEREBRAL ISCHEMIA

Chung, Tae Nyoung[1], Kim, Jin Hee[2], Kwon, Sung Won[3], Suh, Sang Won[2]
[1]Emergency Medicine, CHA Bundang Medical Center, CHA University, Seongnam-Si, Gyeonggi-Do, Republic of Korea, [2]Physiology, Hallym University College of Medicine, Chuncheon-Si, Gangwon-Do, Republic of Korea, [3]Surgery, CHA Bundang Medical Center, CHA University, Gyunggi-Do, Republic of Korea

Background: Global cerebral ischemia (GCI) is the most important cause of poor prognosis after successful resuscitation from cardiac arrest. Therapeutic induction of hypothermia (TH) has shown its efficacy in reduction of the neurologic damage from GCI through various laboratory and clinical researches, so it is recommended as a standard post-cardiac arrest care. There were a few recent works which had shown the effect of mesenchymal stem cell (MSC) on GCI. However, any of them did not compare the effect with that of TH, which is a current standard therapy. Moreover, they also did not assess the interaction with TH, which may limit clinical application. We aimed to show the effect of MSC on the neurologic recovery after GCI including the comparison with that of TH, and to assess the interaction in case of combined application of TH and MSC (TH/MSC). Materials and methods: Rats were subjected to 7 min of electroencephalography-confirmed transient GCI and randomized into 4 intervention groups: placebo control, TH (2 hr, 32~34°C), intravenous injection of human adipose derived MSC (1 x 106), and TH/MSC, along with 4 sham groups treated identically. Rats were sacrificed 7 days after insult. Neuronal death was evaluated by Fluoro Jade B staining (FJB). Microglial activation was evaluated using CD11b immunostaining and previously-defined quantification critieria. IgG, myeloperoxidase (MPO), and 4HNE immunostaining were performed to detect blood brain barrier disruption, neutrophil infiltration, and oxidative injury. Time until the animal removed adhesive tapes on both forepaws was measured to test behavioral function, a week after the insult. Analysis of variance (ANOVA) was performed for comparison of outcome measures of experimental groups, and Bonferroni post-hoc analysis (PH) was done. Results: No degenerating neuron was detected by FJB in any of shams. There were differences in degenerating neuron count of each GCI group at CA1, CA3, and hilus region ($p = <0.001$, 0.004, and 0.033). PH detected differences at: control (146.83) vs TH (66.50), MSC (19.85), TH/MSC (12.60) in CA1; control (26.38) vs MSC (1.40), TH/MSC (2.12) in CA3; control (6.78) vs MSC (0.43) in hilus. Difference in microglial activation of subject groups was found ($p<0.001$). PH showed: no difference among 4 shams (0.34, 0.43, 0.24, 0.34); differences at: 4 shams vs control (2.85), TH (1.61); sham MSC (0.24) vs MSC (0.99); control vs TH, MSC, TH/MSC (0.58); TH vs MSC, TH/MSC. Difference in IgG leakage of subject groups was detected ($p<0.001$). PH revealed: no difference among 4 shams(1.19, 1.25, 1.08, 1.09); differences at: 4 shams vs control (3.08); control vs TH (1.55), MSC (1.73), TH/MSC (1.18). There was no difference in MPO(+) cell count of subject groups ($p=0.052$). Difference in 4HNE intensity of subject groups was found ($p<0.001$). PH showed: no difference among 4 shams(173.00, 172.59, 171.79, 173.12); differences at: 4 shams vs control (214.34); control vs TH (192.48), MSC (179.), TH/MSC (175.55); TH vs MSC, TH/MSC. Difference in behavioral function of normal and GCI groups was found ($p<0.001$), and PH showed the differences at control (153) vs other groups (46.25, 49.00, 48.50, 42.17). Conclusions: Administration of MSC after transient GCI has a prominent protective effect on delayed neuron death, even

compared with TH. There was no negative interaction in TH/MSC. The present results may be a base of further clinical application of MSC as a potential therapeutic strategy for intervention of GCI after cardiac arrest.

T-3014
PROSPECTIVE IDENTIFICATION AND PURIFICATION OF QUIESCENT ADULT NEURAL STEM CELLS FROM THEIR IN VIVO NICHE

Codega, Paolo, Silva-Vargas, Violeta, Paul, Alex, Maldonado-Soto, Angel Ricardo, DeLeo, Annina M., Pastrana, Erika, Doetsch, Fiona
Columbia University, New York, NY, USA

Adult neurogenic niches harbor quiescent neural stem cells, however their in vivo identity has been elusive. Here, we prospectively isolate GFAP+CD133+ (quiescent neural stem cells, qNSCs) and GFAP+CD133+EGFR+ (activated neural stem cells, aNSCs) from the adult ventricular-subventricular zone. aNSCs are rapidly cycling, highly neurogenic in vivo and enriched in colony-forming cells in vitro. In contrast, qNSCs are largely dormant in vivo, generate olfactory bulb interneurons with slower kinetics, and only rarely form colonies in vitro. Moreover, qNSCs are Nestin-negative, a marker widely used for neural stem cells. Upon activation, qNSCs upregulate Nestin and EGFR, and become highly proliferative. Notably, qNSCs and aNSCs can interconvert in vitro. Transcriptome analysis reveals that qNSCs share features with quiescent stem cells from other organs. Finally, small molecule screening identified the GPCR ligands, S1P and PGD2, as factors that actively maintain the quiescent state of qNSCs.

T-3015
THE ROLE OF NEUROFIBROMIN IN STEM CELL DIFFERENTIATION

Coleman, Natalia[1], Chakraborty, Adri[2], Barald, Kate F.[3]
[1]Biology, New Jersey City University, Jersey City, NJ, USA, [2]Biological Sciences, University of the Sciences, Philadelphia, PA, USA, [3]University of Michigan Medical School, Department of Cell and Developmental Biology and Biomedical Engineering, Ann Arbor, MI, USA

Neurofibromin is a product of the tumor suppressor gene Nf1 which is responsible for neurofibromatosis type 1. When NF1 is mutated, Ras is constitutively active, enabling affected cells to proliferate extensively. This proliferation can result in tumor formation, particularly in neural crest-derived cells in the peripheral nervous system. While it is common knowledge that neurofibromin is involved in tumor formation, little is known about its function in neural stem cell proliferation and differentiation in normal and pathological settings. It has been shown that Nf1 loss effects neural stem cell proliferation and differentiation in vivo. Here we study the effects of neurofibromin on differentiation of mouse embryonic stem cells expressing different levels of neurofibromin. We have shown that the mouse embryonic stem cells of all NF1 genotypes in our possession retain their pluripotency and can be differentiated into neuronal cells. Systematic study of complex molecular events in signaling pathways that occur in mouse embryonic stem cells that have one (Nf1+/-), both (Nf1+/+) or neither (Nf1-/-) functional allele during in vitro differentiation are critical for a better understanding of the biology of neurofibromatosis and identification of potential targets.

T-3016
HUMAN NEURAL PRECURSOR CELLS PROMOTE NEUROLOGIC RECOVERY IN A VIRAL MODEL OF MULTIPLE SCLEROSIS

Coleman, Ronald[1], Chen, Lu[2], Leang, Ronika[2], Tran, Ha T.[1], Peterson, Suzanne[1], Kopf, Alexandra[2], Walsh, Craig M.[2], Sears-Kraxberger, Ilse[2], Steward, Oswald[2], Macklin, Wendy[3], Lane, Tom[2], Loring, Jeanne F.[1]
[1]The Scripps Research Institute, La Jolla, CA, USA, [2]University of California, Irvine, Irvine, CA, USA, [3]University of Colorado, Aurora, CO, USA

Multiple Sclerosis (MS) is an immune-mediated chronic disease of the central nervous system (CNS) that is characterized by demyelination and progressive debilitation. Using mice infected with a neurotropic JHM variant of mouse hepatitis virus (JHMV) as a model of MS, we injected human neural precursor cells (hNPCs) derived from human pluripotent stem cells (hPSCs) to explore treatment options for the disease. Two weeks after JHMV infection, hNPCs were transplanted into the spinal cords of mice that exhibited clinical symptoms of the disease. The mice were then scored daily on a clinical diagnostic scale. Mice that were treated with the hNPCs showed significant improvement over mice that received a control treatment as well as mice that received human dermal fibroblast transplants. Additional experiments showed continuing improvement for 3 months post transplantation and sustained recovery for at least 6 months. Spinal cords of mice sacrificed 21 days post-hNPC transplantation showed remyelination and decreased macrophage and T-cell infiltration. The decrease in inflammatory cells was correlated with the increase in remyelination. In order to determine the fate of the transplanted hNPCs, WA09 hES cells were transduced with lentivirus containing an integrating vector with the P. pyralis luciferase gene driven by the constitutive cytomegalovirus (CMV) promoter. These cells retained expression of the reporter after differentiation to hNPCs. Mice transplanted with the labeled hNPCs were injected with luciferin and imaged daily. No luciferase-expressing hNPCs were detected after day 7 post-transplantation, indicating that the cells were rejected. To examine the mechanism of hNPC-mediated recovery, we performed whole genome expression profiling studies on the transplanted cells. We identified genes that were differentially expressed in hNPCs relative to fibroblasts and undifferentiated cells. Because of the short survival time of the transplanted cells, we focused on differentially expressed genes coding for secreted proteins. The expression of these genes was verified through quantitative real-time PCR (qRTPCR) and further validated in in vitro and in vivo systems. Our results show that hNPC transplantation can mediate recovery in a mouse model of MS. Although the hNPCs are rapidly cleared, the mice showed a robust and stable recovery that included remyelination, decrease of inflammation and a lessening of pathological symptoms. We are currently working toward identifying the factor(s) produced by hNPCs that are responsible for the phenotypic recovery. Long term, we hope to develop novel stem cell-based therapies to help recovery of MS patients.

T-3017

A NOVEL SCREEN IDENTIFIES A DOSE-DEPENDENT ROLE FOR AN EPIGENETIC MODIFIER IN NEUROGENESIS AND GLIOBLASTOMA

Coutinho, Fiona[1], Remke, Marc[1], Gallo, Marco[1], Fortier, Simon[2], Head, Renee[1], Clarke, Ian D.[1], Sauvageau, Guy[3], Taylor, Michael D.[1], Dirks, Peter B.[1]

[1]Developmental and Stem Cell Biology, Hospital for Sick Children, Toronto, ON, Canada, [2]Institute for Research in Immunology and Cancer, Montreal, QC, Canada, [3]Institute for Research in Immunology and Cancer, Montreal, QC, Canada

Glioblastoma is the most common and deadliest adult malignant brain tumour. Primary glioblastoma cultures derived in our lab exhibit neural precursor properties but show a block in differentiation, often incapable of achieving a true post-mitotic state. We set out to identify genes that may contribute to this block. We screened a collection of 1300 mouse embryonic stem cell clones carrying haplodeletions spanning over 25% of the mouse genome to identify haploinsufficient genes that impair neurogenesis. We identified several clones haplodeficient in a common region of the mouse genome that achieve a neural progenitor state but fail to further differentiate to neurons. One gene in this region is an epigenetic modifier (EM) that has previously been shown to be important in developmental processes and is highly expressed in the adult mammalian brain. Interestingly, we show that a large subset of Grade IV adult human glioblastomas show genetic loss of this gene as well as reduced expression. This lower expression corresponds with significantly poorer survival. Primary glioblastoma cultures derived in the lab also segregate into high and low expressing groups, with high EM expression roughly corresponding to levels seen in control normal neural stem cells. In vitro, EM-low glioblastoma primary cultures show reduced propensity to undergo neuronal differentiation and preliminary data also show a reduction in differentiation of EM-low tumours in vivo. Expression subgroups have a corresponding gene signature that may be used as a predictor of outcome and suggest possible downstream mechanisms. We hypothesize that loss of expression of EM impairs the ability of glioblastoma stem-like cells to differentiate. This, together with the loss of tumour suppressor function, enables cancer cells to remain in a stem-like state and propagate the tumour. Further experiments are underway to probe the potential cooperation of EM with known tumour suppressors in glioblastoma.

T-3018

CGMP COMPLIANT PROCESS TO PRODUCE DAY-14 TRANSPLANTABLE DOPAMINERGIC NEURON PRECURSOR

Couture, Sylvana M.[1], Lin, Chih- Min[1], Hall, Christine[2], John, Gutierrez[1], Lopez, Patricia[1], Huang, Patricia[1], Krishnan, Aparna[1], Kong, Derek[1], Javier, Heather[1], Dang, Wei[1], Zeng, Xianmin[3], Hsu, David[1], Couture, Larry[4]

[1]Center for Applied Technology Development, Center for Biomedicine and Genetics, Beckman Research Institute of City of Hope, Duarte, CA, USA, [2]Beckman Research Institute of City of Hope, Duarte, CA, USA, [3]Buck Institute for Research on Aging, Novato, CA, USA, [4]CATD, Beckman Research Institute, City of Hope, Duarte, CA, USA

One of the key issues to bringing human embryonic stem cell (hESC)-based technology from bench to bedside is developing defined hESC culture processes and differentiation processes to produce sufficient amount of therapeutically relevant cells under Current Good Manufacture Practice (cGMP). Here we report a successful adaptation of a laboratory procedure into a scalable and cGMP compliant process, focusing on optimization of the process for day-14 transplantable dopaminergic (DA) neuron precursor production and the selection of cGMP-compliant reagents. We showed the equivalency between laboratory procedure and the cGMP process generated DA progenitors. The cells generated under cGMP compliant process exhibited similar culture growth characteristic and marker expression as the cells produced by the laboratory procedure as demonstrated by the following analysis: FACS analysis of PAX6 expression as a marker for neural stem cell (NSC); immunocytochemistry staining of TH and B-tubulin-III as marker for dopaminergic neuron; RT-qPCR of DBH and SERT as contaminant markers of noradrenergic and hindbrain neurons. In addition,we demonstrated the feasibility of generating a large intermediate cell bank (ICB) of neural stem cell for the production of DA neuron precursors. A cryopreserved ICB would greatly streamline the manufacturing process and reduce the lot-to-lot variability. In conclusion, we have successfully optimized and adapted a laboratory procedure for producing day-14 transplantable DA neuron precursor into scalable cGMP-compliant manufacture process which would provide great potential for clinical application.

T-3019

ELECTROACTIVE POLYMER POLYPYRROLE PROMOTES NEURONAL DIFFERENTIATION OF HUMAN NEURAL STEM CELLS: A BIOCOMPATIBLE PLATFORM FOR ADVANCED NEURAL TISSUE ENGINEERING

Crook, Jeremy M., Stewart, Elise, Kobayashi, Nao, Higgins, Michael J., Quigley, Anita Frances, Kapsa, Robert MI, Wallace, Gordon G.

ARC Centre of Excelence for Electromaterials Science, University of Wollongong, Wollongong, Australia,

The advent of stem cell medicine has bolstered interest in the development of biocompatible conductive polymer (CP)-based devices for *in vitro* and *in vivo* cell-support, drug discovery and tissue engineering. We have demonstrated the differentiation of novel clinically relevant human neural stem cells (hNSCs) on the electroactive CP Polypyrrole (PPy) containing the anionic dopant dodecylbenzenesulfonate (DBS). Electrical stimulation of PPy(DBS)-based hNSCs resulted in differentiation to predominantly Tuj1 expressing neurons, with lower induction of GFAP expressing glial cells. In addition, electrically stimulated PPy(DBS)-neurons exhibited a greater total neurite length per cell, mean neurite length and maximum neurite length compared to other treatments. Although neurite number and branching was decreased for hNSCs differentiated on PPY(DBS) compared to standard glass-based differentiation, electrical stimulation of PPy(DBS) film resulted in a greater number of neurites and increased neurite branching compared to unstimulated cells on film. Conversely, standard glass-based differentiation of unstimulated hNSCs had greater numbers of shorter neurites with less branching. Finally, unlike unstimulated cultures, stimulated PPy(DBS)-based cultures comprised nodes or clusters of neurons joined by neurite networks. Topography studies of PPy films support the notion that physical cues provided by the substrate can affect stem cell differentiation, with smoother surfaces potentially conducive to neuronal induction. Also, cyclic voltammetry was consistent with reports of an effect of reduction potential on cell support, with PPy(DBS) having the lowest redox potential compared to other films tested. In conclusion, our findings support the biocompatibility of electroactive PPy(DBS) with human stem cells and use for human neural tissue engineering. CP(dopant) formulations and their electrostatic property may be used to promote specific differentiation outcomes in human stem cells and neuronal networking. Although we have limited our studies to the use of PPy(DBA) as a 2 dimensional (D) culture substrate, a next major priority and challenge is to identify CPs that can be configured into electroactive 3D scaffolds for promoting more specific cell phenotypes,

larger-scale cell culture and refined tissue engineering. Such advanced constructs will inevitably integrate additional biomolecules to fine tune temporospatial control, greater neuronal subtype selectivity, and optimal *in vivo* neural tissue integration and function.

T-3020
GENETICALLY CORRECTED HUMAN IPSC-DERIVED NEURAL PROGENITOR CELLS ENGINEERED TO OVEREXPRESS ARYLSULFATASE A REDUCE CENTRAL NERVOUS SULFATIDE STORAGE IN A MOUSE MODEL OF METACHROMATIC LEUKODYSTROPHY

Doerr, Jonas[1], Böckenhoff, Annika[2], Ewald, Benjamin[1], Eckhardt, Matthias[2], Gieselmann, Volkmar[2], Matzner, Ulrich[2], Ladewig, Julia[1], Brüstle, Oliver[1], Koch, Philipp[1]
[1]*Institute of Reconstructive Neurobiology, Bonn, Germany,* [2]*Institute of Biochemistry and Molecular Biology, Bonn, Germany*

Metachromatic leukodystrophy (MLD) is an inherited lysosomal storage disorder resulting from a deficiency of arylsulfatase A (ARSA), an enzyme that catalyzes the first step in the degradation of 3-O-sulfoglactosylceramide (sulfatide). Deficiency of ARSA leads to the accumulation of sulfatides in oligodendrocytes, Schwann cells and some neurons and triggers progressive demyelinization, the neuropathological hallmark of MLD. Several therapeutic approaches have been explored, including direct enzyme replacement, autologous hematopoietic stem cell-based gene therapy, intracerebral gene therapy or cell-based gene delivery into the central nervous system (CNS). Although positive effects were reported for some tissues and the peripheral nervous system, long-term treatment of the blood-brain-barrier protected CNS remains challenging. Here we used neural and glial progenitor cells generated from induced pluripotent stem cells (iPSCs) of a patient suffering from MLD and introduced high ARSA activity by lentiviral overexpression. Following transplantation of neural and glial precursors into a mouse model of MLD we observed a significant reduction of sulfatide storage up to a distance of >300 µm from grafted cells. Our data indicate that neural stem cells generated via reprogramming from MLD patients can be engineered to ameliorate sulfatide accumulation and might serve as an autologous cell-based vehicle for long-term supply of ARSA in MLD-patients' brains.

T-3021
SPATIO-TEMPORAL HETEROGENEITY IN THE CENTRAL CANAL OF THE MOUSE SPINAL CORD

Douglas, Kathryn[1], Roskams, Jane I.[2]
[1]*Neuroscience, University of British Columbia, Vancouver, BC, Canada,* [2]*University of British Colombia, Vancouver, BC, Canada*

The Allen spinal cord atlas (ASCA) reports the expression of > 20,000 genes throughout both the juvenile (post natal day 4; PND 4) and adult (PND 56) mouse spinal cord (SC). We originally used these data to mine for cell-specific expression to enhance our understanding of the identity and heterogeneity of spinal cord progenitors. Using a combination of olfactory ensheathing cell and embryonic radial glia proteins as a target "signature" profile, we have found that a sub-population of Spinal cord Radial Glia (SCRG) persist into adulthood that are distinct from astrocytes in both morphology and gene expression. Using Brain Lipid Binding Protein (BLBP, aka FABP7) and Glial Fibrillary Acidic Protein (GFAP) to demarcate the anatomical location of putative progenitors, we identified a novel spinal cord radial glial cell (SCRG), anchored laterally at the SC margin and arrayed radially toward the central canal (CC), with the distinct morphological appearance of radial glia (RG). Here, we turn our attention to the CC, where we have found a temporal and spatial segmentation of cells expressing distinct groups of genes. Gene ontology analysis suggests that different regions of the CC may contribute in different ways to spinal repair, and that P4 and P56 spinal cord may have some shared and some distinct mechanisms of regulation. This analysis also reveals potential regulatory pathways that are distinct from other adult progenitors that may be manipulated to enhance repair in the context of injury and SC disease. We are currently testing if subsets of CC progenitors in mouse spinal cord preferentially respond to different lesion scenarios after EAE, spinal cord injury and at the onset of ALS-like symptoms in a Tg mouse.

T-3023
SOLUBLE FACTOR-MEDIATED DIFFERENTIATION OF HUMAN NEURONAL PROGENITORS TOWARDS RETINAL PIGMENT EPITHELIUM-LIKE CELLS

Enzmann, Volker[1], Trepp, Carolyn[1], Tamò, Luca[2], Wolf, Sebastian[1]
[1]*Department of Ophthalmology, University of Bern, Bern, Switzerland,* [2]*Department of Pulmonary Medicine, University of Bern, Bern, Switzerland*

Purpose**:** Degeneration of the retinal pigment epithelium (RPE) is the main etiology of several retinal diseases. The use of stem/progenitor cells to replace the damaged tissue has been proposed recently. The aim of the study was to investigate whether immortalized human neuronal progenitor cells were able to differentiate towards RPE-like cells. Methods: ReNcells, human cortical neuronal progenitor cells (Millipore, Temecula, CA, USA), were used for differentiation. The cells were incubated with RPE-conditioned medium (CM), pigment epithelium-derived factor (PEDF) or retinoic acid (RA) for up to 21 days. Gene and protein expression of stem/progenitor (nestin, Sox2), neuronal (βIII-tubulin), glial (GFAP) and RPE (RPE65, bestrophin) markers were analyzed by qRT-PCR and immunohistochemistry (IHC). Additional retinal genes (MITF, CRALBP, PAX-6, CHX-10) were investigated by qRT-PCR. Morphological changes were tracked by live cell imaging and RPE-related function after differentiation was tested by an *in vitro* phagocytosis assay. Results: In comparison to undifferentiated ReNcells**,** nestin and SOX-2 were downregulated on the protein level but slightly upregulated or unchanged on the gene level, respectively. On the other hand, βIII-tubulin was upregulated on both levels under all treatment conditions, whereas GFAP gene and protein levels were increased significantly in RA-treated cells only. RPE65 and bestrophin gene expression was upregulated after treatment with RA or under all conditions, respectively. However, IHC showed increased protein expression only for bestrophin. Gene expression of CHX-10, MITF and CRALBP was upregulated in all differentiated cells, whereas PAX-6 was upregulated only in RA-treated samples. Following an initial proliferation phase where the cell morphology did not change, cell processes were elongated and the volume of the somas was reduced. Furthermore, ReNcells cultured with RA showed higher phagocytosis rate than cells at the undifferentiated stage. Conclusions: The most efficient substance for differentiation towards RPE-like cells appeared to be RA. Therefore, these cells might be a new source for regenerative treatment of degenerative retinal diseases.

T-3024
HIGHLY EFFICIENT NEURAL CONVERSION OF HUMAN PLURIPOTENT STEM CELLS IN ADHERENT AND ANIMAL-FREE CONDITIONS

Erceg, Slaven, Lukovic, Dunja, Bhattacharya, Shomi S., Diez, Andrea
CABIMER, Seville, Spain

Human pluripotent stem cells (hPSCs) which encompass human embryonic stem cells (hESCs) and human induced pluripotent stem cells (ihPSCs), have a wide appeal for numerous basic biology studies

and for therapeutic applications due to their potential for renewal and to give rise to almost any cell type in the human body. Derivation of neural progenitors from pluripotent stem cells holds promise for investigation of human neurogenesis, studying the development of the central nervous system (CNS) and neural diseases and has potential for cell therapy applications to treat various neurodegenerative diseases such as Parkinson's disease or spinal cord injury. The majority of these cell lines are differentiated in the presence of animal feeder cell lines or animal components, which bears risk of xenogenetic pathogen cross-transfer, limiting thus their medical applications. For these reasons, efforts have been initiated to develop animal ingredients-free conditions for culturing and differentiation of hESC. Protocols with EBs yield a small fraction of neural lineage cells due to the presence of other cell lineage of mesodermal or endodermal origin. Controlled conversion into homogeneous population of neural progenitors in animal-free conditions avoiding a formation of EB is therefore a desirable approach for basic and applied scientific research. Due to many similarities and differences between hESC and ihPSC it very important to compare their potential to differentiate toward specific cells concretely neural cells what could be critical for many applications in both basic and applied research. Here we propose a simple protocol which includes the use xeno-free differentiation medium and extracellular matrix to successfully convert the hESC and ihPSC cells to regional specific and transplantable neural progenitors. The neural progenitors give rise in vitro and in vivo to progeny representing the three major neural lineages: oligodendrocytes, astrocytes and mature electrophysiologically functional neurons. Our protocol is first to our knowledge to be both xeno-free and adherent to differentiate hPSC toward high purity neural progenitors most suitable for cell therapeutic application.

T-3025
THE ORIGIN OF CORTICAL STROKE-INDUCED NEURAL STEM CELLS

Faiz, Maryam[1], Sachewsky, Nadia[2], Morshead, Cindi M.[2], Nagy, Andras[1]
[1]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada

Under physiological conditions, adult neural stem cells (NSCs) in the subventricular zone (SVZ) of the mammalian brain generate neuroblasts that migrate to the olfactory bulb and give rise to new neurons. Previous studies have shown that brain injury elicits a response from these SVZ NSCs and changes the migration of progenitor cells to the sites of damage. Furthermore, resident astrocytes contribute to the process of ongoing gliosis at injury sites. Recently, it has been suggested that reactive astrocytes found at the injury site have the potential to convert to multipotent NSCs capable of neurosphere formation and multilineage differentiation in vitro. However, both endogenous NSCs from the SVZ or de-differentiated reactive astrocytes have so far been limited in their potential to regenerate the stroke damaged brain compartment. In order to increase the availability of cells present at the site of injury, recent effort has focused on using the power of cellular reprogramming to convert astrocytes to NSCs or neurons. Here, we investigated the origin of cortical-derived stem cells following a mild cortical stroke. Lineage tracing, using inducible Nestin-CreERT2 and Cre conditional tdtomato reporter mice, showed that cortical-neurospheres are derived from SVZ precursor cells that migrate to the lesion site early after an insult. A small population of stem cells persists at later time points post stroke, while the majority of cells rapidly differentiate and give rise to a subpopulation of reactive astrocytes (derived from SVZ cells) that contribute to the ongoing gliosis. Lastly, we show that forced expression of transcription factors, N-Myc and Ascl1 can efficiently reprogram SVZ-derived reactive astrocytes to neurons, a novel way to boost endogenous neurogenesis.

T-3026
PROGENITOR CELLS SHED SINGLE-MEMBRANE AUTOPHAGOSOMES INTO THE SURROUNDING CULTURE MEDIUM IN RESPONSE TO VIRAL INFECTION

Feuer, Ralph[1], Robinson, Scott M.[1], Tsueng, Ginger[1], Sin, Jon[2], Mangale, Vrushali[1], Rahawi, Shahad[3], McIntyre, Laura L.[1], Williams, Wesley[1], Kha, Nelson[1], Cruz, Casey[1], Hancock, Bryan M.[1], Nguyen, David P.[1], Sayen, M. Richard[2], Hilton, Brett J.[1], Doran, Kelly S.[1], Segall, Anca M.[1], Wolkowicz, Rolan[1], Cornell, Christopher T.[4], Whitton, J. Lindsay[5], Gottlieb, Roberta A.[2]
[1]Biology, San Diego State University, San Diego, CA, USA, [2]Donald P. Shiley BioScience Center, San Diego State University, San Diego, CA, USA, [3]Biology, San Diego State University, San Diego, CA, USA, [4]Immunology and Microbial Scineces, The Scripps Research Institute, La Jolla, CA, USA, [5]Immunology and Microbial Sciences, The Scripps Research Institute, La Jolla, CA, USA

Recent studies have identified a number of pathogenic viruses which preferentially infect progenitor and stem cells. Coxsackievirus B3 (CVB3), a member of the picornavirus family and enterovirus genus, preferentially targets neural progenitor cells (NPCs) leading to developmental defects in the central nervous system. We genetically engineered a unique molecular marker, "fluorescent timer" protein, within our infectious CVB3 clone and isolated a high-titer recombinant viral stock (Timer-CVB3) following transfection in HeLa cells. "Fluorescent timer" protein undergoes slow conversion of fluorescence from green to red over time, and Timer-CVB3 can be utilized to track virus infection and dissemination in real time. Upon infection with Timer-CVB3, NPCs and myoblast progenitor cells slowly changed fluorescence from green to red over 72 hours as determined by fluorescence microscopy or flow cytometric analysis. Time-lapse photography of partially differentiated progenitor cells infected with Timer-CVB3 revealed substantial intracellular membrane remodeling and the assembly of discrete virus replication organelles which changed fluorescence color in an asynchronous fashion within the cell. Intriguingly, these infected progenitor cells released abundant extracellular microvesicles (EMVs) containing matured "fluorescent timer" protein in addition to infectious virus. CVB3 virions were readily observed within purified EMVs by transmission electron microscopy, and infectious virus was identified within low-density isopycnic iodixanol gradient fractions consistent with membrane association. The preferential detection of the lipidated form of LC3 protein (LC3 II) in released EMVs harboring infectious virus suggests that the autophagy pathway during progenitor cell differentiation plays a crucial role in microvesicle shedding and virus release, similar to a process previously described as autophagosome-mediated exit without lysis (AWOL) observed during poliovirus replication. We suggest that CVB3 may stimulate an autophagosomal ejection pathway inherent within progenitor and stem cells, and these virus-associated EMVs represent a novel route for virus dissemination in the host. Through the use of this novel recombinant virus which provides more dynamic information from static fluorescent images, we hope to gain a better understanding of CVB3 tropism for progenitor cells, intracellular membrane reorganization, and virus-associated EMV dissemination during progenitor cell migration and differentiation within the host.

T-3027
THE ROLES OF MCL-1 AND BCL-X IN DEVELOPMENTAL NEUROGENESIS.

Fogarty, Lauren Caroline[1], Martin, Hiliary[1], Song, Beibei[1], Parrill, Allison[1], Hasan, S M Mahmud[2], Xiong, Jieying[1], Opferman, Joseph T.[3], Vanderluit, Jackie L.[4]
[1]Biomedical Sciences, Memorial University of Newfoundland, St. John's, NL, Canada, [2]Memorial University of Newfoundland, St John's, NL, Canada, [3]St. Jude Children's Research Hospital, Memphis, TN, USA, [4]Biomedical Sciences, Memorial University, St. John's, NL, Canada

During development of the murine embryonic nervous system cells progress through different stages from neural stem cells to progenitors to neuroblasts and finally differentiated neurons. Cell survival signaling changes during neurogenesis however, are still poorly understood. Here, we have examined the roles of anti-apoptotic Bcl-2 proteins myeloid cell leukemia-1 (Mcl-1) and B-cell lymphoma related gene (Bcl-x) in promoting survival as cells progress through the stages of neurogenesis. Nestin-mediated conditional deletion of Mcl-1 (Mcl-1 CKO) or Bcl-x (Bcl-x CKO) in neural precursor populations results in extensive cell death in the forebrain, brainstem and spinal cord at different time points during embryonic development. In the Mcl-1 CKO, apoptosis is initially observed within the proliferating cell populations at embryonic day 10 (E10), coinciding with the start of neurogenesis. In the Bcl-x CKO, apoptosis is first observed in post mitotic cells in the spinal cord and brainstem at E11, however in the cortex apoptosis is not observed until E17 as neurogenesis is concluding. The differences in cell death in the CKO models demonstrate that Mcl-1 and Bcl-x are critical for cell survival during the different stages of neurogenesis. We are currently determining which specific cell populations require Mcl-1, Bcl-x or both and investigating whether changes in the expression of Bcl-2 pro-apoptotic proteins can account for the different requirements for Mcl-1 and Bcl-x. qPCR and in situ hybridization are in progress to examine the expression profiles of the pro-apoptotic Bcl-2 family members during nervous system development. This study will elucidate the distinct and overlapping roles of survival factors, Mcl-1 and Bcl-xL through the different stages of neurogenesis within the developing mouse embryo.

T-3028
MARKERS OF SYNAPSE FORMATION APPEARING IN NEURAL STEM CELLS AFTER TRANSPLANTATION IN MOUSE BRAIN AFFECTED BY ISCHEMIC STROKE

Mitrecic, Dinko, Kosi, Nina, Alic, Ivan, **Gajovic, Srecko**
Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia

Synaptic connections of transplanted stem cells with both host neurons and in-between themselves are necessary for their sufficient level of survival and integration in the host brain circuitry. To estimate the rate of connectivity of neural stem cells (NSCs) we analyzed expression of proteins responsible for synaptogenesis after their transplantation into ischemic mouse brain and compared it to in vitro NSC differentiation. NSC were obtained from E 14.5 C57/Bl6 embryos and cultured as neurospheres. In vitro differentiation was promoted by withdrawing growth factors and plating the cells on lysine and laminin coated coverslips. Ischemic brain injury was done by transient middle cerebral artery occlusion (MCAO). Anesthetized C57/Bl6 mice (n=32) were subjected to 60 minutes of ischemia by intraluminal left MCAO (n=16) and subsequent reperfusion or the surgery was done without ischemic injury (sham operated group, n=16). NSCs were labeled prior to transplantation with a fluorescent dye (PKH26) and stereotactically injected (250000/μL) after 5 days of reperfusion at the dorsal cortico-callosal boundary into either ipsilateral or contralateral hemisphere. Animals were sacrificed at 2, 4, 8 and 14 weeks following transplantation and following synaptic proteins were analyzed on brain sections by immunofluorescence: Cell adhesion molecule 1 (CADM1), Neuroligin 1 (NLGN1) and Synapsin I (SYN1). This was compered to in vitro differentiation of NSCs. In vitro both CADM1 and Neuroligin 1 were detected early on in the cytoplasm of undifferentiated cells. As a late synapse differentiation marker SYN1 could be detected in vitro after the 3rd day. It immediately acquired a punctate pattern indicating the beginning of synapse assembly in the culture. Transplanted NSCs showed significant survival and migration from the implantation site to the site of the lesion at all time-points analyzed (2, 4, 8 and 14 weeks posttransplantation). They ended in the periinfarct region, and mainly aggregated in the ischemic core. Most of them were positive for Nestin, multipotent NSC marker. Some of the transplanted cells showed positivity in the cytoplasm for CADM1 and Neuroligin 1, indicating that the cells produced actively these cell adhesion proteins. On the other hand no SYN1 staining was present at all time points. In conclusion, transplanted NSCs produced cellular adhesion molecules at the site of the lesion, thus exhibiting an early potential for interaction with other cells and extracellular matrix. Late synaptic marker SYN1 was present after in vitro differentiation, still it was not detected after transplantation in vivo.

T-3029
THE RELATIONSHIP BETWEEN PERIODIC NEUROGENSIS AND ANXIETY LEVELS IN MICE

Geum, Dongho[1], Lee, Ja-Myong[1], Kim, Hyun[2], Son, Gi-Hoon[3]
[1]Biological Sciences, Korea University, Medical School, Seoul, Republic of Korea, [2]Anatomy, Korea University, Medical School, Seoul, Republic of Korea, [3]Legal Medicine, Korea University, College of Medicine, Seoul, Republic of Korea

Recent reports suggest that neurogenesis in the hippocampus is not only involved in learning and memory but also mood such as anxiety and depression. However, the relevance between hippocampal neurogenesis and anxiety is still controversial. Here, we generated Nestin-CreER; Rosa26-FloxedlacZ-DTa (Nestin-DTa) mice of which nestin-positive adult neural stem cells (NSCs) were ablated by tamoxifen treatment, and tested the relationship between hippocampal neurogenesis and anxiety level. Five-consecutive daily injections of tamoxifen (5mg/injection) ablated NSCs, and BrdU-positive proliferating cells were decreased to 50% in Nestin-DTa mice. Four-week later tamoxifen injections, mood-related behavior tests such as safety learning, open field test (OFT), elevated plus maze (EPM) and novel feeding test were performed. The Nestin-DTa mice could not learn safety learning which clearly shows the inhibition of hippocampal neurogenesis. The Nestin-DTa mice spent more time in the center of the open field during OFT, and stayed more time in the closed arms in EPM than those of control mice, which clearly demonstrated the higher anxiety level in Nestin-DTa mice. During novel feeding test, Nestin-DTa mice showed the helplessness and could not showed the interest in sucrose. Eight-week later tamoxifen injections, however, behavior tests showed that higher anxiety level and helplessness of Nestin-DTa mice were recovered to normal level. Histological analyses of new born neurons well match with behavior tests. Four-week later tamoxifen injection, new born mature neurons that incorporate into the circuit were decreased, but new born young neurons which are positive for PCNA, Dcx and calretinin were repopulated. Eight- week later tamoxifen injection, both new born mature and young neurons were recovered to normal conditions. Behavior tests in concordance with histological analyses clearly suggest that hippocampal neurogenesis is important for anxiety control.

T-3030
LONG-TERM FUNCTIONAL ASSESSMENT AND HISTOLOGICAL CHARACTERISATION OF TRANSPLANTS OF DOPAMINE NEURONS DERIVED FROM HUMAN EMBRYONIC STEM CELLS IN A RAT MODEL OF PARKINSON'S DISEASE

Grealish, Shane[1], Diguet, Elsa[2], Mattsson, Bengt[1], Kirkeby, Agnete[1], Bramoulle, Yann[2], Van Camp, Nadja[2], Perrier, Anselme[3], Hantraye, Philippe[2], Björklund, Anders[1], Parmar, Malin[1]
[1]Lund University, Lund, Sweden, [2]Molecular Imaging Research Center (MIRCen), Commissariat à l'Energie Atomique (CEA), Fontenay-aux-Roses, France, [3]INSERM, Evry, France

The motor complications of Parkinson's disease (PD) manifest due to the loss of mesencephalic dopamine (mesDA) neurons that reside in the substantia nigra and innervate their main target structure, the striatum. The current strategy for cell replacement therapy for PD relies on the transplantation of mesDA neurons into the striatum. Clinical trials have proven that mesDA neurons from human fetal tissue are effective in relieving motor symptoms. We have developed a xeno-free and feeder-free protocol that allows for the generation of authentic mesDA neurons from human embryonic stem cells (hESCs) (Kirkeby et al. 2012, Cell Rep). To further validate their authenticity and therapeutic potential, we performed long term assessment of grafted hESC-mesDA neurons in the striatum and substantia nigra of a rat model of PD, and assessed their ability to specifically innervate relevant projection areas in the forebrain and to restore DAergic transmission. We transplanted human mesDA neurons of fetal or hESC origin, into an adult rat model of PD using two paradigms. Firstly, we transplanted hESCs into the striatum, and monitored graft maturation and DAergic transmission in vivo using MRI and PET imaging. In the second paradigm, we transplanted mesDA neurons from hESCs and fetal tissue into the substantia nigra, in order to assess the cells' ability to specifically innervate appropriate target structures in the parkinsonian adult rat brain. We did not observe any signs of over-growth in the hESCs grafts 6 months post-transplantation. In the first experiment, we did not observe any neuroinflammatory response to the transplant up to 5 months post-transplantation, and the grafts were rich in mature DAergic neurons. They normalised the pre- and post-synaptic DAergic transmission within the striatum, and thus provide a long-term preclinical evaluation demonstrating that hESC-mesDA neurons can survive for up to 6 months in the adult rat brain without any adverse events. We demonstrate that DAergic functions, with a direct clinical relevance, were restored upon transplantation of hESC-mesDA neurons. In our second experiment, we demonstrate that hESC-mesDA neurons are able to extend axons in a target-specific manner over long distances within the adult rat brain. We observed human DAergic fibres innervating the host striatum, over 8mm from the graft site. The innervation pattern was similar to grafts of fetal mesDA neurons. This confirmed that hESC-mesDA neurons perform in a similar manner as their fetal counterparts, and that hESC-mesDA neurons are able to reform damaged midbrain dopamine pathways. The ability of hESC-mesDA neurons to extend axons through the adult rat brain and precisely innervate key structures that are essential for functional recovery following transplantation has, to our knowledge, not been described before. In conclusion, we have demonstrated that in a long-term rat model of PD, transplants of hESC-mesDA neurons can survive and mature to a correct phenotype and restore DAergic function in the absence of adverse events such as tumor formation. We demonstrate that when grafted in the substantia nigra, hESC-mesDA neurons can seek out and appropriately innervate target structures within the adult brain. Keeping in mind current efforts to bring cell replacement therapy for PD using hESCs into clinical trial, this study expands on the potential that hESC-derived neurons have in future developments of cell replacement therapy in the clinical setting.

T-3031
MUTATIONS IN ADPRHL2, ENCODING ADP-RIBOSYLHYDROLASE LIKE 2, LEAD TO A NOVEL, POTENTIALLY TREATABLE, LETHAL EPILEPSY SYNDROME

Guemez Gamboa, Alicia[1], Dixon-Salazar, Tracy[1], Huang, He[2], Nguyen, Maria[2], Udpa, Nitin[3], Bielas, Stephanie[4], Silhavy, Jennifer L.[1], Marsh, Sarah E.[2], Vaux, Keith[2], Gabriel, Stacey[5], Vernon, Hilary[6], Bafna, Vineet[3], Hertecant, Jozef[7], Haddad, Gabriel[2], Gleeson, Joe[4]
[1]Neurosciences, University of California, San Diego, La Jolla, CA, USA, [2]Pediatrics, University of California, San Diego, La Jolla, CA, USA, [3]Computer Sciences, University of California, San Diego, La Jolla, CA, USA, [4]Neurosciences, University of California, San Diego, La Jolla, CA, USA, [5]Broad Institute, Massachusetts Institute of Technology, Cambridge, MA, USA, [6]McKusick Nathans Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MA, USA, [7]Pediatrics and Genetics, United Arab Emirates University, Al Ain, United Arab Emirates

Poly-ADP-ribose (PAR) modification of proteins occurs as a response to cellular stress such as oxidative or hypoxic insults. PAR modification is mediated by the PARP gene family, and reversed by the PARG gene family. Here we report the exome sequence identification of an inactivating mutation of the PARG gene, ADPRHL2, leading to an autosomal recessive syndrome characterized by seizures, neurological deterioration, and premature death. Here we generated a disease model from human induced pluripotent stem cells (iPSCs) to characterize phenotypes relevant to cell death. Fibroblasts donated from healthy and affected children (with ADPRHL2 mutations) were reprogrammed into iPSCs and differentiated into neural precursor cells. Patient cells lack ADPRHL2 protein, display increased basal and stress-associated PAR-protein modifications, and enhanced susceptibility to apoptosis-inducing factor (AIF)-mediated cell death following insult. The importance of neuronal ADPRHL2 is emphasized by a lethal phenotype following stress from both patient-derived neural precursors and in a neural-specific knockdown of the Drosophila orthologue, which is rescued by PARP inhibition. Several pharmacological PARP inhibitors in clinical trials rescued lethality, suggesting that this class of drugs may be used to treat this lethal epilepsy disorder.

T-3032
ENGINEERED COPY NUMBER VARIATIONS OF 16P11.2 IN HUMAN IN-CELLS TO MODEL PSYCHIATRIC DISORDERS

Haag, Daniel[1], Danko, Tamas[1], Davila, Jonathan[2], Sudhof, Thomas C.[3], Wernig, Marius[2]
[1]Pathology, Stanford School of Medicine, Stanford, CA, USA, [2]Stanford University, Palo Alto, CA, USA, [3]University of Texas Southwestern Medical Center, Dallas, TX, USA,

Autism describes a group of neurodevelopmental disorders phenotypically characterized by impairment of social interaction, communication disability, repetitive patterns of behavior, and limited interests. Recently, genome-wide association studies have revealed regions of rare de novo copy number variations (CNVs) as significant risk factors for the conditions subsumed under autism spectrum disorders (ASD). In particular, a deletion of ~600kb on chromosome 16p11.2, harboring 28 genes, greatly increases susceptibility to develop autism. Interestingly, a microduplication of the very same region was also associated with ASD making 16p11.2 CNV the most recurrent, non-syndromic genomic event for this disorder. In addition, a significant association with schizophrenia was reported for the microduplication but not for the deletion of this region. Modeling both events in mice

recently demonstrated opposing phenotypes in behavior and brain architecture when directly comparing the two CNV genotypes. While a resulting gene dosage effect and a neurodevelopmental implication of the affected genes could clearly be shown, ASD-specific features could not be represented. Here, we attempt to complement the findings from the mouse model by investigating the same genomic lesion in the genetic background of human neurons in vitro. For this purpose, we apply a Cre-lox-based strategy to generate both a hemizygous deletion and a reciprocal duplication of the ~600kb region on 16p11.2 in human pluripotent cells. Subsequently, lentivirus-mediated exogenous over-expression of specific neuronal transcription factors will be performed in parental and mutated cells to derive induced neurons (iNs). Potential differences in morphology, electrophysiology, synapse formation, growth and maturation behavior will then be quantified. In parallel, phenotypic analyses will be conducted on patients-derived iPSCs harboring the CNV and primary cortical cultures of the CNV-mice. Finally, recurrent patterns will be examined to find a mechanistic link between the 16p11.2 CNV and the development of psychiatric disorders.

T-3033
PROGRANULIN EXPRESSION IS NECESSARY FOR THE DOPAMINERGIC NEURONAL DIFFERENTIATION FROM HUMAN NEURAL PRECURSOR CELLS

Hao, Hsiao-Nan[1], Pei, Haitao[2], Peduzzi, Jean[1], Guan, Fangxia[3], Hao, Peter[1], Liu, Longxi[4], Tang, Yunlin[5]

[1]Wayne State University, Detroit, MI, USA, [2]Neurology, Qingdao University, School of Medicine, Qingdao, China, [3]Bioeng, Zhengzhou University, School of Life Sci., Zhengzhou, China, [4]Neurosug, Qingdao University, School of Medicine, Qingdao, China, [5]Neurosurg, PLA 458 Hospital, Guangzhou, China

Progranulin (PGRN) has been found at both neuronal and microglial cells predominantly in central nervous system (CNS). The reported biological functions of PGRN indicate that failure to express normal PGRN associates highly with neural degenerative disorders such as frontotemporal dementia, prominent language disorders and Parkinson's disease. Although PGRN locates variously in the different areas of the fetal brain, the possibility of PGRN to participate in dopaminergic neuronal differentiation (DND) in the developing CNS remains to be explored. Our previous reported results indicate that the DND occurs only among fetal nestin+/CD133+ brain cells rather than the same marker cells isolated from adult neural cells. We also notice that the PGRN expression of fetal neural precursor cells is more than that of adult neural precursors. Granulins are important for CNS development during fetal life, but they also play a critical role of demoting degeneration under normal circumstances. To explore the molecular mechanisms of PGRN in regulating DND, both PGRN antisense deoxynucleotides (DON) and human recombinant PGRN protein were used for this study. Prior to the incubation of the nestin+/CD133+ human fetal brain cells (HFBC) in dopaminergic neuron inducible medium, cells were cultured with PGRN antisense nucleotides contained in growth medium for six hours. With PGRN antisense DON treatment, the level of PGRN expression of the nestin+/CD133+ HFBCs was reduced. The significant decrease of PGRN expression was confirmed using RT-PCR, Northern blotting and protein assays. Interestingly, inhibition of PGRN expression reduces DND from nestin+/CD133+ HFBCs. There are no apoptotic signal to be detected after the cultures treated with PGRN antisense and nonsense DON. In addition, it is likely reducing of PGRN expression increases astrogliosis dramatically. Compared with antisense ODN treated nestin+/CD133+ HFBC cells, no remarkable reduction of PGRN mRNA signal and protein expression have been detected in nonsense treated cells. The potentiality of DND is unattacked after cells incubated nonsense nucleotides. In order to examine whether the PGRN is necessary for DND, exogenous recombinant PGRN protein was administered to the antisense pretreated cultures. The results of this salvage strategy have shown minor effect on the DND. Although few cells were stained positively with both tyrosine hydroxylase (TH)-Nurr1, the portion of the dopaminergic neuronal cells was raised to 3% to 5% after recombinant PGRN was added to the medium in antisense treated cultures. It is unlikely the exogenous PGRN can rescue the DND capacity of cells from antisense ODN treatment. Whereas, the nonsense ODN incubated cells remains same differentiation potentiality to generated more than 64% TH/Nurr1/Tuj1 positive cells. The results indicate that the reduction of PGRN expression significantly inhibits the DND from nestin+/CD133+ HFBCs. Replacement of intracellular PGRN to ensure the DND capacity requests further studies because the internalization pathway of recombinant PGRN is not cleared. Taken together, these results suggest that the PGRN expression is necessary for the DND of the nestin+/CD133+ HFBCs, and also implies to prevent the neural precursors from gliogenesis in vitro. Understanding the mechanism of PGRN in neural differentiation will benefit to generate high quality dopaminergic neuronal precursors for the cell therapy of Parkinson's disease.

T-3034
DYNAMIC MONITORING OF NANOG AND NESTIN GENE EXPRESSION IN NEUROGENIC DIFFERENTIATION PROCESS OF MOUSE STEM CELLS BY BIOLUMINESCENCE MICROSCOPY

Hatta-Ohashi, Yoko, Hayashi, Taro, Takahashi, Takeo, Suzuki, Hirobumi

Corporate R and D Center, Olympus Corporation, Tokyo, Japan

It has been reported that the embryonic stem (ES) cells consist of various cell subsets that express different levels of specific gene markers and continuously convert into each other. Although conventional flow cytometry analysis provides the end-point data of gene expression in ES cell populations, it cannot monitor the temporal changes within individual cells. To monitor differentiation process of ES cells dynamically (temporally and spatially), a new method that can visualize the exact heterogeneity of gene expression in ES cell populations is required. In this study, a bioluminescence microscope that enables us to image promoter activity at single live cells was developed using luciferase as a reporter. We applied it for gene expression monitoring of a pluripotent gene marker *Nanog* and a neural marker *Nestin* in precursor neurogenic cell differentiation of mouse ES cells. Mouse ES cells were co-transfected with *Nanog* and *Nestin* promoter vectors that were reported by two color luciferases, Eluc (green) and CBR (red), respectively. The ES cells stably expressing the two reporter vectors were seeded at 1000 cells per well in the plate (EZSPHERE, AGC) with DMEM medium containing 1uM retinoid acid (RA). Then, the bioluminescence imaging was performed in spherical cell aggregation during embryoid body (EB) formation for four days. In the temporal analysis, *Nanog* expression was dominant initially and then *Nestin* expression was increased gradually via EB formation, however, the timing of differentiation was not homologous among EBs. The present dynamic monitoring of gene expression of specific markers by bioluminescence imaging would be a potential tool for evaluation of cell differentiation in morphogenesis.

T-3035
EPIGENETIC MECHANISMS UNDERLYING THE GENERATION AND DIFFERENTIATION OF NEURAL STEM CELLS

Hitoshi, Seiji[1], Hayashi, Yoshitaka[1], Fuchigami, Takahiro[1], Fuke, Satoshi[1], Ishino, Yugo[2], Ikenaka, Kazuhiro[2]
[1]Department of Integrative Physiology, Shiga University of Medical Science, Otsu, Japan, [2]Division of Neurobiology and Bioinformatics, National Institute for Physiological Sciences, Okazaki, Japan

Epigenetic regulation of gene expression plays pivotal roles in early development of mammalian embryos and in the generation of central nervous tissue. We have been studying epigenetic mechanisms that modify the activation of Notch signaling, which is critical for the generation, maintenance, and differentiation of neural stem cells, and we have identified two epigenetic modifications; DNA demethylation by Glial cells missing (Gcm) genes and histone H2B monoubiquitylation by Ring finger protein 20 (Rnf20). Gcm genes are required for the transition from primitive to definitive neural stem cells. Interestingly, the transient expression of Gcm genes in mouse embryos at around E7.5 induces the demethylation of the promoter of Hes5 gene, one of Notch effector genes, in a genome duplication-independent fashion. On the other hand, we have recently found that Rnf20 is indispensable for embryonic development beyond the blastocyst stage and that it regulates the cell cycle time of ES as well as neural stem cells by activating the expression of several genes including Hes5. We will present recent progress of our research on these topics.

T-3036
EZH2 CONTROLS NEURAL PROGENITOR POOL SIZE AND REGIONAL IDENTITY IN THE DEVELOPING MOUSE MIDBRAIN

Zemke, Martina[1], Draganova, Kalina[1], Schoeler, Anne[2], Koseki, Haruhiko[3], Schuebeler, Dirk[4], Sommer, Lukas[1]
[1]Institute of Anatomy, University of Zürich, Zürich, Switzerland, [2]German Research Center for Environmental Health, Helmholtz Zentrum München, Neuherberg, Germany, [3]RIKEN Research Center for Allergy and Immunology, RIKEN Yokohama Institute, Yokohama, Japan, [4]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Precise temporal and spatial control of gene expression is essential for the determination of the size of the developing brain as well as for establishment of correct cell identities in different brain regions. The polycomb group protein Ezh2, enhancer of Zeste homolog 2, is the catalytic subunit of polycomb repressive complex 2 (PCR2) and is primarily responsible for trimethylation of histone H3K27 (H3K27me3). This epigenetic mark contributes to repression of many genes, which are pivotal for neural development. Here we show that Ezh2 is essential for midbrain development in a region-specific manner. After Wnt1-Cre-mediated ablation of Ezh2 in the midbrain we performed whole-genome transcriptome analysis of mutant and control midbrains as well as H3K27me3 ChIP. In the caudal midbrain loss of Ezh2 results in decreased neural progenitor (NP) proliferation due to negative regulation of Wnt/β-catenin signaling and precocious exit of NP from the cell cycle leading to increased neuronal differentiation. Most intriguingly in the dorsal midbrain Ezh2 ablation not only leads to a loss of midbrain identity markers Pax3 and Pax7 but also to aberrant upregulation of forebrain transcription factors FoxG1 and Pax6 by direct de-repression. Together our data reveal a role of Ezh2 in regulating NP fate decisions and brain area identity by direct and indirect mechanisms.

T-3037
A TRANSCRIPTION FACTOR CODE FOR BRAIN-WIDE DISCRIMINATION OF NEUROANATOMIC IDENTITY AND DEVELOPMENTAL STAGE IN MOUSE

Menon, Vilas[1], Martinez, Salvador[2], Ng, Lydia[1], Lee, Changkyu[1], Glattfelder, Katie[1], Sunkin, Susan M.[1], Henry, Alex[1], Dang, Chinh[1], Raquel, Garcia-Lopez[2], Almudena, Martinez-Ferre[2], Pombero, Ana[2], Rubenstein, John L.R.[3], Wakeman, Wayne B.[1], Hohmann, John[1], Nguyen, Thuc-Nghi[1], Hawrylycz, Michael[1], Puelles, Luis[4], Jones, Allan R.[1], Thompson, Carol[1]
[1]Allen Institute for Brain Science, Seattle, WA, USA, [2]Instituto de Neurociencias, Alicante, Spain, [3]University of California, San Francisco, San Francisco, CA, USA, [4]University of Murcia, Murcia, Spain

To provide a temporal framework to understand the genoarchitecture of brain development, in situ hybridization data were generated for embryonic and postnatal mouse brain at 7 developmental stages for 2109 genes, processed with an automated informatics-based pipeline and manually annotated. This resource comprises 434,946 images of ISH data, 7 novel reference atlases, and tools to explore co-expression of genes across neurodevelopment. Using these data, we observed a shift in the principles governing the molecular organization of the brain across embryonic development, with a transiently detectable neuromeric and dorsoventral plate-based organization of the brain observed in gene expression signatures from E11.5 through E18.5. The analysis of a subset of genes, 800 transcription factors, also captured these broad changes in gene expression; these data were used to identify a minimal set ("code") of 83 transcription factors that can discriminate between brain regions and identify the developmental age of a tissue (between E11.5 and E18.5), providing a possible basis for genetic manipulation or tracking of specific brain regions over development. This approach may eventually provide a useful means to assess the identity and developmental stage of in vitro-differentiated neurons. The resource is available as the Allen Developing Mouse Brain Atlas (developingmouse.brain-map.org).

EYE OR RETINAL CELLS

T-3038
RPES DERIVED FROM IPSCS FOR CELLULAR REPLACEMENT THERAPY

Karumbayaram, Saravanan[1], Lopes, Vanda C.[2], Dimashkie, Anu[1], Byrne, James A.[3], Gomperts, Brigitte[4], Clark, Amander T.[3], Pyle, April[1], Kohn, Donald B.[3], Zack, Jerome A.[5], Williams, David[2], Lowry, William[3]
[1]Broad Stem Cell Research Center at University of California, Los Angeles, Los Angeles, CA, USA, [2]Jules Stein Eye Institute, Los Angeles, CA, USA, [3]University of California, Los Angeles, Los Angeles CA, USA, [4]Mattel Children's Hospital University of California, Los Angeles, Los Angeles, CA, USA, [5]David Geffen School of Medicine at University Of California, Los Angeles, Los Angeles, CA, USA

Age related macular degeneration (AMD) is characterized by the deterioration of the macula of the eye. Presently no successful therapeutic intervention exists for AMD. Patient-specific retinal pigment epithelial cell (RPE) replacement therapy is a promising approach to replace the defective RPE cells (RPEs) and restore vision. With allogeneic transplantation of human embryonic stem cell-derived RPEs for the treatment of AMD, poor compliance by the patients with the immunosuppression regimen can be expected. Hence, our aim is to utilize the iPSC technology for patient-specific cellular therapies

to treat AMD by replacing damaged RPEs. Currently the field lacks a GMP-compliant procedure for patient-specific generation of RPEs and suitable CMC documentation for clinical translation. We recently developed standard operating procedures (SOPs) under good manufacturing practice (GMP) conditions for the derivation of patient-specific iPSCs. We have also differentiated lines of the transplantable grade iPSCs into functional RPEs under defined conditions. Based on the success of our reprogramming and differentiation procedures, and the potential clinical application of these cells, we are working towards the pre-clinical setting for its early translation to the clinic. This is done by validating the RPE cell derivation process, studying the pre-clinical pharmacological and toxicological status of iPSC-derived RPEs and developing documents related to Chemistry, Manufacturing and Control (CMC) procedures for transplantable grade iPSC-derived RPE cell generation for patient specific stem cell treatment for macular degeneration.

T-3039
DIFFERENTIATION OF ADULT RETINAL STEM CELLS INTO ROD AND CONE PHOTORECEPTORS

Khalili, Saeed[1], Ballios, Brian G.[2], Grise, Kenneth[1], Bernier, Gilbert[3], van der Kooy, Derek J.[1]

[1]*Molecular Genetics, University of Toronto, Toronto, ON, Canada,* [2]*Institute of Medical Science, University of Toronto, Toronto, ON, Canada,* [3]*Department of Ophthalmology, University of Montréal, Montreal, QC, Canada*

Adult retinal stem cells (RSCs) are rare pigmented cells in the retinal ciliary epithelium (CE) of many mammals. Multipotent RSCs can give rise to retinal neurons including photoreceptors as well as retinal pigment epithelial (RPE) cells. Photoreceptors are required for vision and are lost in retinal degenerative diseases. RSCs may be a good source to generate photoreceptors for cell therapy/transplantation. We previously applied combinations of taurine, retinoic acid and FGF2/heparin (T+RA+FH) to differentiating clonal RSC monolayer colonies (over 40 days of differentiation) and found that the numbers of rods increased to over 90% of all progeny. In contrast, pan-retinal differentiation conditions (1% fetal bovine solution (FBS)+FH) produced only 10% rods. In order to test the effect of T/RA on early RSC progeny, we incubated primary cultures of dissociated CE from adult mice with T+RA+FH or FH-only (control RSC growth conditions) for the 7 days of clonal sphere growth. When T+RA+FH-derived spheres then were differentiated in 1%FBS+FH for 40 days, the percentage of cells expressing rhodopsin and RPE65 were increased and decreased, respectively, compared to FH-derived spheres differentiated in 1%FBS+FH. There were no differences in total cell numbers. To further investigate the cell biological mechanisms underlying the T/RA effects on RSC differentiation, we used FACS sorting to isolate single non-pigmented and single pigmented cells in wells, which then were treated with T/RA for 28 days of differentiation. All surviving clones derived from single non-pigmented progenitors were rod-only clones (100% rhodopsin-positive; n=34), while those derived from single pigmented progenitors were predominantly no-rod clones (no rhodopsin-positive cells; n=47/48 clones). Surprisingly, one rod-only clone was derived from a single pigmented cell (the largest of the single pigmented cell-derived clones), suggesting the possibility of lineage plasticity in the earliest pigmented progenitors or an action on a single pigmented RSC. We suggest that T/RA is having an instructive effect on rod differentiation. In pan-retinal differentiation conditions, less than 1% of RSC progeny differentiated into cone photoreceptors (cone arrestin-positive). We found that combining TGFβ, Wnt and BMP antagonism, as well as 1%FBS+FH, on differentiating clonal RSC progeny increased the cone differentiation rate to 41% of progeny. Further studies are underway to characterize the cell biological mechanisms underlying this RSC-derived cone differentiation. Together, this research may enhance our understanding of photoreceptor development and be an effective strategy to generate photoreceptors for cell therapy.

T-3040
OXYGEN IS REQUIRED FOR RPE PROGENITOR CELL DIFFERENTIATION

Kharazi, Ludmila, Vertelov, Grigory, Fernandes, Sarah, Kharazi, Alexander

Stemedica Cell Technologies Inc, San Diego, CA, USA

Oxygen (O2) concentration can affect the growth rate of cultivated retinal pigment epithelium (RPE) cells. In this study we investigated its role in differentiation of RPE progenitors in vitro. METHODS: Human fetal eyes (18 weeks gestation age) were obtained from Advanced Bioscience Recourses (ABR) Inc. Primary cells were propagated in dishes either under normal (20%) O2 or low (5%) O2 conditions in MEM medium containing 5% of Fetal Bovine Serum (FBS) until passage 3. For differentiation purposes, expanded RPE progenitors were cultivated in transwell permeable inserts under normal or low O2 conditions in RPE medium containing 1% FBS. Barrier function was assessed after two weeks in transwell culture by measuring transepithelial resistance (TER) using epithelial voltameter. Cell morphology and expression of RPE specific markers ZO-1 and Na/K/ATPase were evaluated by phase contrast and immunofluorescence microscopy. Both hypoxic and normoxic RPE progenitors differentiated under normal oxygen conditions showed gradually increased TER which reached a plateau after approximately 8 weeks in culture. The cells demonstrated characteristic uniform hexagonal morphology and re-pigmentation as well as well-developed tight junctions and clear presence of ZO-1and Na/K ATPase. In contrast, RPE cells similarly differentiated in low oxygen did not upregulate the TER values. The cells appeared heterogeneous with no signs of pigmentation. In addition, the expression of both ZO-1 and Na/K/ATPase markers were dramatically reduced. Interestingly, hypoxia-grown RPE progenitors differentiated under normal O2 developed and maintained higher TER compared to normoxia-grown cells differentiated under the same conditions. These results show that (a) O2 is required for RPE progenitor cells differentiation in vitro, and (b) RPE progenitor cells grown permanently in hypoxia have a greater differentiation potential compared to normoxic cells.

T-3042
IMPROVING DERIVATION OF RETINAL PIGMENT EPITHELIUM FROM HUMAN PLURIPOTENT STEM CELLS USING FLUORESCENT REPORTERS

Leach, Lyndsay Logan, Buchholz, David E., Lowenstein, Stefan E., Clegg, Dennis O.

Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA, USA

In the developed world, age-related macular degeneration (AMD) is the leading cause of blindness in people over the age of 60. AMD is characterized by progressive loss of central visual acuity due to death of the retinal pigment epithelium (RPE), a highly specialized cell type that plays a critical role in the visual cycle and maintenance of photoreceptor health and function. At present, few therapeutic options exist for treating the more common, dry form of AMD. Therapies using transplanted RPE derived from human embryonic stem cells (hESC) are being pursued by multiple groups; however, the process of generating RPE from pluripotent cells has remained inefficient. While improvements in RPE production have been made, we believe better

protocols can be developed by further elucidating signals important for deriving RPE from pluripotent cells, a process which is poorly understood. To address this question, we have developed molecular tools to visualize RPE differentiation by generating fluorescent reporter stem cell lines under control of early eye field and RPE-specific gene promoters: RAX, OTX2, MITF, and RPE65. RAX and OTX2 are expressed earliest in development, during eye field specification, while MITF is expressed during optic vesicle formation. MITF along with OTX2 expression is also maintained in developing and mature RPE. RPE65, a protein necessary for the visual cycle, is a late-stage signature of mature RPE. Together, these are ideal markers for visualizing different lineages as cells differentiate from pluripotency, to the early eye field, to RPE. Using these molecular tools, we can target specific signaling pathways during differentiation and monitor the effects on RPE derivation in real time. Here we report that inhibiting glycogen synthase kinase 3 beta (GSK3ß) during differentiation improves RPE derivation from pluripotent cells, as monitored by fluorescent reporters. RPE generated by this improved protocol were characterized with respect to RPE gene expression and function. A better understanding of the signaling mechanisms that underlie RPE differentiation will result in more efficient methods for deriving RPE from pluripotent cells for the purposes of making the highest quality RPE for emerging therapies.

T-3043
MODULATION OF WNT SIGNALLING BY TGF-BETA IN HUMAN MÜLLER GLIA WITH STEM CELL CHARACTERISTICS

Limb, G. Astrid[1], Wu, Na[2], Eastlake, Karen[1], Lei, Yuan[2], Angbohang, Angshumonik[1], Sun, Xinghuai[2]
[1]Ocular Biology and Therapeutics, University College London, London, United Kingdom, [2]Eye and ENT Hospital, Fudan University, Shanghai, China

Background: Adult human retina harbours a population of Müller glial cells with stem cell characteristics. These cells have the ability to grow and neurally differentiate in vitro, but there is no evidence that they have a regenerative ability in vivo. Transforming growth factor-beta (TGF-β), a cytokine known to be highly upregulated during retinal gliosis, plays an important role in the control of retinal regeneration in adult zebrafish and may also be partly responsible for the inhibition of endogenous regeneration of the adult mammalian retina. It was therefore the aim of this study to investigate the role of this cytokine on the regulation of the Wnt signalling pathway in Müller stem cells from the adult human eye. Methods: We examined the expression of various Wnt signalling molecules by Müller stem cells in vitro, and investigated the effect of TGF-β on the gene and protein expression of Wnt 2b, Wnt 5b and β-catenin following culture of three different human Müller stem cell lines with this cytokine. Expression of Wnt signalling molecules following Notch inhibition by the γ-secretase inhibitor DAPT and the effect of TGF-β in this inhibition was also investigated. Inhibitors of the TGF-β signalling pathway, including the ALK5 receptor inhibitor SB431542, the SMAD inhibitor SIS3 and the JNK inhibitor SP600125 were examined for their effect on the modulation of Wnt signalling molecules by TGF-β. Results: Muller stem cell lines cultured under baseline conditions expressed mRNA transcripts for Wnt2b, Wnt5b, Frizzled 1, 4, 7, β-catenin and Lef1. They did not express Wnt3a or Frizzled 5. Culture of three different cell lines with TGF- β1 and TGF- β3 caused a significant downregulation of the Wnt signalling ligand Wnt2b, whilst inducing a significant upregulation of the Wnt signalling ligand Wnt5b. However, it did not modify the gene expression of the canonical Wnt signalling intracellular component β-catenin. Interestingly, Notch inhibition caused a decrease in Wnt2b and Wnt5b expression, and whilst TGF-β1 did not inhibit this effect on Wnt2b, it significantly inhibited the effect of Notch inhibition on Wnt5b expression. Addition of the TGF-β type I receptor (ALK5) antagonist SB431542 and the SMAD3 inhibitor SIS3 to cells cultured with TGF-β1 significantly reduced the effect of this cytokine on the downregulation of Wnt 2b. Conclusions: These results provide evidence that TGF-β may play an important role in the control of neural differentiation by human Müller stem cells in vitro. Based on these results, we suggest that TGF-β may mediate a crosstalk between the Notch and Wnt signalling pathways, resulting in conflicting signals being activated to prevent endogenous proliferation and differentiation of these cells and this merits further investigations.

T-3044
A SIMPLE, RAPID AND EFFICIENT METHOD FOR PURIFYING LENS EPITHELIAL CELLS FROM DIFFERENTIATING HUMAN PLURIPOTENT STEM CULTURES PROVIDES A NOVEL APPROACH FOR LENS AND CATARACT RESEARCH

O'Connor, Michael D., Murphy, Patricia
The University of Western Sydney, Penrith South DC NSW, Australia

An inability to access large numbers of human lens cells has obstructed investigations into the molecular mechanisms of lens and cataract formation, as well as high-throughput drug screening for anti-primary and secondary cataract agents. Previous work from our group showed that 3-dimensional, focusing lenses with correct cellular arrangement and protein expression can be regenerated in vitro from primary rat lens epithelial cells (LECs). These functional in vitro lenses ultimately develop a cataract similar to age-related nuclear cataract, and provide a non-human model of age-related cataract. We hypothesized that human pluripotent stem cells might be used to produce large numbers of purified human LECs for developmental biology, drug discovery, and in vitro lens formation. By using a combination of transcriptomics and in situ hybridization we developed a cell culture and purification strategy for purification of human LECs from differentiating pluripotent stem cell cultures. The purified human LECs express expected phenotypic markers and can be differentiated into lens fibre cells. Expansion culture of the purified LECs allowed seeding in 96 well-plates, and subsequent exposure to control or hydrogen peroxide led to rapid and total cell death with hydrogen peroxide but not control treatment. These data indicate the purified human LECs have potential for large-scale anti-cataract drug screening. Further transcriptome analyses predicted novel transcription factors to be involved in LEC maintenance, and expression of at least one of these transcription factors was confirmed by PCR and Western Blotting in human lens cells. The purified human LECs are being used by our group to further investigate the role of the novel lens transcription factor we identified, mechanisms of in vitro human lens regeneration, and anti-cataract drug screening.

MUSCLE CELLS

T-3050
THE CHANGES OF PAX3 AND PAX7 EXPRESSION DURING DISUSE AND DENERVATION INDUCED MUSCULAR ATROPHY

Hsi, Chang, Yeh, Geng-Chang
Pediatrics, Taipei Medical University, Taipei, Taiwan

Background: Pax genes (Pax3 and Pax7) are known as member of paired box transcription factors which are crucial regulators for myogenesis.

At the same time, these transcription factors are also well recognized satellite cell markers. Satellite cells, which lie under the basal lamina of muscle fibres are responsible for postnatal muscle regeneration. Here we presented to you the data of experiments which compared the Pax gene expression during the skeletal muscle regeneration triggered by different type of injuries, the denervation and disused model. Methods: Male Sprague Dawley rats (190 -210 g) were used for whole experiment. The denervation model and disused model of rat were developed and evaluation were performed by Footprint analysis, Electrimyogram (EMG), HE staining, immunocytochemical staining, real time PCR. Results: The difference in expression of Pax7 and Pax3 was observed between damage models but within type of muscle (fast or slow muscle type) respectively. Both damage models revealed a remarkable decreasing of muscle mass accompanied with satellite cell number. Through Tunnel assay, apoptosis had been confirmed to be relevant to the decrease of satellite cells. Conclusion: First, we had successfully established the damage model of both denervation and disuse also its evaluation system. The results indicated that there were no difference between denervation and disused caused atrophy in the expression of Pax3 and Pax7 during the atrophy. In other ward, the Pax7 and Pax3 expression during regenerative myogenesis was destined by type of muscle but not type of damage which induces atrophy.

T-3051
ACUTE INFLAMMATION IS REQUIRED FOR MUSCLE STEM CELL PROLIFERATION AND MUSCLE REGENERATION

Hu, Ping, Fu, Xin, Xiao, Jun, Sheng, Li, Yin, Jie, Liu, Yan, Hongyan, Wang

Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China

Skeletal muscle regeneration involves a series of physical responses after injury, including activation of quiescent satellite cells (muscle stem cells), proliferation of satellite cells and myoblasts, differentiation of myoblasts, and formation of new myofibers. In recent years, more and more evidences suggested that inflammation plays important roles during muscle regeneration process. However, how inflammation affects muscle regeneration remains to be elusive. Here we focused on acute inflammation and found that it is a required positive regulator at early stage of skeletal muscle regeneration. Upon muscle injury, we observed large amount of T cell infiltrated at injury site. In immunodeficient mice, where the T cell infiltration is diminished while other limphocytes such as macrophage infiltration remains normal, reparation of muscle injury was dramatically delayed. To further investigate the relationship between acute inflammation and muscle regeneration, we characterized the protein profile of activated T cells. A combination of four factors was identified to be able to promote satellite cell proliferation and long term expansion in vitro. The cultured expanded satellite cells continue to express muscle stem cell marker, and were able to regenerate functional myofiers in vivo. Furthermore, muscular injection of the four factor cocktail could rescue the muscle regeneration defects caused by T cell deficiency. Our results demonstrate that inflammation provides a favorable micro-environment for muscle regeneration.

T-3052
REGULATION OF MUSCLE STEM CELLS BY WNT SIGNALLING INVOLVES HOMEOBOX FACTORS BARX2 AND PAX7

Hulin, Julie-Ann[1], Nguyen, Tran[1], Makarenkova, Helen[2], Meech, Robyn[1]

[1]Flinders University, Adelaide, Australia, [2]The Scripps Research Institute, La Jolla, CA, USA

Satellite cells are the resident stem cells of skeletal muscle. Upon receiving cues from their myofibre-adjacent niche, activated satellite cells undergo asymmetric division to generate a transit amplifying population called myoblasts, as well as to replenish the satellite cell pool. Wnts are key signals that control both satellite cell and myoblast behaviour. The central effectors of the canonical Wnt signalling pathway are TCF/LEF factors and beta-catenin that together bind to Wnt target genes and transduce transcriptional responses. We have shown that the homeobox protein Barx2 is expressed in satellite cells and myoblasts and, by analysis of Barx2 null mice, revealed its important role in muscle growth and repair. The Pax7 homeobox protein is another satellite cell marker essential for muscle growth and repair, most likely by controlling satellite cell self-renewal. We recently reported the novel finding that Barx2, Pax7, and muscle regulatory factors (MRFs) such as MyoD, interact with the core Wnt effector complex to regulate a Wnt reporter gene. We now show that Barx2 regulates the endogenous canonical Wnt target genes Axin2 and CyclinD1 among an array of other canonical Wnt targets. Using luciferase reporter assays we demonstrate that the proximal promoters of CyclinD1 and Axin2 are activated by expression of both beta-catenin and Barx2, whilst Pax7 suppresses this activation. Down-regulation of endogenous Pax7 using siRNA allows greater activation of the Axin2 promoter by beta-catenin and Barx2, supporting the view that the normal role of Pax7 is to attenuate Wnt target gene activation. The CyclinD1 and Axin2 proximal promoter regions contain one or more consensus TCF/LEF sites. Mutation of the CyclinD1 and Axin2 promoter TCF/LEF elements disrupts both beta-catenin- and Barx2-mediated activation, consistent with our observations that Barx2 is recruited to TCF/LEF sites and physically interacts with both beta-catenin and TCF4. Chromatin immunoprecipitation (ChIP) experiments confirm that both beta-catenin and Barx2 are recruited to the Axin2 promoter after treatment of primary myoblasts with Wnt3a. Characterization of whole muscle and primary myoblasts from wildtype and Barx2-null mice reveals that Barx2 expression is induced by Wnt ligands, and that loss of Barx2 downregulates canonical Wnt targets. These data suggest a positive feed-forward loop between Barx2 and Wnt signalling. Taken together with our previous work, our new findings support a model in which Barx2 and MyoD are key transcriptional mediators of Wnt-driven myoblast expansion and differentiation (myogenesis), while Pax7 antagonizes Wnt signaling, consistent with its role in promoting long-term satellite cell self-renewal.

T-3053
PAX3 AND ESRRB CONVERT MEFS TO IMMORTALIZED CELLS

Hwang, Meeyul

Kyungpook National University, Daegu, Republic of Korea

Background: Recently, combination of a defined factors induced mouse fibroblasts to direct somatic stem cells with or without pluripotency. Esrrb (Estrogen related receptor β) has been known to activate Oct3/4 transcription factor, sustaining self-renewal and pluripotency of ES (embryonic stem) cells. Pax3 is a key factor to play a central role in myogenesis during muscle development. Aim: Based on the previous

report, we hypothesized that Esrrb and Pax3 could reprogram mouse embryonic fibroblasts (MEF) into myogenic progenitors. Method: We first constructed the viral vector expressing two factors (Pax3, Esrrb) which are transduced into MEFs. Results: Reprogrammed cells (RCs) were appeared around 2 or 3 weeks after transduction. The morphology of RCs was totally different from MEFs and similar to colony forming unit (CFU) cells. To identify the RCs, the myogenic gene expression such as Pax7, Myf5, MyoD and myogenin was examined using RT-PCR. None of them was expressed in the RCs. But the RCs expressed the transduced genes, Pax3 and Esrrb. We checked the myogenic differentiation potential of RCs by culturing at low serum condition (a differentiation media). RCs did not exhibit the myogenic fusion or the formation of myotube. Next, we used FACs analysis to identify the RCs. The FACs results showed the RCs has mesenchymal cell surface marker such as CD44, CD29, but did not show myogenic progenitor markers. However, RCs can proliferate into more than 40 generation maintaining its characteristics, whereas MEF can sustain until passage 5. To figure out what kind of cell is RCs, further study is required. Conclusion: introduction of Pax3 and Esrrb induced morphological changes of MEF and give immortality.

T-3054
DEVELOPMENT OF HIPSC DERIVED SKELETAL MUSCLE PROGENITORS FOR REGENERATIVE AND PRE CLINICAL APPLICATIONS FOR DMD

Jan, Majib[1], Xi, Haibin[1], Young, Courtney[1], Wakana, Fujiwara[1], Wilson, Matt[1], Zhang, Ruixue[1], Malone, Cindy[2], Pyle, April[3]
[1]MIMG, University of California Los Angeles, Westwood, CA, USA, [2]Biology, California State University Northridge, Northridge, CA, USA, [3]University Of California, Los Angeles, Broad Stem Cell Center, Los Angeles, CA, USA

Duchenne Muscular Dystrophy (DMD) is one of the most severe childhood muscular disorders, resulting in progressive muscle deterioration and ultimately death. This disease is caused by a lack of the protein dystrophin, often due to large genomic deletions that shift the reading frame. No effective treatment is currently available, but one potential therapy involves deriving skeletal muscle progenitor cells (SMPC) from human induced pluripotent stem cells (hiPSCs) derived from DMD patients. Once expanded, these SMPCs could be corrected for proper dystrophin expression and then integrated into patient muscle. We have generated hiPSCs from healthy and DMD-patient fibroblasts to evaluate their use as a pre-clinical tool and in regenerative applications. It has been shown that SMPCs can be generated through the overexpression of specific genes – however, a method that does not require additional genetic manipulation would be required for clinical applications. To obtain a robust SMPC population, we tested 1) overexpression of key skeletal muscle transcription factors using non integrating platforms, 2) addition of factors known to induce skeletal muscle progenitor cell marker expression during embryogenesis in model organisms and 3) evaluation of SMPC potential of genetically targeted cell lines with enhanced mesoderm potential. In addition we are developing a pre-clinical screening platform for evaluating combination therapies using SMPCs and cardiomyocytes derived from DMD-hiPSCs. The refinement of these differentiation protocols for producing and maintaining progenitor populations will aid in the development of regenerative therapies for muscle disorders. This work will lay the groundwork for future studies aimed at using combination therapies in muscular dystrophies in regenerative medicine.

T-3055
BLOCKAGE OF TGF-BETA SIGNALING BY SMALL MOLECULES MAKES FAVORABLE NICHE FOR MUSCLE REGENERATION

Kim, Ah-Young[1], Mi-Ran, Ki[1], Lee, Eun-Mi[2], Lee, Eun-Joo[3], Min, Chang-Woo[1], Kang, Kyung-Ku[4], Lee, Myeong-Mi[1], Kim, Sang-Hyeob[1], Sung, Soo-Eun[1], Hwang, Meeyul[1], Jeong, Kyu-Shik[5]
[1]Kyungpook Natl University, Daegu, Republic of Korea, [2]Kyung-book Natl University, Daegu, Republic of Korea, [3]Kyungpook National University, Daegu city, Republic of Korea, [4]Kyungpook National University, Daegu, Republic of Korea, [5]College of Veterinary Medicine, Kyungpook National University, Daegu city, Republic of Korea

Satellite cells are resident stem cells in mature muscles. They play a key role in the muscle regeneration based on their potentials to give rise to satellite cells (self-renewal) and differentiated skeletal muscle cells. Previous studies have shown that Tgf-beta negatively affects skeletal muscle regeneration by hampering satellite cell proliferation. Tgf-beta also inhibits fusion of muscle fibers and expression of specific genes crucial for normal myogenic differentiation. Moreover, the harmful effect of Tgf-beta in muscle regeneration potentiated in the injury state via transformation of myogenic cells into myofibroblasts responsible for pathologic fibrosis. Thus, the control of Tgf-beta signaling is really important to regenerate skeletal muscle. Here we investigated the effect of two small molecules (Angiostensin II type 1 receptor blocker, losartan; ALK5 inhibitor, LY-364947) on satellite cell functions and possible mechanism of occurred phenomena. Those small molecules are known as inhibiting Tgf-beta signaling pathway by working on different target sites. Regardless of their action sites, they both exhibited positive effects on muscle regeneration not only by boosting the self-renewal activity of satellite cells but also assisting proper differentitation of the precursor cells into functional myofibers. Based on our RT-PCR results, these favorable effects on muscle regeneration might be ascribed to the elevation of both Notch and Wnt signals by these molecules. Because the Notch signaling promotes the proliferation of satellite cells and Wnt/β-catenin signaling induces myogenic specification of muscle stem cells, treatment of these small molecules seems to help enrichment of the muscle stem cell pool and subsequent differentiation simultaneously. In addition, we revealed the involvement of Klf4 on the myogenic differentiation with relevance to Notch signaling.

T-3056
JMJD2C DEMETHYLATES MYOD METHYLATED BY G9A AND REDUCES THE METHYLATION-DEPENDENT MYOD DEGRADATION

Kim, Chang-Hoon, **Kim, Kye-Seong**
Hanyang University, Seoul, Republic of Korea

Histone methylation is an epigenetic modification that regulates gene expression through the recruitment or exclusion of histone writers, erasers, and readers. However, studies on the lysine methylation of non-histone proteins remain limited. Here, we demonstrate that JMJD2C demethylates MYOD and potentiates muscle differentiation by protecting MYOD from methylation-dependent degradation. The expression of JMJD2C protein increased with muscle differentiation. JMJD2C associated with MYOD in vitro and in vivo and directly demethylated MYOD. JMJD2C decreased the G9a-mediated methylation of MYOD in a dose-dependent manner. G9a-methylated MYOD was more highly ubiquitinated than MYOD that was hypomethylated due to JMJD2C activity. Therefore, the level of MYOD methylation correlated with ubiquitin-mediated degradation. CUL4/DDB1/DCAF1 pathway was essential for G9a-methylated

MYOD degradation instead of CUL1/SKP/FBXO32. JMJD2C potentiated muscle differentiation by preventing MYOD degradation. Collectively, our results indicate that JMJD2C plays a pivotal role in MYOD stabilization by preventing the methylation-dependent MYOD degradation.

CARDIAC CELLS

T-3058
IDENTIFICATION AND ISOLATION OF CHAMBER SPECIFIC CARDIAC PROGENITORS

Andersen, Peter, Uosaki, Hideki, Kwon, Chulan
Johns Hopkins University, Baltimore, MD, USA

The heart primordium develops from two distinct pools of Cardiac Progenitor Cells (CPCs), which contributes to different parts of the developing heart, referred to as the first- and the second heart field. Yet, it is still unclear whether these CPC populations are molecular distinct and can be isolated from differentiating pluripotent stem cell cultures. By a candidate approach based on CPC expression patterns in mice combined with lineage trace analyses, we identified differential expression of surface candidates that marks the two distinct CPC pools. Using these surface candidates we were able to isolate distinct pools of CPCs from differentiating pluripotent stem cell (PSC) cultures. Gene expression patterns of these two PSC-derived CPC populations correlate with in vivo expression patterns of the developing right and left ventricle in mice. Microarray and gene expression analyses of the two distinct PSC derived CPC populations revealed functional characteristics and differential expression of previously reported candidates (Tbx5, HCN4 and Tbx1, FGF10, respectively) of the first- and the second heart field. Our results demonstrate that distinct chamber-specific CPCs that give rise to the right- and left ventricle can be identified and isolated directly from differentiating PSC cultures.

T-3059
SELECTIVE RELEASE OF CARDIAC TROPONIN T INTO THE MEDIUM IN CULTURES WITH MURINE AND HUMAN PLURIPOTENT STEM CELL-DERIVED AND MURINE NATIVE NEONATAL CARDIOMYOCYTES

Burkert, Karsten, Ivanyuk, Dina, Baudis, Birte, Hescheler, Jürgen, Saric, Tomo
Institute of Neurophysiology, Medical Center, University of Cologne, Cologne, Germany

Cardiomyocytes (CMs) derived from embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) hold great promise for in vitro disease modeling, drug discovery, toxicity testing and heart regeneration. In order to achieve the production of viable and functional CMs, suitable strategies for monitoring the quality of stem cell differentiation bioprocess are mandatory. Cardiac troponin T (cTnT) represents a highly sensitive and specific serum marker of myocardial injury. We hypothesized that monitoring the release of cTnT into the cell culture medium would represent a simple means for assessing the quality and viability of CMs in differentiating PSC cultures. Surprisingly, in spontaneously differentiating cultures of murine iPSCs and ESCs high levels of cTnT were detectable by electro-chemiluminescence immunoassay (ECLIA) in the medium concomitant with the first appearance of CMs in embryoid bodies and cTnT concentrations in these cultures correlated well with the proportion of CMs in the total cell population. The cTnT release was unlikely a consequence of CM death that may have occurred in the course of differentiation, because we also detected rapid accumulation of cTnT in monolayer cultures of purified viable iPSC-derived CMs. In 24 hours the cTnT released by these cells equalled to around 54 % of the intracellular cTnT content in the cell lysate indicating a high turnover rate of the protein. Measurements of CM viability with a resazurin-based assay, lactate dehydrogenase activity in the medium and caspase activation in the purified murine iPSC-derived CMs revealed that cTnT was not released from apoptotic or necrotic cells. Based on immunoblot analysis, cTnT appeared to be secreted as a full length protein, which was not detectable in the medium by ECLIA after immunoprecipitation with a cTnT-specific antibody. Robust cTnT release was also detected in the medium of pure CMs derived from murine ESCs, in medium of native CMs isolated from murine neonatal hearts, as well as in the medium of cardially differentiating cultures of human iPSCs. Besides cTnT, we have also detected release of NT-proBNP but not of creatine kinase MB isoenzyme (CK-MB) in cultures containing beating clusters derived from human iPSCs, which is suggestive of a selective secretion of only specific markers from viable CMs. Collectively, these observations suggest that sustained release of specific cardiac markers, such as cTnT and NT-proBNP, into the cell culture medium from viable immature neonatal and PSC-derived CMs can be exploited as a method for monitoring of CM differentiation in the course of bioprocess optimization or screening of novel cardioinductive substances that is highly specific, simple and broadly applicable to any cell line without requirements for a specific genetic cell modification. The finding that immature CMs in vitro release a marker that is normally associated only with myocardial injury opens the question of biological meaning of this phenomenon, its in vivo significance and relationship to clinical situations in which elevated serum cTnT levels are found without any signs of heart disease. These issues and the mechanism of the cTnT secretion by viable CMs remain to be further explored.

T-3060
SAFETY AND FEASIBILITY OF INTRAMYOCARDIAL INJECTION OF AUTOLOGOUS CORD BLOOD DERIVED-MONONUCLEAR CELLS IN PIGS FOR RIGHT VENTRICLE REGENERATION WITHIN CONGENITAL HEART DISEASES

Cantero Peral, Susana[1], Burkhart, Harold M.[2], Oommen, Saji[1], Yamada, Satsuki[1], Terzic, Andre[1], Patrick, O'Leary W.[1], Cannon, Bryan C.[1], Nelson, Timothy J.[1]
[1]Mayo Clinic, Rochester, MN, USA, [2]University of Nebraska Medical Center, Omaha, NE, USA

Background and Objectives: Hypoplastic left heart syndrome (HLHS) is a severe form of congenital heart diseases (CHD) that consists of multiple obstructions to flow through the left heart and aorta, as well as hypoplasia of left ventricle. This requires the affected children to undergo in a multistage surgical palliation within the first days of life and most of them end in a cardiac transplantation. The study of stem cell therapies to address heart diseases has advanced steadily over the last decade. Umbilical cord blood (UCB) is rich in multipotent stem/progenitor cells with enhanced potency for angiogenic and myogenic differentiation and proliferative characteristics and can easily be obtained during birth. Our ultimate goal is to re-engineer the native right ventricle (RV) of HLHS children with stem cell-based regenerative strategies using autologous umbilical cord blood-derived mononuclear cells (UCB-MNC) and delivered into the RV myocardium. We designed and executed a pre-clinical long-term study using a porcine model in order to assess the safety and feasibility of intramyocardial transplantation of autologous UCB-MNC into the RV. Material and Methods: Piglets were born at Mayo Clinic animal house facility after C-section from two litters and had autologous UCB collected, processed, and analyzed within 24 hours. Upon meeting clinical-grade release criteria, the oldest animals (n=12) that passed a veterinarian medical

exam were randomized in a double-blinded procedure before surgical delivery of test article or placebo injection into the RV at age of 4 weeks. UCB-MNC (3 x 10^6 cells/kg) or the same volume of vehicle (DMSO 10%) were injected intramyocardially into 5-10 sites radially to RV under visualization by chest surgery. After 3-month of cardiovascular and biochemical follow-up, animals were sacrificed for terminal necropsy with comprehensive histology. We also maintained a satellite group of animals that contained labeled cells to allow for cell-tracking and biodistribution data. Results: The study was technically feasible, and was conducted in the spirit of GLP to mimic future clinical studies. No complications were encountered upon UCB collection. Cord blood volume ranged from 20-59 ml (mean 31.5 ml, SE 2.03 ml). Of the cord blood collected from 26 piglets none demonstrated insufficient viability (mean 92.3%, SE 0.71%) or endotoxin contamination. White blood cell counts (WBC) ranged from 8.9 x 10^6 to 80.4 x 10^6 (mean 40.2 x 10^6, SE 4.68 x 10^6), with a mean mononuclear cell percentage of 96.9%. A single cord blood unit was contaminated in the 26 cord blood units collected and 8 animals were not included in the randomization schedule due to failure of meeting release criteria. All animals remained healthy without evidence for adverse events that included clinical monitoring, cardiovascular performance, clinical chemistry, and terminal multi-system histological analysis. The final analysis revealed no statistical differences between the cohorts receiving the test article and placebo injections as all animals survived the procedures without medical complications. Conclusions: The overall large animal long-term safety and toxicity study demonstrated no evidence of adverse risk due to intramyocardial injection of UCB-MNC. Demonstrating that autologous UCB-MNC can be safely administered will establish the foundation to advance new therapeutic modalities leading towards FDA-approval for clinical testing within CHD.

T-3062
DELIVERY OF MIR181A WITH CATIONIC POLYURETHANES-SHORT BRANCH PEI PROMOTE IPSC DIFFERENTIATED INTO FUNCTIONAL CARDIOMYOCYTE

Chien, Chian-Shiu[1], Chiou, Shih-Hwa[2]
[1]Taipei Veterans General Hospital, Taipei City, Taiwan, [2]Department of Med Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan

Heart diseases, especially myocardial ischemia, remain one of the leading causes of mortality worldwide and usually result in irreparable cardiomyocyte damages and severe heart failure. Inducible pluripotent stem cells (iPSC) generated through reprogramming somatic cells are considered as a potential platform providing an alternative source for regenerative medicine without encountering ethics problems or immunological rejection. Recent advances on iPSC researches have demonstrated promising liver and neuronal cells generated from induced differentiation of iPSC, rendering iPSC-derived cardiomyocytes a rising hope for heart repair. However, despite the low induction efficiency, the driver molecules of myocardial differentiation and the functional reconstruction capacity of iPSC-derived cardiomyocytes is still questionable. Here, we demonstrated that mouse iPS-differentiated cardiomyocytes, like primary-cultured ones, expressed cardiac markers including Hox-A11, HCN4, Connexin 43 (Cx43). A microRNA array showed that miR181a is upregulated during the process of cardiomyocyte differentiation; overexpression of miR181a using a cationic polyurethane (PU-PEI), a biodegradable non-viral vector, delivery system increased the expression of carciomyocyte-specific genes as well as the phosphorylation of Cx43, which marks a state of enhanced resistance of ischemic injury. Functional analysis indicated that miR181a increased the beating area of the iPSC-differentiated cardiomyocytes without altering the beating frequency, indicating that miR181a may not only promote the cardiomyocyte differentiation. Mechanism investigation revealed that miR181a could elevate MyoD expression and enhanced cardiomyocyte differentiation. Our study demonstrated that miR181a regulates the expression of cardiac specific genes and promotes iPSC differentiation into functional cardiomyocytes. Delivery of PU-PEI-miR181a may host clinical potential to enhance the efficiency of cardiac differentiation from iPSC and increase the beating function of these differentiated cardiomyocytes.

T-3063
ASSESSMENT OF CARDIOTOXIC COMPOUND EFFECTS ON INTRACELLULAR CA^{2+} FLUX DURING HUMAN IPSC-DERIVED CARDIOMYOCYTE CONTRACTIONS

Cromwell, Evan F., Crittenden, Carole, Sirenko, Oksana
Molecular Devices, Sunnyvale, CA, USA

Assessing cardiotoxicity is important in the early stages of drug discovery to eliminate potentially toxic compounds from further development. There is a need for cardiotoxicity assays that use more biologically relevant cell-based models to aid development of new chemical entities and ensure drug safety. Induced pluriopotent stem cell (iPSC)-derived human cardiomyocytes are an attractive model for studies of molecular mechanisms of cardiotoxicity and intracellular signalling because they express GPCRs and ion channels, and demonstrate spontaneous mechanical and electrical activity similar to native cardiac cells. An emerging assay in this area employs fast kinetic fluorescence imaging to monitoring spontaneously contracting cardiomyocytes with calcium sensitive dyes. Contraction of cardiac muscle is induced by the influx of Ca^{2+} and release of Ca^{2+} from the sarcoplasmic reticulum in a series of signal transduction steps known as excitation-contraction coupling. By using fluorescent signal to monitor the changes in intracellular Ca^{2+} concentration, it is possible to detect concentration-dependent modulation of beating rate, atypical patterns, as well as subcellular distribution and levels of calcium ions. We have used fast kinetic imaging systems for dynamic monitoring of the coupling between intracellular Ca^{2+} localization and mechanical contraction in cardiomyocytes. We measured the intracellular distribution of Ca^{2+} during a contraction cycle using high spatial resolution and image acquisition up to 200 frames per second. We compare differences in temporal distributions and levels of Ca^{2+} for a variety of cardioactive and cardiotoxic compounds including agonists and antagonists of b-adrenergic signalling, cardiac steroids (digoxin), kinase inhibitors (staurosporine), hERG channel blockers (cispride), anti-cancer agents (idarubicin), and other compounds. The phenotypic patterns observed are consistent with expected mechanisms of action of the compounds. We conclude that human iPSC-derived cardiomyocytes are a useful model for cardiotoxicity testing and can be potentially used to determine specific mechanisms of toxicity.

T-3064
CARDIAC PROGENITOR CELLS AND HEART REGENERATION: DEVELOPMENT AND VALIDATION OF A PHENOTYPIC SCREEN FOR MULTI-LINEAGE DIFFERENTIATION

Drowley, Lauren[1], Jonebring, Anna[1], Andersson, Henrik[1], Kattman, Steven[2], Koonce, Chad[2], Anson, Blake[2], Swanson, Bradley[2], Plowright, Alleyn[1], Wang, Qing-Dong[1], Brolen, Gabriella[1]
[1]AstraZeneca, Mölndal, Sweden, [2]Cellular Dynamics International, Inc, Madison, WI, USA

The loss of functional cardiomyocytes that occurs after myocardial infarction and heart failure cannot be repaired in adult myocardium

and is the focus of significant research. Several progenitor/stem cell populations exist in the heart, some of which can play a role in normal turnover and/or repair. However, despite the presence of these cells within the myocardium, true functional repair after a myocardial infarction does not occur. Identification of compounds aimed at enhancing repair through directed differentiation of transplanted or endogenous progenitor cells is a promising approach to enable regeneration of cardiac tissue. Cardiac progenitor cells (CPCs) represent the earliest stages of mesodermal commitment to cardiac lineages and stem cell-derived CPCs are capable of generating highly enriched cultuResearch of cardiomyocytes and other cardiac cell types. This potential has allowed drug discovery efforts aimed at enhancing proliferation, differentiation, and cell death in this cell population. An enriched population of human iPSC-derived CPCs has been generated that are KDRlow/c-Kitneg/PDGFR-αpos. These CPCs have been characterized for expression of progenitor cell and cardiac markers throughout the differentiation process. Using the CPCs, we have developed a multi-lineage differentiation assays that is suitable for high-throughput screening. The CPC to cardiomyocyte differentiation protocol is robust and routinely yields cultuResearch of up to 30-40% cardiomyocyte purity as assessed by troponinT immunocytochemistry at four days post thaw, increasing to 60-80% by day 8. In addition, the CPCs have the capacity to differentiate down multiple lineages, including cardiac, smooth muscle, and endothelial. In order to identify molecules that can differentially affect the fate decision of the CPCs, we have developed a multi-lineage differentiation assay in a 384-well format. Using this assay, we have screened a small compound library and have identified Wnt and MAPK inhibitors, which have a positive regulatory role in cardiac differentiation from the progenitor cell population. In addition, we have identified compounds that have not been previously described in the literature as positive regulators cardiac differentiation. Here, we have applied high-content phenotypic screening to assess multi-lineage differentiation with a relevant cell population. This assay enables screening campaigns directed at identifying novel therapeutics which could be applied to regeneration of cardiac tissue.

T-3065
EFFECTS OF EXTRACELLULAR MATRIX ON HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTE PHENOTYPE

Gluck, Jessica M., Khan, Ambereen, Chiamvimonvat, Nipavan, Lieu, Deborah K.
University of California, Davis, Sacramento, CA, USA

Statement of Purpose: Due to the limited regenerative ability following myocardial injury, one promising therapeutic approach is to repopulate the heart with human pluripotent stem cell-derived cardiomyocytes (CMs); however regardless of the cell line origin, epigenetics and the extent of genetic manipulation, the resulting CMs are characteristically immature relative to the adult CMs, making them unsafe and unable to immediately improve cardiac function. Extracellular environment is known to direct cellular differentiation and maturation. Our study investigates the effects of extracellular matrix on the myofilament development that can improve their contractile properties in human induced pluripotent stem cell (hiPSC)-derived CMs. Methods: hiPSCs were maintained in mTeSR media on matrigel before being subjected to a published CM differentiation protocol. Elastic dishes (μ-Dish ESS, ibidi) with various Young's moduli (1.5, 15, 28 kPa) were coated with extracellular matrix (ECM) proteins (collagen IV, laminin, fibronectin, or tropoelastin). hiPSC-derived CMs were then seeded on the dishes. At various time points the cells were evaluated for their myofilament development. Results: hiPSC-derived CMs on tropoelastin have small and round morphology. Well-developed myofilaments were never observed in these cells for all Young's moduli tested. Myofilaments were most abundant and developed for hiPSC-derived CMs plated on collagen IV followed by fibronectin and laminin. The myofilaments were more established along with large and spread out cell morphology for hiPSC-derived CMs on Young's moduli of 15 kPa or higher. Our results indicate there is a distinct variation in hiPSC-derived CM phenotypes between the different moduli evaluated as well as exposure to different ECM proteins. Conclusions: Extracellular matrix protein and the substrate elasticity can affect the cell morphology and myofilament development in hiPSC-derived CMs. Studies elucidating the full role of the microenvironment on CM development may develop a protocol for directing the phenotypes of hiPSC-derived CMs.

T-3066
UNDERSTANDING THE MOLECULAR BASIS OF BAG3-RELATED CARDIOMYOPATHY VIA INTERACTION PROTEOMICS AND GENOME-ENGINEERING IN IPS-DERIVED CARDIOMYOCYTES

Judge, Luke M.[1], Perez-Bermejo, Juan[1], Yoo, Jennie[1], Truong, Annie[1], Spindler, Matthew[1], Salomonis, Nathan[2], Lizarraga, Paweena[1], Miyaoka, Yuichiro[1], Johnson, Tasha[1], Johnson, Jeffrey[1], So, Po-Lin[1], Krogan, Nevan[1], Conklin, Bruce R.[1]
[1]Gladstone Institutes, San Francisco, CA, USA, [2]Department of Pediatrics, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, USA

Genetic association studies are generating a wealth of information about genetic variants associated with cardiovascular disease, a leading cause of mortality worldwide. The BAG3 gene is an important example, with >20 variants, half of which are associated with cardiomyopathy. The clinical outcomes associated with different variants range from life threatening dilated cardiomyopathy (DCM) to reduced risk of developing DCM. To study the molecular and cellular consequences of these variants, we need to develop robust human cell-based models. We are using a combination of affinity purification/mass spectrometry (AP-MS), genome editing in human induced pluripotent stem cells, and directed differentiation to identify the functional consequences of BAG3 mutations in human cardiac tissue. We used AP-MS to compare different variants of the BAG3 gene, obtaining a list of interactions that seem affected by the presence of a disease-related mutation. This set of experiments suggests that disease-related mutations of BAG3 are associated with a loss of interaction with a number of heat shock proteins, among others. It also suggests that one specific mutation (P209L) produces pathological gain-of-function due to an increased interaction with tubulin beta. Furthermore, we found that transcription of BAG3 and known interactors increased during directed differentiation of human iPS cells to cardiomyocytes (iPS-CMs). In order to test the mechanism of BAG3 function in iPS-CMs, we generated multiple isogenic disease lines using Transcription Activator-Like Effector Nuclease (TALEN) assisted homologous recombination. We have prepared an allelic series encompassing a spectrum of BAG3 mutations: loss-of-function mutations, the P209L putative gain of function mutation, and a common variant associated with decreased risk of cardiomyopathy. We are also introducing tagged alleles of BAG3 into iPS-CMs so that AP-MS can be performed in the relevant cell type. Functional studies of this allelic series of mutant cardiomyocytes will elucidate the role of BAG3 in cardiac disease. We also believe our approach will provide an important resource towards a broader understanding of the role of genetic variation in cardiac disease.

T-3067

THE LET-7 FAMILY OF MIRNAS PROMOTES MATURATION OF HUMAN STEM CELL DERIVED CARDIOMYOCYTES BY INDUCING A METABOLIC SUBSTRATE SWITCH

Kuppusamy, Kavitha[1], Sperber, Henrik[2], Madan, Anup[3], Chudin, Eugene[3], Rodriguez, Marita[4], Fischer, Karin[1], Fugate, James[5], Van Biber, Benjamin[5], Pabon, Lil[5], Tulloch, Nathaniel L.[6], Cook, Savannah[7], Ware, Carol B.[6], Sniadecki, Nathan[7], Laflamme, Michael[7], Murry, Charles[8], Ruohola-Baker, Hannele[9]

[1]Biochemistry, University of Washington, Seattle, WA, USA, [2]Chemistry, University of Washington, Seattle, WA, USA, [3]Labcorps, Seattle, WA, USA, [4]Mechanical engineering, University of Washington, Seattle, WA, USA, [5]Pathology, University of Washington, Seattle, WA, USA, [6]University of Washington, Seattle, WA, USA, [7]University of Washington, Seattle, WA, USA, [8]University of Washington - Center for Cardiovascular Biology, Seattle, WA, USA, [9]Institute for Stem Cell and Regenerative Medicine University of Washington, Seattle, WA, USA

Human embryonic stem cells have the ability to provide an unlimited source of cardiomyocytes. But the therapeutic potential of these stem cell derived cardiomyocytes is largely hampered by their lack of physiological maturity. Currently, it is not possible to direct/accelerate hESC derived cardiomyocytes towards functional maturation since the factors that drive the process are largely unknown. In this study, , using large scale miRNA and mRNA sequencing, we identified let-7 family of miRNAs to be one of the most highly up regulated in cardiomyocyte samples that were matured using bioengineering strategies and long term culturing techniques. Over expression of members of let-7 family (let-7 OE) in stem cell derived cardiomyocytes results in increased cell size, perimeter, sarcomere length, force contraction and respiratory capacity. Interestingly, genome wide mRNA sequencing of let-7 OE cardiomyocytes revealed several let-7 targets in PI3/AKT/Insulin pathway to be significantly down regulated while a number of fatty acid transporters were significantly up regulated. Furthermore, these cardiomyocytes that are enriched for specific members of the let-7 family also show higher usage of palmitate in a metabolic flux assay using Seahorse analyzer clearly demonstrating that this increase in cardiomyocyte maturation by let-7 could be a result of down regulation of PI3/AKT/Insulin pathway and an up regulation of fatty acid metabolism. Thus we propose let-7 to be an important mediator in inducing adult-like metabolic energetics in maturing hESC derived cardiomyocytes. This approach of generating mature cardiomyocytes with let-7 over expression from hESCs/hIPSCs will be highly significant for basic and applied research thereby increasing the value of stem cell cardiogenesis for medical applications.

T-3068

LINEAGE REPROGRAMMING OF MOUSE FIBROBLASTS TO PROLIFERATIVE AND MULTIPOTENT INDUCED CARDIAC PROGENITOR CELLS BY DEFINED FACTORS

Lalit, Pratik A.[1], Salick, Max R.[2], Lea, Martin[1], Jackson, Steven A.[2], McIntosh, Brian E.[2], Crone, Wendy C.[2], Kyba, Michael[3], Thomson, James A.[4], Garry, Daniel J.[5], Kamp, Timothy J.[1]

[1]Medicine, University of Wisconsin, Madison, Madison, WI, USA, [2]University of Wisconsin, Madison, Madison, WI, USA, [3]University of Minnesota, Minneapolis, MN, USA, [4]Morgridge Institute for Research, Madison, WI, USA, [5]Medicine, University of Minnesota, Minneapolis, MN, USA

Several studies have reported reprogramming of mouse fibroblasts to induced cardiomyocytes (iCMs). However, reprogramming to cardiac progenitor cells (CPCs), which may be more favorable for cell therapy because of the proliferative properties and multipotency of the cells, remains to be accomplished. This study aims to reprogram mouse fibroblasts to induced CPCs (iCPCs) by transcription factor (TF)-mediated reprogramming. We have cloned a library of 22 genes including cardiac-related TFs and chromatin remodeling agents into a novel, inducible lentivirus vector. Fibroblasts were derived from a Nkx 2.5- YFP cardiac progenitor reporter mouse model that expresses YFP in developing heart only between E7.5 - E12 during embryogenesis. We first confirmed efficient transcription/translation of our defined factor library. Next, as a control experiment, neonatal cardiac fibroblasts were infected with Gata4, Mef2c, Tbx5 or Gata4, Mef2c, Tbx5, Hand2 (published factors) and monitored for appearance of beating cells. Spontaneously beating, YFP- iCMs were observed only after application of doxycycline (25 days) for both combinations of factors, demonstrating a robust reprogramming system. We then infected adult cardiac fibroblasts with the entire library of 22 factors and observed YFP+ cells on doxycycline treatment. The factor pool was subsequently narrowed down to 11 essential factors. After infection with 11 factors, YFP+ cells were first observed 4 days after doxycycline treatment. By day 20 YFP+ cells developed into two-dimensional, highly proliferative YFP+ colonies of cells that lost their parental fibroblast morphology and exhibited a high nucleus to cytoplasmic ratio. Uninfected/iPSC factors infected control fibroblasts yielded no beating or YFP+ cells. RNA seq/qPCR analysis of YFP+ cells revealed up-regulation of CPC markers including Mesp1, Mef2c, Irx4, Gata6, Tbx5, Tbx 20 accompanied with down regulation of fibroblast specific genes such as Fsp1, Thy1. Immunocytochemistry revealed that YFP+ cells have nuclear localization of CPC transcription factors including Gata4, Nkx 2.5 and Irx4. The YFP+ cells could be differentiated into cardiac lineage cells like cardiomyocytes (alpha-actinin, alpha-MHC, cardiac-actin), smooth muscle cells (SM-MHC) and endothelial cells (CD31) based on expression of lineage specific markers via immunocytochemistry. Even after doxycycline withdrawal for over 25 passages, YFP+ cells expressed CPC markers, retained proliferative ability and multipotency indicating stable reprogramming to iCPC state. Similar reprogramming was demonstrated using non-cardiac fibroblasts and PiggyBac transposon based delivery system. Recently, iCPC reprogramming was achieved using a 6-factor library. These results demonstrate the ability of inducible defined factors to reprogram mouse fibroblasts into proliferative and multipotent iCPCs.

T-3069

NOVEL ANTI INFLAMMATORY AGENTS IN CELL BASED THERAPY FOR MYOCARDIAL INFARCTION

Sirish, Padmini[1], Li, Ning[1], Lopez, Javier E.[1], Lee, Kin Sing S.[1], Wu, Joseph C.[2], Hammock, Bruce[1], Chiamvimonvat, Nipavan[1]

[1]University of California, Davis, CA, USA, [2]Stanford University School of Medicine, Stanford, CA, USA

Background: Cardiovascular disease is the leading cause of death for both men and women in the United States. Since cardiac myocytes have limited ability to regenerate, their malfunction or significant loss due to aging or diseases can lead to lethal consequences. Recent studies have provided exciting evidence to support the notion that stem cells may offer an enormous potential for regenerative therapy. However one intractable barrier to cell therapy in all tissues is overcoming the confounding hurdle of scars that ensue from acute and robust inflammatory responses arising during tissue injury. One of the most conserved inflammatory responses is the activation of phospholipase A2 and the release of arachidonic acid, which is metabolized through the cyclooxygenase, lipoxygenase, and cytochrome P450 (CYP450) pathways. Our laboratory was the first to demonstrate the beneficial effects of a novel class of inhibitors of the enzyme soluble epoxide hydrolase (sEH) in the CYP450 pathway in clinically relevant models

of cardiac hypertrophy and failure. Treatment with sEH inhibitors prevents ventricular myocyte hypertrophy, decreases cardiac fibroblast proliferation and fibrosis and electrical remodeling in pressure overload and myocardial infarction (MI) models. The CYP450 pathway represents the robust frontier in the inflammatory pathway that we targeted using specific and novel sEH inhibitors (sEHIs). Methods: To test our hypothesis that the suppression of pro-inflammatory cytokines and chemokines at the injury site using sEHIs can provide optimal conditions for the integration of cells, human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) were transplanted into mouse myocardium with MI and treated with sEHIs. Multidisciplinary in vivo and in vitro techniques were used to assess the survival and engraftment of hiPSC-CMs. Results: In vivo bioluminescence imaging showed a significant improvement in the survival of hiPSC-CMs in mice with sEHI treatment compared to no sEHI treatment at 4 weeks. Functional analysis showed a significant improvement in the fractional shortening (62±2.6%, n=4) in the sEHI treated mice compared to the non-treated mice (55±0.6%, n=3, $p<0.05$). Histological analysis demonstrated that treatment with sEHI resulted in a decrease in infarct size and prevented the development of cardiac dilatation post-MI. The mechanistic basis for improved stem cell engraftment in the treated hearts were studied by examining the cellular environment and the engrafted hiPSC-CMs. Single-cell based flow cytometric assays showed a decrease in apoptotic cardiomyocytes, non-myocytes and a decrease in reactive oxygen species production in the sEHI treated mice. NF-κB represents one of the critical players in the cytokine-mediated inflammation. To determine if sEHI treatment prevents the activation of NF-κB in hiPSC-CMs, we examined the nuclear translocation of NF-κB in hiPSC-CMs in culture. Our data demonstrates an increased nuclear translocation of NF-κB in the cultured hiPSC-CMs upon TNF-α stimulation that was inhibited by the treatment of sEHI. Conclusions: Taken together, our data demonstrate an improved cardiac function, prevention of adverse cardaic remodeling and enhanced engraftment of hiPSC-CMs in the injured myocardium with sEHI treatment. Our findings suggest the suppression of inflammation and resolution of pre-existing fibrosis using sEHIs may become an adjuvant to cell therapy independent of cells and tissue types.

T-3070
N-CADHERIN REGULATES SECOND HEART FIELD CARDIAC PROGENITOR STEM CELL NICHE BY WNT SIGNALING

Soh, Boon Seng[1], Xu, Huansheng[1], Buac, Kristina[1], Ng, Shiyan[1], Li, Edward[1], Wu, Hao[1], Chmielowiec, Jolanta[1], Jiang, Xin[1], Bu, Lei[1], Li, Ronald Adolphus[2], Cowan, Chad[1], Chien, Kenneth R.[3]
[1]Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA, [2]Stem Cell and Regenerative Medicine Consortium, Hong Kong University, Hong Kong, [3]Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden

The cardiac progenitor cells (CPCs) in the anterior heart field (AHF) can proliferate and differentiate toward the major cell types found in the heart, primarily cardiomyocytes. The mechanism by which their proliferation and differentiation are regulated remains to be elucidated. Here we demonstrate a dynamic interaction between N-cadherin and β-catenin in the regulation of the AHF-derived CPCs and the differentiated cardiomyocytes. Through in-silico data mining and genetic loss-of-function studies we identified N-cadherin as a component of the microenviroment for the AHF CPCs. In the CPCs, N-cadherin is expressed at a low level and plays a positive role in the activation of Wnt/β-catenin signaling to promote the proliferation and prevent pre-mature differentiation of the progenitor cells.

T-3071
REPORTER-BASED ISOLATION AND COMPARISON OF HUMAN INDUCED PLURIPOTENT STEM CELL- AND EMBRYONIC STEM CELL-DERIVED CARDIAC PROGENITORS AND CARDIOMYOCYTES

van den Berg, Cathelijne W.[1], Okawa, Satoshi[2], Casini, Simona[1], Elliott, David A.[3], del Sol Mesa, Antonio[2], Passier, Robert[1], Mummery, Christine L.[1], Davis, Richard P.[1]
[1]Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, Netherlands, [2]Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg, [3]Murdoch Childrens Research Institute, Parkville, Australia

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have the potential to differentiate into multiple cell types of the human body, including cardiomyocytes. These pluripotent stem cells (PSCs) offer great opportunities for studying early development, modelling genetic cardiac diseases, drug discovery and screening, as well as toxicity testing. While a lot of comparisons have been performed on undifferentiated ESCs and iPSCs, few studies have looked at the differentiated cell types. It is therefore largely unknown how much variability exists between human iPSC- and ESC-derived cardiomyocytes (hiPSC-CMs and hESC-CMs), particularly in terms of gene and protein expression. Analysis of hiPSC-CMs has been hampered by the unavailability of cardiac-specific fluorescent reporter lines that would enable isolation of cardiac progenitors. Generation of hiPSC lines containing the same lineage-specific reporter as a hESC line would facilitate quantification and isolation of comparable progenitor populations to determine the true degree of similarity between lineages derived from hESCs and hiPSCs. We therefore generated a hiPSC line in which expression of NKX2-5 was mirrored by GFP. NKX2-5 marks a cardiac progenitor that subsequently becomes a more committed cardiovascular population. This matches a similar line we have previously generated in hESCs. Using completely defined culture systems for both maintaining the human PSCs and inducing cardiac differentiation, we could generate cultuResearch comprising of more than 50% GFP+ cells from both cell lines. Cell surface proteins we had previously identified as markers of human cardiomyocytes were expressed in the differentiating NKX2-5 GFP hiPSC line, indicating that hiPSCs quantitatively had similar cardiomyogenic potential as hESCs. With these unique reporter lines, we have now been able to isolate comparable cardiac populations derived from both ESCs and iPSCs, allowing us to investigate their phenotypic and epigenetic stability and further assess their potential usefulness as an in vitro model system.

T-3072
REGULATION OF CARDIOMYOCYTE PROLIFERATION BY CNOT3

Wang, Li, Hu, Guang
National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

Stem cell therapy has been considered as a promising strategy to repair damaged myocardium caused by infarction and ischemic heart diseases. Despite breakthrough in deriving cardiomyocytes from human pluripotent stem cells, the survival and maturation of those cells after transplantation into infarcted myocardium are still major obstacles hindering their clinical application. Thus, it is critically important to find novel molecular targets participating in cardiac development to improve the therapeutic effects of stem cell transplantation. Cnot3 has been identified as a factor required for normal heart function in an RNAi screen in *Drosophila*. Herein, we sought to determine its role in

cardiac development and evaluate its potential in improving outcomes of cardiac stem cell therapy. We show that Cnot3 is highly expressed at the cardiac progenitor stage, both during human embryonic stem cell (hESC) cardiac differentiation and mouse heart development. Cnot3 silencing inhibits cardiomyocytes differentiation and represses their proliferation, while Cnot3 overexpression has the opposite effects. Importantly, the pro-proliferative effect of Cnot3 is also evident in mature cardiomyocytes. In vitro, Cnot3 expression promotes proliferation in hESC-derived mature cardiomyocytes. In vivo, Cnot3 heterozygous deletion inhibits proliferation of cardiomyocytes in adult mice. Furthermore, Cnot3 expression in human heart decreases during the natural course of aging, correlating with the loss of proliferation of cardiac cells. Taken together, our data suggest that Cnot3 plays a key role in regulating cardiomyocyte proliferation both during development and in the adult stage. We are further testing the potential therapeutic benefits of Cnot3 overexpression in the treatment of myocardial infarction and other heart diseases.

T-3073
PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS AS A MODEL FOR FAMILIAL CARDIOMYOPATHY

Yang, Daniel[1], Futakuchi-Tsuchida, Akiko[1], Xu, Joy[1], Jiao, Alex[1], Boucek, Robert[1], Kim, Deok-Ho[2], Regnier, Michael[1], Lil, Pabon[1], Reinecke, Hans[1], Murry, Charles[3]
[1]University of Washington, Seattle, WA, USA, [2]University of Washington Department of Bioengineering, Seattle, WA, USA, [3]University of Washington - Center for Cardiovascular Biology, Seattle, WA, USA

Background: Mutations in sarcomeric proteins are frequent causes of familial cardiomyopathies. We identified a family with a cardiomyopathy that was characterized by a normal sized left ventricle and moderately severe systolic dysfunction that developed in the fourth decade of life. Exon sequencing of the sarcomeric genes revealed a missense myosin heavy chain 7 (MYH7) E848G mutation that segregated with the clinical phenotype. Animal modeling of MYH7 associated genetic cardiomyopathies have been difficult because many studies have yielded conflicting results, likely because MYH6 is the primary isoform in rodents. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are a new platform that can be used to model genetic cardiomyopathies. We hypothesized that MYH7 E848G results in impaired contractile function in patient-specific iPSC-CMs. Methods and Results: We obtained dermal biopsies from two family members with the MYH7 E848G mutation. Another two dermal biopsies were obtained from normal individuals. Fibroblasts were reprogrammed to hiPSC colonies with episomal factors containing Oct4, SOX2, Klf4, l-Myc, Lin28, p53 shRNA, and EBNA1. A third normal patient hiPSC line was previously generated with lentivirus. Flow cytometry demonstrated greater than 50% double positive staining of these cells with pluripotency markers, SSEA4 and GCTM2. Using a differentiation protocol containing activin A, BMP4, and WNT signaling agonist/antagonists, we generated hiPSC-CMs with over 70% cTNT positive staining from all 5 patient hiPSC lines. Frozen stocks of the hiPSC-CMs from all 5 patients were created by freezing the cells with a rate-controlled bio-freezer at day 20 of differentiation. Human iPSC-CMs were thawed and plated onto nanopatterned slides to form aligned myofibrils. Analysis of the contractile function of individual hiPSC-CMs with Ionoptix, a video-based fluorescence-microscopy imaging device, identified no significant change in the contractile properties of the cells 30 days after differentiation. Interestingly, after 50 days, the maximal fractional shortening of the mutant hiPSC-CMs were ~30% less than the age-matched wild-type hiPSC-CMs (Kruskal-Wallis $P<0.05$; $P<0.05$ for all post-hoc analysis between mutant and wild-type lines except for one comparison that was $P<0.07$; $n>10$ in all groups). Analysis of calcium transients with the ratiometric dye, fura-2, did not identify any differences in the day 30 hiPSC-CMs. To further test the hypothesis that the E848G mutation is the cause of impaired systolic function in this family, we constructed adenoviruses that overexpress flag-tagged MYH7 or MYH7 E848G and demonstrated that full-length MYH7 and MYH7 E848G incorporate into the sarcomeResearch of wild-type hiPSC-CMs. Ongoing studies with these viruses will investigate the impact they have on the contractile function of wild-type hiPSC-CMs.Conclusion: Patient-specific hiPSC-CMs from a family with MYH7 E848G associated systolic dysfunction develop impaired contractile function over time, which parallels the clinical phenotype.

T-3074
REGENERATION OF CARDIOMYOCYTES FROM FIBROBLASTS IN VIVO USING HUMAN CARDIAC REPROGRAMMING FACTORS IN A PIG MODEL

Yu, Pengzhi (Palmer)[1], Huang, Yu[1], Vega, Felix[2], Metzler, Scott A.[1], Chin, Albert[2], Fu, Jidong[1], Srivastava, Deepak[1]
[1]Gladstone Institutes, San Francisco, CA, USA, [2]ISIS, San Carlos, CA, USA

Because adult cardiomyocytes have little regenerative capacity, myocardial infarction from coronary occlusion typically leads to the permanent loss of cardiomyocytes and collagen scarring of the infarcted region. We previously reported that the forced expression of three transcription factors (Gata4, Mef2c and Tbx5, or GMT) is capable of directly reprogramming cardiac fibroblasts into cardiomyocyte-like cells in vitro and in vivo in the mouse model. In human cells, the addition of two other transcription factors, ESRRG and MESP1, were sufficient to initiate cardiac reprogramming in vitro. We investigated whether these five factors could induce conversion of fibroblasts to cardiomyocytes in vivo in pigs as a pre-clinical model. Three days after myocardial infarction created by catheter occlusion of the left ventricular descending artery, retroviral vectors expressing DsRed only, or together with genes encoding the human cardiac reprogramming factors, were delivered intra-myocardially in the peri-infarct region. Pig hearts were explanted 6 weeks after the retroviral infection of dividing cells and analyzed histologically. Newly generated cardiomyocytes were detected in the peri-infarct regions by immunostaining of cardiac Troponin T and DsRed and were characterized by well-formed sarcomeric structuResearch and morphology. These findings establish the ability of human cardiac reprogramming factors to transform endogenous non-cardiomyocytes into cardiomyocyte-like cells in a large animal model after injury. Functional improvement from this approach and safety are currently being evaluated.

MESENCHYMAL CELL LINEAGE ANALYSIS

T-3076
POLYMER NANONEEDLE TEMPLATES WITH USER-DEFINED FEATURESEARCH FOR CONTROL OF STEM CELL FUNCTION

Balikov, Daniel[1], **Crowder, Spencer William**[1], Terekhov, Alexander[2], Costa, Lino[2], Lewis, Holley[1], Hofmeister, William[2], Sung, Hak-Joon[1]
[1]Vanderbilt University, Nashville, TN, USA, [2]University of Tennessee Space Institute, Tullahoma, TN, USA

A variety of synthetic matrix systems with coating, mixing, and conjugation of biological molecules have been exploited to explore the effect of the microenvironment on in vitro cell behavior and fate in

a 3D context. Unfortunately, these systems lack the ability to tightly control heterogeneous, multi-directional physical interactions that the cell experiences with the matrix, and to decouple cell-matrix or cell-cell interactions. Thus, while previous studies elucidated the essential role of cell-matrix interaction on cell lineage commitment, the importance of cell-cell interaction, which competes with cell-matrix interaction in determining the cell phenotype, has been underexplored. Using a recently-developed nanomachining technique, we have produced user-defined templates of polymeric nanoneedles with variable parameters, including needle spacing (single to hundreds of microns), needle size (tens to hundreds of nanometers), and polymer composition. These samples are pseudo-three-dimensional due to the protrusion of needles from the two-dimensional polymer "floor," and serve as a more relevant culture system than conventional tissue culture polystyrene or three-dimensional protein gels. No other machining method to date has been able to provide such reproducible templates in order to eliminate batch-to-batch variability and control nanoscale topology in order to study cell behavior. Because we were able to utilize this new technology for cell substrate development, we hypothesized that variation of these parameters would incrementally bias cells towards either a cell-matrix or cell-cell dominant phenotype, which we have been able to show via immunostaining and quantitative PCR. Using human mesenchymal stem cells (hMSCs) and the highly biocompatible polymer, poly(ε-caprolactone) (PCL), we observed 2x3 um needle spacing (x-y spacing) substrate to significantly increase cell-cell interactions while concurrently decreasing cell-matrix interactions, when compared to a flat, no needles control substrate or tissue culture plate. Cells were more aligned and had significant cell-cell membrane interactions, while cells on flat substrates had a more prominent cell spreading morphology. Quantitative PCR also showed that focal adhesion associated proteins like SRC and Vinculin were significantly down regulated on 2x3 um needle spacing substrate while cell-cell adhesion associated proteins like intracellular adhesion molecule (ICAM) and platelet endothelial cell adhesion molecule (PECAM) were significantly upregulated on the same needle substrate. Predictably, the opposite trends were seen when hMSCs were cultured on substrates with larger needle spacing, such as 10x10 um, illustrating the utility of these substrates for biasing cell-cell versus cell-matrix interactions.Given these results, this study is the first of its kind to specifically manipulate nanotopological aspects of cell substrates to effectively decouple the competition of cell-cell and cell-matrix interactions that is experienced within the cellular microenvironment. Moreover, we continue to explore additional combinations of the outlined parameters in the hope of pinpointing a series of substrates that can be used for large-scale culture purposes needed for complex cell studies in the biomedical sciences.

T-3077
EFFECTS OF LOW OXYGEN TENSION ON STEMNESS OF EX VIVO EXPANDED HUMAN YOUNG CARTILAGE-DERIVED STEM CELLS

Kim, Jiyoung[1], Min, Byoung-Hyun[2], Park, So Ra[3], Choi, Byung Hyune[4]
[1]Inha Research Institute for Medical Sciences, Inha University College of Medicine, Incheon, Republic of Korea, [2]Department of Orthopaedic Surgery, Ajou University School of Medicine, Suwon, Republic of Korea, [3]Department of Physiology, Inha University College of Medicine, Incheon, Republic of Korea, [4]Division of Biomedical and Bioengineering Science, Inha University College of Medicine, Incheon, Republic of Korea

Most of adult stem cells reside in defined hypoxic microenvironments termed niches which regulate stem cell fate. The embryonic stem cells (Esc) are derived from embryos which exist 3-5% oxygen condition. This environment is physiologically normal not only for ESc but also for many other types of stem cells and plays an important role in maintenance of cell stemness. However, it is not clear whether hypoxic condition is critical for stem cell function. In this study, we successfully isolated stem cells from human young cartilage tissue and these cells not only expressed mesenchymal stem cell markers (CD73, CD90, and CD105) but also showed increased expression of SSEA-4 under hypoxic condition. Cultivation at 5% oxygen improved a colony forming capacity and cell proliferation rate of human young cartilage stem cells (hCSCs) and resulted in a significantly increased gene expression of OCT4, SOX2, and NANOG compared with normoxic condition. hCSCs under hypoxic condition could be successively passaged 15.5±0.9 times over a period of 4-5 months and underwent an average of 45.5±3.9 population doublings. However, hCSCs under normoxic condition reached senescence after 9.5 ± 1.2 passages and underwent an average of 28.3 ±2.7 population doublings. Under appropriate differentiation culture conditions, they differentiated into adipocytes, osteocytes, and chondrocytes, as revealed by oil red O, alizarin red, and alcian blue staining, respectively. Long-term cultivation of hCSCs in normoxic condition significantly induced the expression of p53, p21 and BAX, whereas hCSCs in hypoxic condition markedly enhanced the expression of BCL2. Indeed, senescence significantly decreased in hypoxic cells, as evaluated by the expression of senescence-associated beta-galactosidase. These results strongly demonstrate that hypoxia can affect stem cell function and could elucidate hypoxic niche function.

T-3078
IDENTIFICATION OF FUNCTIONALLY DISTINCT DIFFERENTIATION-COMPETENT AND IMMUNOMODULATORY MESENCHYMAL STROMAL CELL SUBPOPULATIONS

Knight, Charlotte Anne, James, Sally R., Clough, Sally L., Lee, Jennifer, Afsari, Farinaz, Ashmore, James, Ashton, Peter, Genever, Paul
Department of Biology, University of York, York, United Kingdom

Mesenchymal stromal cells (MSCs) offer broad therapeutic options in regenerative medicine, which include exploiting their tissue forming capacity and immunomodulatory characteristics. MSCs are frequently examined as heterogeneous cell populations that are likely to contain a true, rare stem cell subset as well as additional, functionally-important non-stem cell fractions. To enable in-depth analyses of MSC heterogeneity, we adopted a telomerase (hTERT) immortalisation and cloning strategy to identify functional variance in human MSC subpopulations. Four clones (Y101, Y102, Y201 and Y202) with different behavioural traits were selected from >100 single cell-derived MSC colonies following hTERT lentiviral transduction. Importantly, all lines expressed markers typically assigned to MSCs, with positive CD29, CD44, CD73, CD90, CD105 and negative CD34 and CD45 expression. When subcloned at low seeding densities, Y101/Y201 lines formed dispersed colonies and maintained a fibroblastic morphology typical of primary MSCs, whilst Y102/Y202 demonstrated a flattened, rounded and enlarged morphology with compact non-migratory colonies. Y101 and Y201 MSC lines exhibited tri-lineage (osteo-, adipo-, chondrogenic) differentiation potential, whilst Y102/Y202 appeared nulli-potent under typical differentiation induction stimuli. Following global gene expression profiling, over 6,000 transcripts were differentially expressed between the four lines and parental MSCs ($P<0.05$, >2-fold) with clustering algorithms grouping Y101 with Y201, closest to primary MSCs, whilst Y102 and Y202 clustered independently. Y101 and Y201 were enriched in genes representative of mesodermal MSCs (PRRX1, PDGFRA, KITLG, GDF5) whereas nulli-potent Y102/Y202 were enriched in angiohematopoietic genes (FLT1, KDR, TEK, EDN1, VWF, MCAM) suggesting alternative developmental origins. Significant differences in metagenes representing cell-ECM interactions, RTK signalling, adipogenesis

and endochondral ossification were identified. Immunomodulatory genesets such as cytokine/inflammatory and interferon/TNF signalling were significantly enriched in the non-differentiating Y102/Y202 lines and strikingly, 61% of genes altered upon exposure of primary MSCs to proinflammatory cytokines were also differentially expressed in unstimulated Y202 compared to parental MSCs, suggesting an intrinsic immunomodulatory function in rare, poorly-differentiating MSC subpopulations. Using ELISAs and immunocytochemistry we confirmed significantly increased secretion of IL-7, a cytokine essential for lymphopoiesis, in Y102/Y202 compared to Y101/Y201. Heterogeneous primary MSCs secreted low levels of IL-7, with negligible expression in dermal fibroblasts. To identify IL-7-positive MSC subpopulations *in vivo*, we used an IL-7Cre Rosa26-eYFP lineage-tracing mouse model. YFP-positive cells were found in bone marrow at perivascular and endosteal-lining locations with an approximate 4% frequency. 90% of femoral osteocytes, terminally-differentiated cells of the osteogenic lineage, and all adipocytes examined were YFP-negative, indicating they derived from an IL7-negative MSC progenitor. These findings provide strong evidence for the existence of bone marrow subsets of differentiation-competent MSCs and resident, immunomodulatory MSCs, which will instruct functional analyses of MSCs and selection criteria for therapy.

T-3079
ANALYSIS OF LINE1 EXPRESSION IN HUMAN MESENCHYMAL STEM CELLS

Kono, Ken
Division of Medical Devices, National Institute of Health Sciences, Tokyo, Japan

Human mesenchymal stem cells (hMSCs) can differentiate into a variety of cell types such as osteoblasts, chondrocytes, and smooth muscle cells. Therefore, hMSCs have been used in studies such as the differentiation to bone and cartilage and their regeneration. Long interspersed nuclear element 1s (LINE-1s) are retrotransposons that comprise ~17% of the human genome. Although most of LINE-1s have been rendered inactive by mutations, the average human genome contains an estimated 80-100 retrotransposition (RTP)-competent LINE-1s which may induce genome instability. Cytidine deaminase enzyme, APOBEC3B (A3B), was known to restrict LINE-1s RTP. A previous study demonstrated that there is a deletion polymorphism in A3B gene. For safety evaluation of the tissue engineered products, it is very important to maintain the genome integrity of stem cells. Although LINE-1s mRNA expression was observed in ES and iPS cells, it is unknown whether LINE-1s mRNA was expressed in hMSCs. In this study, we examined the LINE-1s mRNA expression in hMSCs and the relationship between the LINE-1s mRNA expression and A3B genotype. First, we measured LINE-1s mRNA expression in 2 kinds of hMSCs purchased from Lonza by quantitative RT-PCR and found that LINE-1s mRNA was expressed in the hMSCs. Because A3B deletion polymorphism is more common in Japanese, we analyzed the genotype of A3B in 25 Japanese hMSCs purchased from JCRB Cell Bank. Fourteen, nine, and two of hMSCs were homozygous for A3B wild type (WT), heterozygous, and homozygous for A3B deletion, respectively. Next, we examined the relation between A3B genotype and LINE-1s mRNA expression, but no relation was found. However, we found that hMSCs from homozygous for A3B deletion express LINE-1s that have high homology to RTP-competent LINE-1s. These results indicated that A3B genotype may affect LINE-1s RTP and the genome integrity was threatened in homozygous for A3B deletion.

T-3080
DIRECT GENESIS OF FUNCTIONAL RODENT AND HUMAN SCHWANN CELLS FROM SKIN MESENCHYMAL PRECURSORS

Krause, Matthew Paul[1], Dworski, Shaalee[2], Feinburg, Konstantin[1], Jones, Karen L.[1], Johnston, Adam[1], Paul, Smitha[1], Peles, Elior[3], Kaplan, David R.[4], Miller, Freda D.[1]
[1]Neurosciences and Mental Health, The Hospital for Sick Children Research Institute, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel, [4]Cell Biology, Hospital for Sick Children, Toronto, ON, Canada

The skin is a highly regenerative organ containing distinct populations of adult precursors that serve to maintain this regenerative capacity. One of these is a Sox2-positive dermal precursor that resides in hair follicles and that can regenerate the dermis and induce hair follicle morphogenesis. These cells, termed skin-derived precursors (SKPs) can be cultured and differentiate into mesenchymal cell types like smooth muscle cells, adipocytes and dermal fibroblasts, and peripheral neural cells such as Schwann cells. However, lineage tracing recently showed that SKPs isolated from facial skin come from the neural crest, while SKPs from dorsal skin derive instead from a somite origin, as does the rest of the dorsal dermis. In spite of these different origins, dorsal and facial SKPs are very similar at the transcriptome level. These findings indicate that cells of different developmental origins can converge to generate somatic tissue precursor cells with highly similar phenotypes. However, they also raise a number of important questions. In particular, while it is generally thought that only the neural crest generates peripheral neural cells, these findings suggest that mesenchymal precursors of non-neural crest origin might have the same capacity. Here, we have addressed this issue. Our data indicates that SKPs have the capacity to generate neural progeny despite their non-neural crest origin. In particular, we show, using a Dermo1Cre/+ mouse for lineage tracing, that SKPs isolated from the dorsal skin originate from dermal mesenchymal cells. These mesenchymally-derived SKPs can, without genetic manipulation, generate functional Schwann cells, a neural cell type that originates from the neural crest developmentally. These SKP-Schwann cells express canonical markers of Schwann cells and myelinate axons when injected into crushed sciatic nerve. Transcriptome analysis shows that the Schwann cells generated from dorsal rodent SKPs are highly similar to Schwann cells isolated from the peripheral nerve, yet distinct from undifferentiated SKPs. Moreover, we demonstrate that this is not a rodent-specific phenomenon, since human SKPs isolated from neonatal foreskin dermis can also generate myelinating Schwann cells. Thus, non-neural crest-derived mesenchymal precursors can differentiate into bona fide peripheral glia in the absence of genetic manipulation, suggesting that developmentally-defined lineage boundaries are more flexible than widely thought.

T-3081
LOW IMMUNOGENICITY OF HUMAN UMBILICAL CORD BLOOD DERIVED-MESENCHYMAL STEM CELLS (HUCB-MSCS) IN HUMANIZED MOUSE MODEL

Lee, Miyoung, Jeong, Sang Young, Ha, Jueun, Choi, Soo Jin, Oh, Wonil, Yang, Yoon Sun, Jeon, Hong Bae
Biomedical Research Institute, Medipost Co, Ltd, Seoul, Republic of Korea

Evaluation of the *in vivo* immunogenicity of allogeneic mesenchymal stem cells (MSCs) for human clinical applications has been hampered by lack of suitable models due to technical and ethical limitations. In this study, we show that allogeneic human umbilical cord blood

derived-MSCs (hUCB-MSCs) maintained low immunogenicity even after immune challenge *in vitro*. To confirm these properties *in vivo*, a humanized mouse model was generated by intravenously injecting isolated hUCB-derived $CD34^+$ cells into immune-compromised NOD/SCID $IL2\gamma^{null}$ (NSG) mice. Subpopulations of engrafted human immune cells including $CD45^+$, $CD3^+$, $CD19^+$, HLA-DR^+, $CD14^+$, and $CD56^+$ cells were confirmed up to 24 weeks post-transplantation with human $CD34^+$ cells in the blood or bone-marrow of humanized mice by FACS analysis. The functionality of the engrafted human immune cells was also verified using a mixed lymphocyte reaction (MLR) to measure T cell proliferation, and ELISA analysis to quantify human IFN-γ, TNF-α and Ig G production. After repeated intravenous injection of human peripheral blood mononuclear cells (hPBMCs) or MRC5 cells into these mice, immunological alterations including T cell proliferation and increased human IFN-γ, TNF-α and human IgG level, were observed; these effects were absent in PBS and hUCB-MSCs injected groups. In addition, while reduction of survival rate and lymphocyte infiltration in the lung and small intestine were observed after 3 weeks of hPBMC or MRC5 transplantation, no adverse events were observed in the PBS- or hUCB-MSC-injected groups. In conclusion, our data suggest that allogeneic hUCB-MSCs have low immunogenicity *in vitro* and *in vivo*, thus providing the immunological safety that is required for allogeneic clinical applications.

T-3082
GENOME-WIDE INTEGRATIVE ANALYSIS UNVEILS CONVERGENT MOLECULAR PATHWAYS AND BIOLOGICAL PROCESS IN MESENCHYMAL STEM CELLS AND TYPE 1 DIABETES

Li, Lisha[1], Yin, Zhuo[2], Lu, Man[1], Ming, Qianwen[1], Li, Yulin[1], Wang, Haijun[3]

[1]Pathology, Jilin University, Changchun, China, [2]Changchun Institute of Technology, Changchun, China, [3]Northeast Normal University, Changchun, China

Type 1 diabetes (T1D) is characterized by the absolute loss of insulin-secreting β- cells due to autoimmune-mediated destruction. T1D has received much attention recently as a potential target for the emerging field of stem cell medicine. Intriguing immunoprivileged and immunomodulatory properties of MSCs and their impressive record of safety in clinical trials make them promising candidates for further investigation. In treatment of T1D, the role of MSCs may not be limited to replace damaged cells, but also could promote the endogenous β-cell regeneration and survival; inhibit immune response to β- cells, restore the balance of immune cells, modify the pancreatic microenvironment and reduce β cell damage. The evidence provided by experiment studies of MSCs in pancreatic regeneration has been documented, but the mechanism of MSCs to treat diabetes has not been fully explored. T1D may involve complex mechanism, so we hope to interpret it by molecular and functional pathways and networks. The correlation and association between human T1D and MSCs were analyzed with genome-wide data. 52 genes associated with T1D were retrieved from the GWAS catalog with a genome-wide significance level of $p<10-08$ (http://www.genome.gov/gwastudies/). 250 genes associated with MSCs were retrieved from the functional genomics data of Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE8954) with the GEO2R, the tools to analyze GEO data. We identified only one overlapping gene CDK2 commonly down-regulated in both T1D and MSCs. CDK2 is involved in the control of the cell cycle, triggers duplication of centrosomes and DNA, controls the timing of entry into mitosis/meiosis, and coordinates the activation of cyclin B/CDK1 at the centrosome and in the nucleus. Consequently, there is no evidence of direct connection between T1D and MSCs at molecular level. By DAVID tools (http://david.abcc.ncifcrf.gov/), we found that while T1D genes were enriched in 2 Gene Ontology biological processes including biological regulation and immune system process, MSCs genes were enriched in 8 Gene Ontology biological processes including developmental process, multicellular organismal process, biological regulation, growth, cellular component organization, cellular process, response to stimulus and biological adhesion. That suggests MSCs may play more complicated roles in treatment of T1D. MSC shared 3 pathway with T1D, namely Jak-STAT signaling pathway, Oocyte meiosis and Progesterone-mediated oocyte maturation pathway. The JAK-STAT signaling pathway transmits information from chemical signals outside the cell, through the cell membrane, and into gene promoters on the DNA in the cell nucleus, which causes DNA transcription and activity in the cell. It affects basic cell functions, like cell growth, differentiation and death. Disrupted or dysregulated JAK-STAT functionality (which is usually by inherited or acquired genetic defects) can result in immune deficiency syndromes and cancers. Integration analysis of microarrays studies and GWAS data suggests that MSC therapy of T1D is complex at molecular and signaling pathway level. We shall further validate JAK-STAT pathway in cell and animal model of T1D treated by MSCs.

T-3083
ESTABLISHMENT OF MESENCHYMAL STEM CELL-LIKE CLONAL STEM CELLS FROM MOUSE SALIVARY GLANDS

Lim, Jae-Yol[1], Yi, TacGhee[2], Song, Sun[3], Kim, Young-Mo[1]

[1]Department of Otorhinolaryngology, Inha University School of Medicine, Incheon, Republic of Korea, [2]Inha Research Institute for Medical Sciences, INHA University School of Medicine, Incheon, Republic of Korea, [3]Translational Research Center, Inha University School of Medicine, Incheon, Republic of Korea

Successful therapy for radiation-induced salivary gland (SG) hypofunction is currently unavailable; however, tissue-specific stem cells are expected to be promising candidates for SG regeneration. In this study, we present our method for establishment of single cell-derived clonal mesenchymal stem cells from mouse SGs and their characterization as compared with bone marrow-derived clonal mesenchymal stem cells (BM-cMSCs). Salivary gland-derived clonal mesenchymal stem cells (SG-cMSCs) were isolated by a modified subfractionation culturing method and expanded *in vitro*. The properties of SG-cMSCs were examined with respect to their marker expression, gene expression, and *in vitro* immunosuppressive activity as compared with those of BM-cMSCs. Many stem cell-like clones were isolated from mouse SGs by a modified subfractionation culturing method. Cell surface marker and gene analyses revealed that SG-cMSCs share the characteristics of MSCs, but they exhibit differential expressions in parts of aquaporin-5, CK-14, and collagen IV. SG-cMSCs showed the ability to differentiate into all 3 mesenchymal lineages after appropriate induction. The immunosuppressive activity of SG-cMSCs appeared to be more potent than that of BM-cMSCs. These results show that homogeneous SG-cMSCs can be isolated from mouse submandibular SGs by a modified subfractionation culturing method. These SG-cMSCs possess the properties of MSCs but also exhibit differential expressions. Their biological functions and relations with SG epithelial progenitor cells need to be further investigated.

T-3084

IFN-GAMMA REGULATES HLA-I SURFACE EXPRESSION OF HUMAN EMBRYONIC STEM CELLS DERIVED MESENCHYMAL STEM CELLS THROUGH MODULATING MULTIPLE FACTORS OTHER THAN HLA-I SYNTHESIS

Liu, Hua[1], Wang, Yafei[1], Lu, Ping[1], Ji, Junfeng[1], Ouyang, Hongwei[1], Zou, Xiaohui[2]
[1]Center for Stem Cell and Tissue Engineering, School of Medicine, Zhejiang University, Hangzhou, China, [2]Clinical Research Center,, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China

Although abundant evidences have indicated that mesenchymal stem cells (MSCs) are immune-privileged, controversy was courted from MSCs lysis by cytotoxic T cells and NK cells, as well as quick removal of MSCs allografts by recipients' immune system. It is proposed to generate hypoimmunogeneic stem cell lines by down-regulation of HLA-I to overcome immune rejection, but an appropriate HLA-I expression level on surface is critical for cell survival from NK cell attack. So far the regulation mechanisms of low HLA-I expression in human MSCs is unclear. Our objective is to investigate the mechanism of HLA-I expression on the surface of human MSCs. Flow cytometry analysis showed low surface and moderate intracellular HLA-I expression levels of MSCs. With IFN-γ treatment, the fluorescent intensity (FI) of HLA-I expression on MSCs surface increased significantly in the first 2 days ($P<0.05$) and slightly decreased, while the FI of intracellular HLA-I kept increasing up to 3 days. Furthermore, based on real-time PCR data, the synthesis of HLA-I increased significantly in the first 3 days, while the synthesis of multiple genes, which function as protein breakdown and peptide transport, changed in the similar manner as the surface HLA-I expression under the IFN-γ treatment. These data suggested that HLA-I expression on MSCs surface was dominantly regulated by transport and protein breakdown after HLA-I synthesis. As a common pro-inflammatory factor, IFN-γ may exert extensive effect on multi-protein expressions to regulate surface expression of HLA-I. Further investigation on these mechanisms will allow the development of strategies to modulate HLA-I expression and MSCs immunogenicity.

T-3085

INFLUENCE OF REACTIVE OXYGEN SPECIES ON THE G1-S PHASE PROGRESSION IN THE HUMAN MESENCHYMAL AND EMBRYONIC STEM CELLS

Lyublinskaya, Olga G., Borisov, Yaroslav G., Pugovkina, Natalia A., Tikhomirova, Natalia V., Obidina, Julia V., Nikolsky, Nikolay N.
Institute of Cytology RAS, St.Petersburg, Russian Federation

It is now well established that reactive oxygen species (ROS) mediate the regulation of the cell cycle progression in somatic cells, being involved in the phosphorylation and ubiquitination of the cell cycle key regulatory molecules. It has been shown that an increase in the steady-state levels of endogenous ROS is observed in the late G1 phase of proliferating cells and that the antioxidant treatment of cells prohibits their G1-S phase transition. Comprehensive molecular analysis (Havens et al., 2006) revealed that the antioxidant-treated cells, arrested in the late G1 phase, had transited across the restriction point from early G1 into late G1, but failed to accumulate the cyclin A protein due to its continued ubiquitination and targeting for subsequent degradation by the anaphase promoting complex (APC/C), an ubiquitin E3-ligase which normally should be inactivated prior to initiation of S phase. These observations pointed to the ROS-dependent G1/S phase checkpoint that coordinates cellular ROS production with cell cycle progression in somatic cells. At the same time, there is no information about this checkpoint in the cycle of stem cells. The present study is aimed at the investigation of ROS-dependent regulation of G1-S phase progression in the human mesenchymal and embryonic stem cells. Mesenchymal stem cells (MSCs) from human endometrium and human embryonic stem cells (ESCs) derived and characterized in the Institute of Cytology RAS were employed for the study. ROS intracellular production and cell cycle dynamics were measured by flow cytometry. MSCs were synchronized in G0/G1 phase of the cell cycle by either contact inhibition or serum deprivation, as well as in G2/M phase by nocodazole. ESCs were synchronized in G2/M phase by nocodazole. Tempol and N-acetyl-L-cysteine (NAC) in the 1 - 10 mM concentration range were used to scavenge ROS. Investigation of asynchronous cultures of MSCs showed that intracellular ROS production positively correlated with the proliferative activity of the cells. Antioxidant treatment resulted in the inhibition of the cell proliferation and G1 phase arrest. Experiments with synchronized MSCs revealed the transient increase in the endogenous ROS before the progression to the S-phase of the cell cycle. Treatment with antioxidants decreased intracellular ROS production and prohibited the MSC transition to the S-phase. Thus, our data evidences about the ROS-dependent G1/S phase checkpoint in the MSC cycle. In contrast to MSCs, experiments with both synchronized and asynchronous cultures of ESCs revealed neither increase in the endogenous ROS nor antioxidant-induced cell cycle arrest before the S-phase entry. We suppose that different role of ROS production in the regulation of G1-S phase progression in MSCs and ESCs may be due to the different dynamics of APC/C activity and cyclins ubiquitination throughout the cell cycle of multipotent and pluripotent cells. While in MSCs APC/C operates in the switch on-off mode, in ESCs the activity of APC/C is suppressed and only slightly modulated during progression through the cell cycle. Our hypothesis needs to be confirmed by the molecular assays which are in progress now.

T-3086

IMMUNOLOGICAL EVALUATION OF HUMAN ADIPOSE STEM CELLS CULTURED IN DIFFERENT SERUM CONDITIONS VERSUS XENOFREE/SERUMFREE CONDITION

Mannerström, Bettina, Patrikoski, Mimmi, Sivula, Jyrki, Miettinen, Susanna
BioMediTech, University of Tampere, University of Tampere, Finland

The potential of human adipose stem cells (ASCs) for regenerative medicine has been noticed due to the ease of isolation and the capacity of the cells to differentiate towards several cell types of mesodermal origin. Additionally, ASCs have low immunogenicity and their immunosuppressive effects make them even more relevant cell source considering clinical applications modulating immunity and inflammation. Also, there is the emerging need for stem cell therapies to treat various tissue defects. Nevertheless, expansion of ASCs is often necessary prior to clinical use, yet standard in vitro cell culture techniques utilize animal-derived reagents such as fetal bovine serum that should be avoided due to safety concerns. Therefore, xeno- and serum-free (XF/SF) cell culture reagents are highly desirable for enhancing the safety and quality of the transplanted ASCs. The immunogenicity and immunosuppressive potential of ASCs was determined through mixed lymphocyte reactions (MLR) post cell isolation and expansion in different culture conditions: fetal bovine serum (FBS), human serum (HS), and XF/SF conditions. Additionally, cytokine secretion and immunophenotype of the cells was analyzed. Our results showed that ASCs are not highly immunogenic when cultured in FBS, HS or in XF/SF condition. Furthermore, the strongest suppression of peripheral mononuclear cells (PBMCs) was observed with ASCs cultured in FBS conditions, whereas ASCs cultured in

HS or XF/SF conditions suppressed PBMC proliferation only with higher cell numbers. Also, differences in cytokine secretion were seen between culture conditions. The characteristic immunophenotype of ASCs was maintained in all conditions, however cells expanded in XF/SF conditions showed significantly lower expression of CD54 and higher expression of CD45. The culture conditions have an effect on immunogenicity, suppressive potential and cytokine secretion of ASCs, albeit ASCs were not highly immunogenic in any condition, and suppressive potential was detectable in every condition with high ASC numbers. Thus, allogeneic ASCs have the potential for future stem cell treatments.

MESENCHYMAL STEM CELL DIFFERENTIATION

T-3088
INDUCTION OF CARDIAC DIFFERENTIATION FROM RATTUS NORVEGICUS ALBINUS ADIPOSE DERIVED STEM CELLS THROUGH THE INHIBITION OF NF-KAPPA-B PATHWAY WITH RECOMBINANT ADENOVIRUSES COMBINED WITH SPECIFIC GROWTH FACTORS AND CARDIAC BIOMATRIX

Ibarra-Ibarra, Blanca Rebeca[1], Gómez-Mondragon, Misael[1], Hernández-Gutíerrez, Salomón[2], Lara-Martínez, Andrés[2], Páez-Arenas, Araceli[1], Varela-López, Elvira[1], Massó-Rojas, Felipe Alonso[1]
[1]Physiology Department, Cellular Biology Section, Instituto Nacional de Cardiología, Ignacio Chávez, Mexico City, Mexico, [2]Molecular Biology Department, Universidad Panamericana, Mexico City, Mexico

Cardiac differentiation from adipose derived stem cells (ASC) has been proven; however, the efficiency of terminal differentiation is very low. Several signaling pathways are involved in the process of proliferation and differentiation of stem cells, and some studies have shown that inhibition of NF-κB pathway participates in the induction of the stem cell differentiation. In the present study, we provide evidence that cardiomyocytes are differentiated from ASC. In present study we demonstrate that the induction of differentiation by transduction of genes that inhibit the classical pathway of NF-κB (*IKB-α* and *A20* genes) combined with specific growth factors and cardiac biomatrix (CB) increase the induction efficiency of cardiac differentiation from ASC. ASC were isolated with mechanical dissociation and collagenase, the cells were seeded in complete medium (low-glucose DMEM, 10% FBS, 100 U/mL penicillin, 10 mg streptomycin and 25 µg amphotericin B per mL) at 37°C in a humid 5% CO2 air atmosphere. We characterized rat ASC with CD markers (CD45, CD44, CD90, CD105, RT1A and CD73) using flow cytometry. CB was obtained through neonatal rat hearts. Recombinant adenoviruses were previously constructed and proved. In order to obtain optimal expression of the desired gene, the viral construct had a MOI of 100. We used recombinant adenoviruses with *IKB-α* or *A20* genes to induce differentiation. At 48 hours post transduction, cells were plated with or without CB and treated with growth factors for 4 weeks: BMP-4+FGF-2 (week 1), IGF-1 (week 2), BMP-4+FGF-2+IGF-1 (week 3) and BMP-4+FGF-2+IGF-1+HAM medium (week 4). We evaluated the presence of cardiac linage proteins (GATA-4, troponin-I and MYH) and a stem cell surface marker (CD90) at 7, 14, 21, and 30 days by flow cytometry. The ASC had a fibroblast-like morphology with irregular processes and were positive for CD90, CD44, RT1A and CD73; and negative for CD45 and CD105. We observed immature cardiomyocytes differentiation at 7 days with or without CB, the induced cardiac differentiation was maintained through 14 days. The highest troponin I level observed were A20 transducted cultures with or without CB (45.6% and 61.8% positive cells for troponin I, respectively). By day 21, we observed a decline in troponin I expression, with a substancial increase of GATA-4 by day 30, the highest value of positive cells with GATA4 was observed in the *IKB-α* transducted cultures with biomatrix, 74.7%. MYH and CD90 were not observed in any of the cultures during experimental process. Cardiac-like cells, under light microscope had multinucleation and extension of their cytoplasmic processes with adjacent cells. The cells not treated maintained their fibroblast-like morphology. We do not observed spontaneous beating in any of the cultures. We have shown that ASC have the capacity to differentiate into immature cardiomyocytes after inhibition of NF-κB pathway with recombinant adenoviruses combined with specific growth factors and CB. *A20* appear to be better differentiation inductor than *IKB-α*. Induction by recombinant adenoviruses can increase the induction of differentiation in ASC; however, it is not sufficient to maintain the differentiated state. We designed a novel and an efficient culture protocol to obtain large amounts of immature cardiomyocytes from ASC, but we need to investigate further in order to obtain functional mature cardiomyocytes.

T-3089
EFFECTS OF THE INTRINSIC MECHANICAL PROPERTIES OF A SINGLE CELL ON DIFFERENTIATION IN ADIPOSE- AND UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS

Ihn, Han, Kim, Kyung Sook
Department of Biomedical Engineering, College of Medicine, Kyung Hee University, Seoul, Republic of Korea

Mesenchymal stem cells (MSCs) are pluripotent; however, their differentiation is limited by several factors including the intrinsic mechanical properties of single cells. We investigated the effects of the mechanical properties of MSCs on differentiation by changes in stiffness during the differentiation. Adipose- (ASCs) and umbilical cord-derived mesenchymal stem cells (UCMSCs) were isolated from fat tissue and human umbilical cord, respectively. The cells were cultured separately in adipogenic and osteogenic differentiation medium. The differentiation into adipocytes and osteoblasts was confirmed by oil-red O and von Kossa staining, respectively, and mechanical properties were analyzed by atomic force microscopy (AFM). UCMSCs were stiffer than ASCs, and had poor ability to differentiate to adipocytes. ASCs were well differentiated into both adipocytes and osteoblasts. In both cases, the stiffness increased during the first week of induction and then decreased. The mechanical properties of the differentiated cells depended significantly on the type of MSCs. These results suggest that it is necessary to identify an appropriate stem cell source for successful tissue regeneration, and provide a better understanding of the ability of MSCs to differentiate in relation to their mechanical properties.

T-3090
CD105 EXPRESSION AS A MARKER TO EXPLAIN OSTEOGENIC POTENTIAL DIFFERENCES OF MESENCHYMAL STEM CELLS OF DIFFERENT SOURCES

Ishiy, Felipe Augusto André[1], Fanganiello, Roberto D.[1], Capelo, Luciane[2], Semedo-Kuriki, Patrícia[1], Morales, Andressa Gois[1], Passos-Bueno, Maria Rita[1]
[1]Genetics and Evolutive Biology, University of São Paulo, São Paulo, Brazil, [2]Institute of Science and Technology, Federal University of São Paulo, São José dos Campos, Brazil

The use of mesenchymal stem cells (MSCs) is a promising therapeutic approach for tissue engineering due to their ability to boost tissue regeneration and it is a safe option regarding the possibility of immune response and teratoma formation. It is still under debate if MSCs of

different origins have variable differentiation outcomes. In this regard, we have observed that MSCs from human exfoliated deciduous teeth (SHED) harbor higher in vitro osteogenic differentiation potential (OD) compared to MSCs derived from human adipose tissue (hASCs). The mechanisms that could explain these differences are unknown. It is already established in hASCs that CD105 (ENG) expression, a transmembrane glycoprotein that functions as a TGF-B1 co-receptor, is inversely proportional to its OD. We have thus tested if expression levels of CD105 (ENG) could contribute to the OD potential differences between SHED and hASCs. SHED and hASCs were obtained from ten healthy subjects for each cell type. Both SHED and hASCs cell populations were positive for MSCs immunophenotype surface markers (CD29+, CD73+, CD90+, CD105+) and negative for hematopoietic and endothelial surface markers (CD31-, CD34-, CD45-; n=9 per cell source). Undifferentiated hASCs showed higher mRNA (6,3-fold $p<0.05$) and protein (7,24-fold $p<0.001$) expression of ENG compared to SHED. During osteoblastogenic differentiation ENG exhibited different expression patterns between SHED and hASCs, with an upregulation of ENG in SHED and downregulation in hASCs during the first 6 days. After 9 days of osteoblastogenic differentiation SHED presented higher alkaline phosphatase activity (AP) with 2.29 fold compared to hASCs ($p<0.001$). Following 21 days of osteogenic induction calcium matrix production (Alizarin Red staining-AR) indicated a 2.37 fold higher calcium deposition in SHED compared to hASCs. Through FACS, we sorted out four lineages of each cell source for Low and High ENG expression, and after expansion we plated the different cell populations (unsorted, low and high ENG expression) for OD. At days 9 and 21 unsorted and low ENG expression populations of SHED and hASCs lineages presented higher osteogenic potential ($p<0.01$) as compared to high ENG expression populations. Using Ingenuity Pathway Analysis software (IPA) we selected 6 MicroRNAS (mirs 1287, 1207, 4781, 4421, 4649 and 4651) that were predicted to suppress ENG. Through quantitative real time PCR we quantified the expression of these microRNAs of 6 lineages of each source. We observed that the expression levels of one of these mir was indirectly correlated with ENG levels both in SHED and hASCs, suggesting that it might regulate ENG levels. We will verify if downregulation of this mir in SHEDs will reduce its OD potential. In this work we observed that the ENG plays an important role to the OD potential in MSCs contributing to the OD differences between different sources, and revealed one microRNA that could be a potential candidate to modulate the expression of ENG. In summary, we are showing a potential molecular mechanism to explain the OP differences of SHED and hASCs.

T-3091
CARDIAC MESENCHYMAL PROGENITORS MIGHT BE INVOLVED IN INTRAMYOCARDIAL ADIPOSITY

Kami, Daisuke[1], Kitani, Tomoya[2], Gojo, Satoshi[1]
[1]*Department of Regeneration Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan,* [2]*Kyoto Prefectural University of Medicine, Kyoto City, Japan*

Background: The deposition of fat within the myocardium after myocardial infarction was proposed to be one of culprits for ventricular tachycardia (VT). Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is an inherited heart disease characterized by fatty accumulation predominantly in the right ventricle, which frequently leads to lethal VT or ventricular fibrillation. We demonstrated that cardiac mesenchymal progenitors (CMPs) have an ability to differentiate into adipocytes in adipogenic culture conditions. It's a great potential to abrogate the intramyocardial adipogenic pathway in these diseases. We examined the effects of transcription factors related to reprogramming process into pluripotency, since there are up-regulations of these transcription factors in myocardial infarction and ARVC/D. Methods: CMPs were isolated from C57BL/6J mouse adult heart. Briefly, the dissected hearts were minced, and digested twice for 30 min at 37°C with 0.2% type II Collagenase and 0.01% DNase I. Isolated Sca-1 positive cells were seeded and cultured on Collagen I coated dish in Dulbecco's Modified Eagle's medium/F-12 supplemented with 40 ng/ml basic fibroblast growth factor. To elucidate the mechanism of adipogenic differentiation, CMPs and NIH3T3 fibroblasts were infected with Oct3/4, Sox-2, Klf-4, c-Myc, or the combinations, which were constructed in Sendai virus. Cells were not cultured in an adipogenic condition, but in a standard cell culture media without leukocyte inhibitory factor directly onto geltrex-coating culture dish, in order to inhibit to proceed into pluripotency. Adipogenic phenotype and pathway was evaluated at each day by Oil red O staining, and qRT-PCR and Microarray methods, respectively. Results:OSKM-induced CMPs were formed small intracellular lipid droplets in the cytoplasm, which were clearly stained with Oil red O, 6 days after the infection. On the other hand, OSKM-induced NIH3T3 fibroblasts did not show any lipid droplets and positive Oil red O staining through 10 days after the infection. To detect time-dependent gene expression of OSKM by qRT-PCR from Day 2 to Day 10, expression of Oct4 and Sox2 were decreased, and c-Myc and Klf4 were increased. Gene expression of Isl1, and adipogenic specific genes (Fas, Pparg) were also gradually increased in CMPs, not in NIH3T3. Expression of C/EBPa, adipogenic transcription factor, transiently increased only in CMPs. We also performed genome-wide gene expression analysis using Agilent mouse microarray chips. Hierarchical clustering analysis showed that OSKM-induced CMPs could be clearly discriminated from CMPs without any treatment. In addition, principal component analysis revealed that Fabp4, Adiponectin, Acsl1, AU018778, Acad1, FATP-1, and Perilipin2, which are involved in the Ppar signaling pathway, were significantly increased in time dependent manner. Next, to determine which of the OSKM were critical in adipogenic differentiation, we examined the effect of withdrawal of individual factors from the pool of OSKM on the formation of lipid droplets and Oil red O staining. Klf4 and c-Myc was key factor of adipogenic differentiation in CMPs. Conclusion: CMPs can differentiate into adipocytes by two overexpression genes c-Myc, and Klf4. Adiposity in post-MI and ARVC/D might be attributed to CMPs, because a stress induce the expression of transcription factors related to reprogramming.

T-3092
MAINTENANCE OF STEMNESS IN MESENCHYMAL STEM CELLS BY MESENCHYMAL-HEMATOPOIETIC INTERACTION

Kanazawa, Sanshiro[1], Mabuchi, Yo[2], Akazawa, Chihiro[2], Takato, Tsuyoshi[1], Hoshi, Kazuto[1]
[1]*The University of Tokyo, Tokyo, Japan,* [2]*Tokyo Medical and Dental University, Tokyo, Japan*

Bone marrow-derived mesenchymal stem/stromal cells (MSC) that can be used to treat the several medical transplantation would be good cell sources for the cartilage regenerative medicine using autologous cells. However, it is difficult to the large and complex tissue regeneration using the cultured MSCs since MSCs which are isolated based on their adherence to plastic conventionally, shows poor growth and differentiation and are heterogeneous in their population, limiting the ability to investigate the intrinsic characteristics of the cells (stem-ness). To solve the problem, it is essential to establish a cell culture techniques for the MSC to maintain the stem-ness. Originally, since many of MSCs are present in the bone marrow, the MSCs may have potential for maintaining the stem-ness by interaction with hematopoietic cells.

Therefore, in this study, we hypothesized that the proliferation ability of MSCs could be enhanced by co-culture with hematopoietic cell, and compared to the pure MSC culture. EGFP labeled mouse bone marrow-derived MSC as the mesenchymal lineage and a C57BL6/J wild derived HSC as the hematopoietic lineage were used for co-culture. MSCs were plated at 3x103 cells in MSC culture media, and incubated for 24 hr. For coculture, at 7x103 cells of HSC were added to MSC-containing wells. For transwell cultures, HSC were plated at 7x103 cells in the upper compartment, while 3x103 cells of MSC were plated in the lower compartment. The signal, closely-involved in stem cell niche was detected and proliferation rate of MSCs by co-culture with time in culture was analyzed. In addition, to examine maintenance of the stem-ness by co-culture, we examined a mesenchymal lineage differentiation, such as the osteoblasts, adipocytes and chondrocytes for mMSC after co-cultured. As the result, we observed MSCs co-culture with HSCs significantly increased the cell proliferation and maintained their stem-ness. We also examined mesenchymal induction analysis that MSCs proliferated with co-culture, and then evaluated mesenchymal maturity. Consequently, MSC co-cultured with HSCs were equally differentiated and matured compared to pure single MSCs. These results suggest that mesenchymal-hematopoietic interaction plays pivotal roles in the proliferation ability and maintenance of stem-ness of MSCs. Finally, we performed a comprehensive screening of putative genes to maintain the stem-ness signal from the HSCs to MSCs.

T-3093
HUMAN MESENCHYMAL STEM CELLS DIFFERENTIATION TO CHONDROCYTE USING SIMULATED MICROGRAVITY ENVIRONMENT

Kawahara, Yumi[1], Kyousuke, Nakata[2], Takahiro, Fukazawa[1], Keiji, Tanimoto[3], Takuma, Furukawa[2], Yunosuke, Ookura[2], Naofumi, Otsuru[2], Yuge, Louis[2]

[1]Space Bio-Laboratories Co. Ltd., Hiroshima, Japan, [2]Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan, [3]Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan

Articular cartilage has rich extra-cellular matrix (ECM) to supply lubrication for joints and to support load bearing quality. ECM is synthesized from chondrocyte in response to mechanical stress loading. Mechanical stress plays an important role to maintain articular cartilage homeostasis and is known the key factor that influences chondrocyte differentiation. Previous reports used dynamic compression loading and static pressure to examine the effect of mechanical stress on chondrocyte differentiation. They reported mechanical stress loading enhanced chondrocyte differentiation and excess mechanical stress caused ECM broken. However, it was unclear mechanical stress unloading affected on chondrocyte differentiation. A clinostat is one of the devices for providing simulated microgravity environment to examine the weightless research *in vitro*. In this study, human mesenchymal stem cells (hMSCs) were induced differentiation to chondrocyte in normal 1G environment (Group 1G) and 10^{-3}G environment (Group MG). Simulated microgravity environment was newly developed three-dimensional-clinostat, the Gravite (KITAGAWA IRON WORKS Co., Ltd., Japan), controlled by rotation of two axes, resulting 10^{-3} G average over time. In 1G environment, hMSCs pellets were differentiated to chondrocyte for 21 days culture. ECM synthesis decreased hMSCs pellets in Group MG compared to hMSCs in Group 1G. In microgravity environment, hMSCs pellets were expressed differentiated markers less than in 1G environment. Transcription factors expression was also lower in Group MG. These results indicated hMSCs inhibited chondrocyte differentiation in microgravity environment. In chondrocyte regeneration, unloading period is required for stem cell engraftment after cell transplantation. Our results suggested that too long unloading period prevents the functional recovery of articular cartilage. Future studies are needed to evaluate the best treatment protocol for stem cell engraftment and the functional recovery of articular cartilage.

T-3094
TRANSCRIPTIONAL PROFILING OF EARLY CHONDROGENIC COMMITMENT IN HUMAN MESENCHYMAL STROMAL CELLS

Keogh, Andrea[1], Schwarzl, Thomas[2], Clayton, Elizabeth[1], Ryan, Caroline[1], Howard, Linda[1], Barron, Valerie[1], Higgins, Desmond[2], Georgina, Shaw[1], Frank, Barry[1]

[1]Regenerative Medicine Institute, National University of Ireland Galway, Galway, Ireland, [2]Systems Biology Ireland, University College Dublin, Dublin, Ireland

Human mesenchymal stromal cells (hMSCs) can be stimulated to differentiate towards the chondrogenic lineage and hence possess great therapeutic potential for the repair of cartilage defects in osteoarthritis and other articular diseases. To date, the fundamental intracellular signalling cascades regulating early chondrogenic lineage commitment in hMSCs is incompletely understood. Many studies have focused attention on the regulation of the key chondrogenic transcription factor SRY (Sex Determining Region Y)-Box 9 and its function in chondrocyte-specific gene expression. The advent of functional genomics in combination with systems biology approaches has strengthened our knowledge and has paved the path to overcoming some of the challenges associated with understanding complex developmental processes. Previously, microarray data in our laboratory has demonstrated that the majority of transcriptional changes occur within the first 16 hours of chondrogenic differentiation in MSCs. To elucidate the key signalling pathways involved and to provide a new understanding of this lineage specific step, a detailed RNA sequencing time course was performed. MSCs derived from bone marrow are limited by their heterogenous phenotype in vitro. In this study we developed a homogenous clonal population of hMSCs by limited dilution cloning to profile lineage commitment. RNA was extracted from MSC-micromass pellets at various time points spanning from the first 15 minutes of commitment up until 16 hours. RNA from monolayer MSCs and 21 day micromass pellets displaying overt chondrogenic differentiation were used as appropriate controls. Using gene ontology and pathway analysis an event map was constructed to demonstrate the sequential events that lead to chondrocyte lineage commitment. Results confirmed that many of the known extracellular matrix proteins involved in chondrogenesis such as proteoglycan 4 and cartilage oligomeric matrix protein are up regulated 7-fold and 4-fold respectively at 16 hours. Notch and members of the Notch signalling pathway which have also been previously shown to be important at the onset of chondrogenesis, are up regulated as early as 30 minutes in our time course. In addition to this confirmatory data, our research has focused on the identification of novel transcription factors and genes that function in regulating early chondrogenic commitment. We hypothesize that the mapping of these transcriptional networks at the onset of chondrogenesis will lead to the development of chondrogenically primed MSCs that will aid in the enhancement of cartilage repair strategies.

T-3095

IN VITRO DIFFERENTIATION OF HUMAN SUPERNUMERARY TOOTH-DERIVED DENTAL PULP STEM CELLS ON NANOSCALE RIDGE/GROOVE PATTERN-ARRAYED SURFACE

Kim, Daehwan
Seoul National University, Seoul, Republic of Korea

The aim of this study was to establish human dental pulp stem cells (hDPSCs) from supernumerary teeth and determine the effects of a 350-nm nanoscale ridge/groove pattern-arrayed (nano-patterned) surface on adipogenic and osteogenic differentiation of hDPSCs. Several surface markers were analyzed by FACS to confirm the isolated cells as hDPSCs. To demonstrate the effect of a nano-patterned surface on the differentiation of hDPSCs, the cells were cultured on a nano-patterned surface with or without adipogenic or osteogenic induction factors. Cells were then stained with Oil red O or Alizarin red, and the lineage specific genes LPL and Runx-2 were analyzed by real-time PCR at 3, 6 and 9 days after culture. Different from hematopoietic and endothelial markers, mesenchymal stem cell markers were highly expressed in hDPSCs. The hDPSCs on a nano-patterned surface showed a linear arrangement compared to irregular cells on a conventional surface. During adipogenic differentiation, more Oil red O stained cells were found in the nano-patterned group than in the conventional group. On the other hand, there was no significant difference in Alizarin red staining between the nano-patterned and conventional surface groups after induction of osteogenic differentiation. Gene expression analyses revealed significantly higher expression of LPL in the nano-patterned group than in the conventional group, whereas Runx-2 expression was higher in the conventional group than in the nano-patterned group. In conclusion, this study showed that a nano-patterned surface may be able to enhance adipogenic differentiation of hDPSCs by altering their morphology and gene expression patterns, whereas the same surface may inhibit or suppress osteogenic differentiation of the cells. This study was supported by a grant from the National Research Foundation of Korea (NRF-2006-2004042, and No. 2013070465 through the Oromaxillofacial Dysfunction Research Center for the Elderly at Seoul National University) and the Technology Development Program for Agriculture and Forestry, Ministry of Agriculture, Food and Rural Affairs (MAFRA; 111160-04), Republic of Korea.

T-3096

EPIGENETIC REGULATORS INCREASE MULTILINEAGE DIFFERENTIATION CAPACITY OF HUMAN MESENCHYMAL STROMAL CELLS

Kim, Hye Joung[1], Kim, Yoo Jin[2]
[1]1Laboratory of Hematological Disease and Immunology, The Catholic University of Korea, Seoul, Republic of Korea, [2]Laboratory of Hematological Disease and Immunology, The Catholic University of Korea, Seoul, Republic of Korea

Disruption of epigenetic regulation such as DNA methylation and histone modification is associated with humandisease, it has emerged as an important modulator of mesenchymal stem cells (MSCs) proliferationand differentiation. However, detailed explanation on epigenetic change by regulator is not enough and the application of MSC therapy research is insufficient to be known. We addressed the effects of these regulations on chondrogenesis, osteogenesis and neurogenesis in MSCs derived from bone marrow (BM-MSCs) using the tricostatin A (TSA), a histone deacetylase inhibitor and 5-aza-2'-deoxycytidine, a demethylating agent. BMMSC has been used for induce of Neurogenesis, chondrogenesis, osteogenesis and during differentiation induction,TSA(100nM) and 5AZA(2uM) has been processed alone or combination. Various differnetitaion analyzed by -cyto/histo chemistry, western, real time PCR and pyrosequencing. In neueogenesis, protein expression has identified with various neuromarker and it has confirmed that most differentiation induce in combination of 5-aza and TSA. Also protein expression has confirmed with osteo or chondro marker in osteogenesisand differentiation potential of chondrogenesis. As the result of that 5-aza and TSA combination is most induce just like in neurogenesis. Moreover, differentiation potential of various lineages has confirmed with each expression of differentiation key regulator gene and 5-aza and TSA combination shows most increasing in gene expression with three lineages. However, pyrosequencing performed toobserve the increase of gene expression is due to epigenetic change that processing with epigenetic regulator. As the result of that, decreasing pattern observed in epigenetic change with BDNF as neurogenic marker and Runsx-2 as ostepgenic marker. But this is not that significant. Methylation pattern of SOX-9 as chondrogenic marker has not shown any different. Our study demonstrates the usefulness of the epigenetic regulator, epigenetic regulator for the promotion of lineage specific differentiation. This finding could be helpful for clinical application from MSCs.

T-3097

STRESS AS AN INSTRUCTIVE DETERMINANT OF MESENCHYMAL STEM CELL FATE

Klepsch, Sebastian[1], Lepperdinger, Guenter[2]
[1]Stem Cell Aging, Leopold-Franzens University, Institute for Biomedical Aging Research, Innsbruck, Austria, [2]Austrian Academy of Sciences, Innsbruck, Austria

Stem cells are vitally involved in tissue regeneration and homeostasis in later life. Mesenchymal stem cells (MSC) are one particular type of tissue specific adult stem cells that can differentiate into mesoderm-type cells, such as osteoblasts, chondrocytes and adipocytes. Osteocytes cannot be cultivated nor is it possible to study their development in vitro. So far no protocol has been established which would allow osteocyte differentiation starting from their natural precursors which are commonly believed to be mesenchymal stem cells (MSC). Treatment of human bone marrow derived MSC with the hyaluronan synthase inhibitor 4-Methylubelliferone (4-MU)not only promotes osteogenesis in vitro but more than that induces a novel cellular phenotype that closely resembles osteocytes in vivo. Investigating the underlying mechanisms of this enhanced differentiation process, we observed that 4-MU specifically triggers the formation of stress granules (SG), which is described as a common stress response in most eukaryotic cells. These RNA-protein complexes contain non-translating mRNAs, translation initiation components, and many additional proteins affecting mRNA function and therefore are believed to serve as a decision point for untranslated mRNAs to become stored, degraded or rerouted for enhanced translational re-initiation. Analyzing the mRNA content of the formed SG via pull down experiments and RT-PCR, revealed the presence of markers (RUNX2, Col1A1, IBSP…) known to be important for osteogenic differentiation. Furthermore we hypothesize that 4-MU caused SG formation is based on a two way mode of action. First we could show that 4-MU treatment leads to elevated UDP-GlcNAc levels, the substrate for O-linked N-acetylglucosamine modification of proteins. O-GlcNAc modification of ribosomal proteins has been linked to stress granule assembly and therefore could explain our findings. Oxidative stress is described as a potent stress granule inducer, we could show in several experiments that 4-MU is a potent ROS inducer and therefore hypothesize this dual mode of mechanism. In parallel we performed 3D culture of human mesenchymal stem cells to mimic the in vivo situation. We could show that 4-MU treatment in combination with osteogenic differentiation, results in pattern formation and

microstructures, closely resembling the in vivo situation of trabecular bone marrow. These micro organoids could serve as a potent tool and surrogate model for research on the Stem cell niche and hematopoiesis.

T-3098
IDENTIFICATION AND CHARACTERISATION OF EPIGENETIC MECHANISMS IN OSTEOBLAST DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

Kramm, Anneke[1], Dunford, James[1], McGarry, David[2], Schuele, Roland[2], Kubicek, Stefan[3], Oppermann, Udo[1]
[1]Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Botnar Research Centre, Oxford, United Kingdom, [2]Klinikum der Universität Freiburg, 1.Universitäts-Frauenklinik und Zentrum für Klinische Forschung, Freiburg, Germany, [3]Ce-M-M- Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

A major therapeutic challenge in regenerative medicine is how to replace bone once it is lost, as mammals have a limited capacity to repair bone defects. Common bone diseases such as osteoporosis and osteoarthritis create a high demand for healthy bone cells and tissue. To meet this demand, stem cell approaches are investigated for application in bone-tissue engineering, specifically human bone marrow-derived mesenchymal stem cells (hMSCs) are used as a source for osteoblasts, the bone forming cells. However, their potential is limited by a lack of knowledge regarding the regulation of transition from hMSCs to osteoblasts. With the aim to improve our understanding and to ensure safe application of these cells, we performed a systematic study of elucidating epigenetic mechanisms underlying osteoblast differentiation. To identify potential genes involved, knock-down experiments with lentiviral shRNAs and inhibitor screens with small molecules targeting a subset of known epigenetic modulators were performed. Measurements of alkaline phosphatase activity as marker for osteoblast formation and cell viability of hMSCs undergoing differentiation were used to identify the effect of the inhibition on bone formation. The screens identified around 90 potential epigenetic modulators involved in osteoblast differentiation, 2/3 of which were downregulating and 1/3 upregulating alkaline phosphatase activity. The range of regulation was spanning from 10fold decrease to 20fold increase of alkaline phosphatase activity. Here we present the deeper analysis of two identified target genes, one upregulating and one downregulating alkaline phosphatase activity. Bromodomain-containing protein 4 (BRD4) was identified as one component of epigenetic regulation; its inhibition led to a decrease in alkaline phosphatase expression, downregulation of osteoblast key transcription factors Runx2 and Osterix as well as impaired bone matrix formation. Further investigation is ongoing and a thorough understanding of this mechanism could potentially provide a target for diseases characterised by extensive bone differentiation and mineralisation. These include for instance heterotopic ossification or calcification of soft tissues.Conversely, we also identified candidate epigenetic modifiers improving bone growth with the aim to discover new approaches and tools for treating bone loss and mending fractures. Knockdown of lysine (K)-specific demethylase 1A (KDM1A/LSD1) was discovered to upregulate alkaline phosphatase activity. Treatment with a small molecule inhibitor targeting KDM1A led to an increase in marker gene expression, namely alkaline phosphatase, Runx2, and bone sialoprotein. Intriguingly, in a transgenic mouse model overexpressing Kdm1a a decrease in bone volume and bone mineral density was observed, thus supporting the hypothesis of KDM1A as a central regulator of osteoblast differentiation. In summary, understanding epigenetic mechanisms controlling osteoblast differentiation offers novel strategies to harness the therapeutic potential of mesenchymal stem cells. We have successfully identified two epigenetic regulators having a profound effect on the expression of osteoblast/bone related genes in an hMSC to osteoblast differentiation model.

T-3100
AN EFFICIENT GENERATION OF FUNCTIONAL MELANOCYTES FROM HUMAN ADIPOSE-DERIVED STEM CELLS

Li, Yumei, Min, Min, Ma, Hong, Liping, Li, Hui, Xu
Jiangsu University, Zhenjiang, China

Objective: Vitiligo is a common acquired depigmentation disorder that results in damage to and loss of melanocytes. Currently, the exact cause of vitiligo remains obscure, but several theories have been proposed to explain the loss of epidermal melanocytes in this disorder, including autoimmune, biochemical oxidant-antioxidant, neural, viral and genetic factors. Mesenchymal stem cells have been widely used in experimental and clinical research because of their unique biological characteristics and advantages. Compared with human bone marrow, human adipose tissue provides abundant source of mesenchymal stem cells, which can be harvested easily and safely. These stem cells have high ability for proliferation and multilineage differentiation. Therefore, human adipose-derived mesenchymal stem cell (hADSC) is becoming an effective source for stem cell bank and for tissue engineering. In this study, we have defined conditions for the efficient derivation of human melanocytes from hADSCs. The resulting hADSC-derived melanocytes were found to express all major melanocyte markers. Method**:** Under the permission of human ethic committee of Jiangsu University Hospital and the informed consent from patients, human subcutaneous fat was collected from abdomen, and hADSCs were isolated and identified by flow cytometry. In this study, we developed an efficient method for inducing hADSCs into a melanocyte in vitro for 10 weeks using Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum with Wnt3a, endothelin-3 (EDN3), basic fibroblast growth factor (bFGF) and stem cell factor (SCF). The markers on hADSCs-derived melanocytes were detected by RT-PCR and identified by immunocytochemistry and immunofluorescence. Results: Antigenic profiling with flow cytometry showed that the isolated cells from fat possess the characters of hADSCs cells. CD29, CD44, CD73, CD90, CD105 and CD166 in hADSCs were high expressed, which the positive rates were all over 95%; in the meanwhile there were low or no expression of CD31, CD34, CD45 and HLA-DR. The cells were also identified to differentiate to osteogenic and adipogenic cells. It showed that the cells had multi-differentiation potential After cultural induction for the cells with our conditional medium, the melanocyte gene expression such as MITF,KIT,DCT,TYR,TYRP1 and SOX10 were significantly upregulated compared with control cells. The hADSCs-derived melanocytes markers were stained positives for MITF, HMB45, TYRP1 and S100 with the analysed of immunocytochemistry and immunofluorescence. Furthermore, in the culture supernatant, the oncogens of melanoma inhibitory activity (MIA), neuron-specific enolase (NSE), soluble intercellular cell adhesion molecule (sLCAM) and soluble vascular cell adhesion molecule (sVCAM) were not detected. Conclusion: We have established an efficient approach to induce human adipose-derived stem cells into functional melanocytes, which of the properties could be kept for long term without promoting tumorigenicity**.** It represents a new source of human melanocytes for research and development in the future as well as in the potentially clinical application. It is hoped that through the study when we put the hADSC-derived melanocytes into a reconstructed vitiligo animal model, they behaved as fetal melanocytes, homed to the basement membrane, and produced melanosomes to treat vitiligo.

T-3101

THE INFLUENCE OF EMBRYONIC CARDIAC MYOCYTES ON THE CARDIOMYOGENIC POTENTIAL OF MARROW STROMAL CELLS IN A THREE-DIMENSIONAL COLLAGEN CELL CARRIER

Li, Jiang, Valarmathi, Mani T.
Comparative Biosciences, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Cardiovascular disease is a leading cause of significant morbidity and mortality in the United States. Bone marrow stromal cells (BMSCs) are capable of differentiating into cardiomyocyte-like cells in vitro and contribute to myocardial regeneration in vivo. Consequently, BMSCs may potentially play a vital role in cardiac repair and regeneration. However, this concept has been limited by inadequate and inconsistent differentiation of BMSCs into cardiomyocytes along with poor survival and integration of neo-cardiomyocytes after implantation into ischemic myocardium. Use of adult BMSCs in the stimulation of mammalian cardiac muscle regeneration is in its infancy, and to date, it has been difficult to determine the efficacy of the procedures that have been employed. In order to overcome these barriers and to explore adult BMSCs based myocardial regeneration, we have developed an in vitro model of three-dimensional (3-D) cardiac muscle using ventricular embryonic cardiac myocytes (ECMs) and BMSCs. When ECMs and BMSCs were co-cultured in a 3-D collagen cell carrier (CCC) engineered from type I collagen fibers for 7, 14, 21, or 28 days, BMSCs underwent maturation and differentiation characteristic of myogenic lineage and acquired the cardiac phenotype. The BMSCs phenotypic induction was confirmed at the morphological, immunological, biochemical and molecular levels. The observed expression of transcripts coding for cardiomyocyte phenotypic markers and the immunolocalization of cardiomyogenic lineage-associated proteins revealed typical expression patterns of neo-cardiomyogenesis. At the biochemical level differentiating cells exhibited appropriate metabolic activity and at the ultrastructural level myofibrillar and sarcomeric organization were indicative of an immature phenotype. Functional analysis of differentiating BMSCs revealed cytosolic calcium transients and electromechanical coupling with ECMs. Our 3-D co-culture system sustains the ECMs in vitro continuum of differentiation process and simultaneously induces the maturation and differentiation of BMSCs into cardiomyocyte-like cells. Finally, the favorable physicochemical microenvironment renders these co-cultured cells, BMSCs and ECMs to reprogramme in synchrony towards cardiomyogenic lineage and commitment. Thus, creating the notion that in the presence of BMSCs, ECMs are capable of undergoing partial dedifferentiation, a step necessary for the further successive but progressive synchronized redifferentiation of both BMSCs and ECMs towards cardiac myocytes. Our study highlights a crucial phenomenon of partial dedifferentiation followed by redifferentiation of in vitro cardiomyocytes in the vicinity of adult marrow-derived stems cells. This novel insight could shed light on many controversies that prevail at present in this emerging field of adult stem cell based myocardial regeneration. This report clearly demonstrates that adult BMSCs under appropriate in vitro combination of physical, chemical and cellular environmental cues could be induced to undergo cardiomyogenic differentiation pathway and recapitulates many aspects of in vivo neo-myogenesis in a 3-D context. Thus, this unique 3-D co-culture system provides a useful in vitro model and a prospect to elucidate various molecular mechanisms underpinning the integration and orderly maturation and differentiation of BMSCs into neo-cardiomyocytes during adult stem cell based myocardial regeneration.

T-3102

FIBRO ADIPOGENIC PROGENITORS AS A TARGET OF ANTI FIBROTIC DRUGS

Low, Marcela, Rossi, Fabio M.V.
University of British Columbia, Vancouver, BC, Canada

Fibro/adipogenic progenitors (FAPs) are multipotent mesenchymal stromal cells that assist in muscle regeneration but also are responsible for the pathogenic changes associated with chronic muscle degeneration upon their differentiation into adipocytes and fibroblasts. FAPs have also been identified as the main collagen producing cells after skeletal muscle damage. Targeting FAPs may provide new opportunities to study the mechanisms regulating extracellular matrix gene expression and to identify therapeutic targets for fibrotic diseases. Due to their known role in extracellular matrix deposition, the TGFβ αvδ Ωντ pathways could have a key role in the switch of FAPs from a pro-regenerative role towards a pro-fibrotic role. The objectives of our work are to characterize collagen1-a1 expression in FAPs and to identify possible inhibitors of FAP differentiation into fibrogenic cells. Our experimental model uses a transgenic mouse expressing EGFP under the control of the collagen1-a1 promoter (collagen1a13.6-EGFP). Using this model, we evaluated GFP/collagen expression by fluorescence activated cell sorting (FACS) and imaging scanner. Initial characterization of collagen expression after notexin-induced skeletal muscle damage showed that FAP collagen expression peaked at day 7, decreasing thereafter. In vivo treatment with TGFβ increased the number of collagen expressing FAPs in the tibialis anterior muscle. This effect was recapitulated in vitro using FACS isolated GFP (-) FAPs exposed for 3 days to TGFβ treatment. Using the same model, the cells were incubated with several Wnt ligands showing that Wnt3a was a potent inducer of collagen expression. In order to identify possible inhibitors of FAP collagen expression, we screened 6 kinase inhibitor libraries; 480 compounds in total. GFP (-) FAPs were treated with TGFβ and the various kinase inhibitors. After 3 days, the effects on GFP/collagen expression were analyzed using an imaging scanner. Our results showed that 100 compounds have the potential to inhibit collagen expression in FAPs. Interestingly, the most potent inhibitors shared a common target, the p38 MAPK pathway. Our results show that the TGFβ and Wnt pathways mediate collagen expression in FAPs and are potential targets for the inhibition of fibrogenic differentiation. The drug screening results suggest that activation of the p38 MAPK pathway mediates the differentiation of FAPs towards a fibrogenic phenotype. The characterization of the specific effectors of these pathways regulating collagen and other extracellular matrix genes will help us to elucidate the molecular mechanisms responsible for the switch of FAPs from a pro-regenerative towards a pro-fibrotic role and inhibit them through pharmacological strategies. In summary, FAPs represent a new target cell type for the inhibition of fibrosis in skeletal muscle.

T-3103

APOLIPOPROTEIN A-I ENHANCES OSTEOGENESIS OF MESENCHYMAL STEM CELLS AND COMPLETELY PREVENTS THE OSTEOPOROSIS

Lu, Joyce (Jean)[1], Liu, Yu-Chuan[1], Huang, Wei-Kai[1], Lin, Kun-Yi[2], Lu, Frank[3], Wu, Han-Chung[1]
[1]Academia Sinica, Taipei, Taiwan, [2]Tri-Service General Hospital, Taipei, Taiwan, [3]National Taiwan University Hospital and National Taiwan University Medical College, Taipei, Taiwan

Apolipoprotein A-I (ApoA-I), a component of human high-density lipoproteins (HDLs), which remove cholesterol from arteries and can prevent cardiovascular diseases. The decrease of ApoA-I expression

levels increases the chances of cardiovascular disease, obesity, or other metabolic syndrome. By a high throughput screen with an overexpression library with 12380 genes in primary mesenchymal stem cells (MSCs), we found ApoA-I can promote activity of early osteogenesis markers alkaline phosphatase (ALP). In addition, we further demonstrated Apo-I enhances the expression of late osteogenesis marker Alizarin red staining upto 40 fold. Consistently, ApoA-I increased the RNA expression of osteogenic markers of ALP and bone sialoprotein (BSP). This is different to the function of other apolipoproteins. The function of ApoA-I in promoting bone formation could be detected both in human and mouse MSCs, but not in mouse cells already committed to late osteogenic cells. However, ApoA-I did not alter the Runx2 expression, which suggests ApoA-I function is not at the very first steps of osteogenesis. Interestingly, ApoA-I can activate both the phosphorylation of signal transducer and activator of transcription 3 (STAT3) and extracellular signal-regulated kinases (ERK). Inhibition of STAT3 activity or ERK activity hampers the effect of ApoA-I in osteogenic differentiation. Of note, ERK inhibitor also blocks STAT3 phosphorylation. Thus ERK may be the upstream regulator of STAT3. Meanwhile, we identified many cytokines stimulated by ApoA-I are regulated by STAT3 or ERK. The knockdown of these cytokines and its receptors blocks the osteogenesis of MSCs. Importantly, we found ApoA1 transgenic mice can completely prevent the osteoporosis in ovariectomized mice, a model mimic osteoporosis occurs in postmenopausal women. Osteoporosis is an important disease that leads to more death in peoples than breast cancer. One-third of the women and one-fifth to tenth of the men experience of osteoporotic fracture and the one year mortality rate of hip fracture is 30%. Taken together, we find an ApoA-I-ERK-STAT3 pathway can promote osteogenic differentiation in vitro, and ApoA-I can prevent the osteoporosis in vivo. This study identified many new ApoA-I-mediated pathways and novel mechanisms in bone formation, and also provides new potential therapeutic targets for bone disease like osteoporosis.

T-3104
THERAPEUTIC APPLICATION OF BONE MARROW DERIVED POOLED ALLOGENEIC MESENCHYMAL STROMAL CELLS (STEMPEUCEL, REGISTERED) IN BUERGER'S DISEASE-SAFETY AND EFFICACY RESULTS OF A NON-RANDOMIZED, OPEN LABEL, MULTICENTRIC, DOSE RANGING, PHASE II STUDY IN INDIA

Majumdar, Anish Sen[1], Balasubramanian, Sudha[2], Thej, Charan[2], Rajkumar, M[3], Krishna, Murali[4], Dutta, Santanu[5], Sarkar, Uday[6], Desai, Sanjay[7], Radhakrishnan, R[8], Dhar, Anita[9], Mathiyazhagan, R[2], Kumar, Uday[1], Baikunje, Umesh[1], KV, Prasanth[1], Sunderraj, Swathi[2], Anthony, Naveen[1], CH, Anoop[1], Gupta, Pawan[10]

[1]Stempeutics Research Private Ltd., Bangalore, India, [2]R and D, Stempeutics Research Private Ltd., Bangalore, India, [3]Madras Medical College, Chennai, India, [4]Sri Jayadeva Institute of Cardiovascular Sciences and Research, Bangalore, India, [5]Nightingale Hospital, Kolkata, India, [6]Health Point Hospital, Kolkata, India, [7]M.S Ramaiah Medical College and Hospitals, Bangalore, India, [8]Sri Ramchandra Medical College, Chennai, India, [9]AII India Institute of Medical Sciences, New Delhi, India, [10]Clinical Research, Stempeutics Research Private Ltd., Bangalore, India

Buerger's disease is a non-atherosclerotic segmental inflammatory disease that most commonly affects the small and medium-sized arteries. Its prevalence among all patients with peripheral arterial disease (PAD) varies from 0.5 to 5.6 percent in Western Europe to 45 to 63 percent in India. Critical limb ischemia (CLI) is a severe form of the disease that results in excruciating rest pain and non-healing ischemic skin ulcers and gangrene of the lower extremity. About 50% patients with CLI lose their affected leg within 6 - 12 months, and approximately 15% will require contralateral amputation within 2 years. Emerging data suggest that mesenchymal stromal cells (MSC) may offer therapeutic benefit to "no revascularization" option patients. Bone marrow derived mesenchymal stromal cells (BMMSC) are known to possess strong immunomodulatotory and anti-inflammatory properties, and promote tissue regeneration through paracrine activity. Stempeucel® is manufactured from BMMSC obtained from healthy volunteers in a GMP facility using a two-tier banking process and the product is comprised of pooled allogeneic BMMSC. Stempeucel® express MSC-associated cell surface markers, differentiate into bone, cartilage and adipose lineages of cells, possess potent immunosuppressive activity in mixed lymphocyte cultures and secrete various angiogenic factors including VEGF. Conditioned medium collected from stempeucel® induce migration, proliferation and differentiation of human umbilical vein endothelial cells (HUVEC) in vitro and the activity is significantly inhibited by anti-VEGF antibody. Results obtained from preclinical and phase I/II clinical trials established the safety of the allogeneic pooled BMMSC-based product. A phase II, non-randomized, multicentric, dose finding study in patients with Buerger's disease is currently ongoing using three different doses of stempeucel® (1, 2, and 4 million cells/kg - 36 patients for each dose). A control arm constituting 18 patients received standard protocol of care. Inclusion criteria include: (i) Buerger's disease as diagnosed by Shionoya criteria (ii) Males or females in the age group of 18-65 years (iii) Patients having infrapopliteal occlusive disease with rest pain and ischemic ulcer/necrosis, who are not eligible for revascularization treatment (iv) Patients in Rutherford- III-6 if gangrene extending maximally up to the head of metatarsal but limited to toes. The primary efficacy end points of the study are relief of the rest pain and ulcer healing in the target limb. Recruitment of first two doses and the control arm patients is completed and the patients are being followed-up for six month after stempeucel® administration. Preliminary analysis of clinical data indicates that allogeneic BMMSCs offer a promising treatment for patients with Buerger's disease.

T-3105
PLATELET RICH OR POOR PLASMA INCREASES PROLIFERATION OF HUMAN PERIODONTAL LIGAMENT STEM/PROGENITORS CELLS

Martinez Cardozo, Constanza E.[1], Gonzalez Itier, Sergio A.[2], Palma, Verónica[2], Smith Ferrer, Patricio C.[1]

[1]Facultad de Medicina - Odontologia, Pontificia Universidad Catolica de Chile, Santiago de Chile, Chile, [2]Facultad de Ciencias, Universidad de Chile, Santiago de Chile, Chile

Periodontal regeneration can be defined as the complete restoration of the tissues that have been lost due to either trauma or inflammatory disorders such as periodontal disease. Tissue engineering has emerged as an attractive tool to reconstitute damaged tissues lost after periodontal disease. Human Periodontal Cells constitute a heterogeneous cell population that contains stem, progenitors, and differentiation cells. In order to regulate the regeneration of periodontal tissues, it is important to identify factors that control cell proliferation and differentiation in stem and progenitor cells. It has been proposed that plasma fractions may constitute an attractive source of autologous growth factors necessary to stimulate periodontal regeneration including Platelet rich (PRP) and Platelet-poor plasma (PPP). Considering that it is critically important to amplify the number of stem cells and to guide the differentiation of progenitor cells, we propose to evaluate the role of PRP and PPP on cell proliferation Periodontal ligament cells were isolated from explants of periodontal ligament of Healthy human impacted third molars from young adults (18-24 years old) who attend

to a student's dental health practice at the Pontificia Universidad Católica de Chile, under approved guidelines set by the Ethics Committee of the Faculty of Medicine. Written informed consent will be obtained from all subjects. Explants from the middle third of the root, will be cultured. PRP and PPP fractions were obtained from 4 healthy volunteers with the GPS® III Platelet Concentration System (Biomet). Both fractions were analyzed by growth factors protein array (Raybiotech). Cell cultures obtained were analyzed by flow citometry to evaluate positive markers for mesenchymal stem cells: CD105, CD90, CD73, CD71 and negative markers: CD14, CD11b, CD34, CD45 and CD79. We analyzed the differentiation potential using condrogenic, adipogenic or bone/cementum differentiation media. Isolated cells were treated with characterized PRP or PPP (5, 10, 20 or 40%) during 48 hours and as a control we used fetal bovine serum (FBS, 10%). Finally we evaluated the expression of proliferation markers Ki67 PCNA and BrdU incorporation by immunostaining. We obtained cell cultures enriched with CD: 90, 73 and 105 positive cells (95%) and only a 1% of negative stem cell markers. These cells were able to differentiate to adipose, bone/cementum and condrogenic lineages. Protein array analyses of PRP and PPP showed a similar expression patron in both fractions with significant expression of PDGF, TGF-beta, IGF and EGF. Finally, we observed an increase of proliferation on periodontal ligament cells after the treatment of PRP or PPP even in the presence of 5% of PPP we obtain a 65% double positive BrdU/ki67 cells. We propose that PPP should be explored as an alternative of FBS in a really serum-free to maintain the in vitro expansion of Human periodontal ligament stem/progenitor cells as a substitute of FBS, towards their use in periodontal regeneration. Funding by Fondecyt grants 11121294(CM) and 1130618 (PS)

T-3106
ROLE OF NOTCH SIGNALING IN THE MAINTENANCE OF HUMAN MESENCHYMAL STEM CELLS UNDER HYPOXIC CONDITIONS

Moriyama, Hiroyuki[1], Moriyama, Mariko[1], Ishihara, Shin[1], Okura, Hanayuki[2], Matsuyama, Akifumi[2], Hayakawa, Takao[1]
[1]Kinki University, Higashi-Osaka, Japan, [2]Foundation for Biomedical Research and Innovation, Kobe, Japan

Human adipose-derived mesenchymal stem cells (hADMSCs) are attractive for cell therapy and tissue engineering because of their multipotency and ease of isolation without serial ethical issues. However, their limited growth potential as a result of cellular senescence in in vitro culture systems hinders their therapeutic application. Some somatic stem cells including hematopoietic stem cells, neuronal stem cells, and mesenchymal stem cells are known to be localized in hypoxic regions; thus, hypoxia may be beneficial for ex vivo culture of these stem cells. These cells exhibit a high level of glycolytic metabolism even under high-oxygen conditions and further increase their glycolysis rate under hypoxia. However, the physiological role of glycolytic activation and its regulatory mechanisms are still incompletely understood. Here we show that Notch signaling is required for glycolysis regulation under hypoxic conditions. Our results demonstrate that 5% O_2 dramatically increased the glycolysis rate, improved the proliferation efficiency, prevented senescence, and maintained the multipotency of hADMPCs. Intriguingly, these effects were not mediated by hypoxia-inducible factor (HIF), but rather by the Notch signaling pathway. 5% O_2 significantly increased the level of activated Notch1 and expression of its downstream gene, *HES1*, but not the level of HIF-1α. In addition, 5% O_2 markedly increased glucose consumption and lactate production of hADMPCs, which decreased back to normoxic levels upon treatment with a γ-secretase inhibitor. Moreover, enzyme activity assay of rate-limiting steps of glycolysis revealed that these enzymes were significantly activated in 5% oxygen condition, which was attenuated by Notch inhibition. Furthermore, mitochondrial mass and activity were decreased in 5% oxygen condition, whereas they were restored when Notch signaling was inhibited. These data suggest that a metabolic shift from mitochondrial respiration to glycolysis occurred in 5% oxygen conditions through Notch signaling. In order to identify the molecular mechanism of the metabolic shift, transcriptome analysis was performed. We found that HES1 was involved in induction of GLUT3, TPI, and PGK1 in addition to reduction of TIGAR and SCO2 expression. Finally, 2-deoxy-glucose, an inhibitor of glycolysis, attenuates the proliferation rate of hADMPCs, whereas the aerobic respiration block by NaN_3 did not decrease the proliferation; rather, it increased proliferation at a low concentration, which may support our data indicating that the metabolic switch from mitochondrial respiration to glycolysis provides a growth advantage to hADMPCs. These results clearly suggest that Notch signaling regulates glycolysis under hypoxic conditions and thus likely affects the cell lifespan via glycolysis.

T-3107
DEVELOPMENT OF A SINGLE TET-OFF LENTIVIRAL VECTOR SYSTEM WITH TIGHTLY REGULATED AND HOMOGENEOUS EXPRESSION OF TARGET GENES IN HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

Moriyama, Mariko[1], Moriyama, Hiroyuki[1], Okura, Hanayuki[2], Matsuyama, Akifumi[2], Hayakawa, Takao[1]
[1]Kinki University, Higashi-Osaka, Japan, [2]Foundation for Biomedical Research and Innovation, Kobe, Japan

Genetic modification of human adipose-derived mesenchymal stem cells (hADSC) is highly valuable for their exploitation in therapeutic applications. Here we have developed a novel single tet-off lentiviral vector platform; the regulator and response elements are combined in a single lentiviral genome. This vector consists of a modified tet-response element composite promoter followed by a Gateway cassette containing attR recombination sites flanking a ccdB gene, which allows an easy and rapid shuttling of gene of interest into the vectors. This vector is also carrying an improved version of the tetracyclin-controlled transactivator (tTA-advanced) and the blasticidin resistance gene, linked by the viral T2A peptide, under a ubiquitous promoter. In this study, we used the cytomegalovirus (CMV) or the elongation factor 1alpha (EF-1α) promoter as the ubiquitous promoter, and EGFP was introduced into the vectors by LR reaction. hADSC transduced with either the lentiviral vectors carrying CMV or EF-1α promoter was effectively selected by blasticidin without affecting their stem cell properties, and EGFP expression was strictly regulated by doxycycline (dox) in these cells. However, we demonstrated the intriguing finding that most (>90%) of the hADSC transduced with lentiviral vectors carrying the EF-1α promoter strongly expressed EGFP in the absence of Dox, whereas >50% of the cells transduced with lentiviral vectors carrying the CMV promoter were EGFP negative, regardless of their blasticidin resistance. The inhibitor of histone deacetylation trichostatin A (TSA) re-induced the expression of EGFP, suggesting that "promoter suppression" might occur by histone deacetylation when using the CMV promoter. Our data also demonstrated that adipocytes, chondrocytes, osteocytes and neuronal cells, differentiated from these dox-responsive cell lines, also constitutively expressed EGFP only in the absence of dox. This single tet-off lentiviral vector thus provides powerful tools for applied research on hADSC.

T-3108

OVEREXPRESSION OF KRÜPPEL-LIKE FACTOR 4 INDUCES A MESENCHYMAL TO EPITHELIAL TRANSITION AND REDIFFERENTIATION OF HUMAN PANCREATIC BETA CELL DERIVED MESENCHYMAL STROMAL CELLS FOLLOWING EXPANSION IN ADHERENT CULTURE

Muir, Kenneth Ross, Lima, Maria Joao, Docherty, Hilary, Docherty, Kevin
University of Aberdeen, Aberdeen, United Kingdom

Cell therapy in the form of islet transplantation is an established treatment modality for Type 1 Diabetes (T1D) however lack of tissue donors is a major factor in limiting widespread adoptance. Development of a replenishable source of insulin-producing cells would provide a solution to this and provide potential even to cure type 1 diabetes. Attempts to culture and expand pancreatic beta cells in vitro have been met with limited success as they do not proliferate to any meaningful extend in their differentiated state. Adherent culture results in their transition from insulin-producing epithelial cells to mesenchymal stromal cells (MSCs) with high proliferative capacity but devoid of any hormone production. The aim of this study was to determine whether the transcription factor, Krüppel-like factor 4 (Klf4), can induce a mesenchymal to epithelial transition (MET) i.e. reversal of the dedifferentiation process that takes place in cell culture. Human islet enriched pancreatic cells were cultured in RPMI with 10% FBS and allowed to dedifferentiate and expand. The resultant population of MSCs were transduced with an adenovirus containing KLF4 (ad-KLF4) and incubated for between 2 and 10 days. Gene expression was assessed by real-time quantitative PCR. Morphological changes and protein distribution were assessed by immunocytochemistry. Lineage traced beta cells were created using two lentiviral vectors expressing cre recombinase under the insulin promoter and a floxed STOP dsred promoter under control of the cytomegalovirus promoter. Treatment with ad-KLF4 resulted in infected cells taking on a more rounded epithelial like phenotype. This was associated with re-expression of epithelial genes E-Cadherin and EPCAM and reduced expression of mesenchymal markers vimentin, snai2 and α-SMA maximally at 48hr post transduction (all p=<0.001). Markers of differentiated pancreatic cells were also up-regulated, including insulin by 891.2% (p=<0.0001), amylase by 1117.9% (p=0.002) and CK19 (p=0.002) by 3844%. Endocrine transcription factors NGN3, MafA, Nkx6.1 and NeuroD1 were all significantly up-regulated. Cells staining for E-cadherin, C-peptide, amylase and CK19 were seen on fluorescence immunocytochemistry at 96hr post ad-KLF4, but not in control cells. Genetic lineage tracing confirmed at least some of these cells were derived from beta cells. Our results show that for the first time that MSCs derived from human pancreatic beta cells can be induced to undergo a mesenchymal to epithelial transition in standard culture conditions by overexpressing KLF4. In addition to this, these cells show evidence of redifferentiation towards mature pancreatic cell types without the use of additional growth factors or small molecules. These findings hold promise that beta cells which have dedifferentiated and expanded ex-vivo can be redifferentiated by directly promoting an MET.

T-3109

INVOLVEMENT OF FGFR-2/PI3-KINASE/AKT/GSK3-BETA SIGNALING PATHWAY IN BASIC FIBROBLAST GROWTH FACTOR-INDUCED NEURONAL DIFFERENTIATION OF CANINE BONE MARROW STROMAL CELLS

Nakano, Rei, Edamura, Kazuya, Kitanaka, Taku, Narita, Takanori, Okabayashi, Ken, Sugiya, Hiroshi
Nihon University, Fujisawa, Kanagawa, Japan

In the veterinary medicine, the clinical trial of spinal regenerative therapy using autologous bone marrow stromal cells (BMSCs) has been performed in dogs. In the previous studies, we reported that basic fibroblast growth factor (bFGF) is effective for differentiation of canine BMSCs into functional neurons. However, the mechanism of bFGF-induced neuronal differentiation of canine BMSCs has not been well elucidated, although which is very important for the clinical application of canine BMSCs. Canine BMSCs was isolated and cultured from the bone marrow of the humerus of healthy beagle dogs. This study was conducted under Nihon University Animal Care and Use Committee approval. On the fourth day of culture, non-adherent cells were removed when the culture medium was replaced, thus isolating the canine BMSCs. For neuronal induction, the canine BMSCs were incubated in Neurobasal-A medium supplemented with 2% B27 supplement containing bFGF (100 ng/mL). Real-time RT-PCR was performed to evaluate the expression of mRNAs of neuronal marker, MAP2. In canine BMSCs, bFGF induced an increase in MAP2 mRNA expression. To determine which subtype of FGFRs in canine BMSCs contributes to the specific neurogenic effects of bFGF, we measured the mRNA and protein expression levels of various FGFRs. Semi-quantitative RT-PCR experiments showed FGFR-1 and -2 mRNAs were detected. The expression of FGFR-1 and -2 proteins in canine BMSCs were confirmed by Western blotting. We then analysed the binding property of bFGF with FGFR subtypes. In canine BMSCs, cross-linking and immunoprecipitation analysis revealed that bFGF bound to the FGFR2. In the presence of the FGFR inhibitor, the PI3-kinase inhibitor or the Akt inhibitor, bFGF failed to induce MAP2 mRNA expression. In bFGF-treated cells, Akt, the downstream target of PI3-kinase, was phosphorylated, and the phosphorylation was inhibited by the FGFR inhibitor, the PI3-kinase inhibitor or the Akt inhibitor. In bFGF-treated cells, GSK3β, one of the substrate of the Akt, was phosphorylated in a time dependent manner. The phosphorylation of GSK3β was inhibited by the FGFR inhibitor, the PI3-kinase inhibitor or the Akt inhibitor. In conclusion, we suggest that FGFR-2/PI3-kinase/Akt/GSK3β pathway is involved in bFGF-induced neuronal differentiation of canine BMSCs.

T-3110

ARRESTED AUTOPHAGY IN HUMAN MESENCHYMAL STEM CELLS/MULTIPOTENT STROMAL CELLS IS MOBILIZED DURING EARLY DIFFERENTIATION AND CAN BE MODULATED TO IMPROVE DIFFERENTIATION EFFICIENCY

Nuschke, Austin[1], Rodrigues, Melanie[1], Stolz, Donna B.[2], Chu, Charleen T.[1], Wells, Alan[1]
[1]Pathology, University of Pittsburgh, Pittsburgh, PA, USA, [2]Cell Biology, University of Pittsburgh, Pittsburgh, PA, USA

Mesenchymal stem cells (MSCs) hold therapeutic potential in regenerative medicine due to their capacity to form multiple cell lineages and support the regenerative environment by secreting beneficial growth factors and cytokines. However, many studies involving implanted MSCs have shown that the cells do not survive implantation in a wound site, where the MSCs are immediately exposed to nutrient starvation, pH changes, or other stressors. To contribute to tissue

formation, MSCs must be able to withstand these challenges to survive long enough to provide their therapeutic benefit. As such, processes involved in how MSCs respond to metabolically demanding conditions have recently become of interest, as such pathways could be exploited to improve the MSC response to these environments. Given that MSCs are involved in normal regenerative processes in wound healing, we have examined innate cellular mechanisms that MSCs might use to combat high metabolic demand. Here, we studied changes in MSC autophagy in response to MSC differentiation, hypothesizing that MSC autophagy would be mobilized during differentiation, and that differentiation itself would be modulated by agents that alter autophagy during the critical early period of differentiation. Transmission electron microscopy revealed that human MSCs present a high concentration of autophagosomes that remain intact in the cytosol not undergoing degradation. This phenotype suggests that MSCs exist in a state that is primed for rapid autophagy activation in times of high energy demand, such as during differentiation. Therefore, using media to induce both osteogenesis and adipogenesis separately, we differentiated human bone marrow-derived MSCs in vitro to monitor the autophagic response over 72 hours. MSCs were differentiated either under normal conditions or with 5 uM rapamycin or 5 nM bafilomycin for the first three hours to induce or block autophagosome degradation, respectively. Immunoblots for LC3, a classic marker of autophagosome turnover, showed that differentiating MSCs rapidly degraded existing autophagosomes within the first 24 hours of differentiation, comparable to rapamycin-stimulated cells. Separately, we examined the effects of early drug modulation of autophagy on long term MSC differentiation efficiency in adipogenic or osteogenic conditions. Surprisingly, we found that treatment with 5 nM bafilomycin during the first three hours of differentiation enhanced both the osteogenic and adipogenic differentiation efficiency over 30 days, as measured by deposition of spicules and generation of fat droplets, and supported by upregulated expression of lineage-specific genes. In contrast, 5 uM rapamycin treatments in a similar study reduced the ability of the MSCs to form bone or fat in culture. Our results strongly implicate autophagosomes as regulatory for MSC activation and differentiation. These structures exist in MSCs at a basal/arrested state, wherein they are able to be rapidly degraded; this could provide sources for energy generation or building blocks of macromolecules. By slowing autophagy mobilization in early MSC differentiation, MSCs seem to be able to balance energy utilization and meet the metabolic demands of differentiation more efficiently. This may ultimately prove to be a phenotype that can be targeted to improve the response of MSCs implanted in wound sites, where the cells must respond to a high number of stressors during the differentiation process.

T-3111
BIOACTIVE GLASS IONS STRONGLY ENHANCE OSTEOGENIC RESPONSE IN HUMAN ADIPOSE STEM CELLS

Ojansivu, Miina Emilia[1], Vanhatupa, Sari[1], Björkvik, Leena[2], Hupa, Leena[2], Miettinen, Susanna[1]
[1]BioMediTech, University of Tampere, Tampere, Finland, [2]Process chemistry center, Åbo Akademi University, Turku, Finland

Human adipose stem cells (hASCs) are a promising autologous cell source for the applications of regenerative medicine. They are multipotent stem cells able to differentiate *in vitro* for example towards bone, cartilage and fat cells. They are also easily accessible with high yield. Of the various biomaterials used in bone tissue engineering, bioactive glass (BaG) has been shown to be especially advantageous due to its osteoinductive properties. However, it is currently not known whether the BaG-induced osteogenic differentiation of hACSs is caused by ionic dissolution products or cell attachment on the BaG surface. To shed light on this, hASCs were cultured in BaG ions containing extracts prepared from different BaG compositions. Bioactive glass extracts were prepared from each glass type using basic medium (BM) and osteogenic medium (OM) as an extraction basis in the protocol. To assess osteogenesis, quantitative alkaline phosphatase (qALP) activity was analyzed after 7, 11 and 14 days of culture. In addition, mineralization was determined quantitatively by Alizarin red staining at 11 and 14 days. Cell proliferation was studied using CyQuant® Cell Proliferation Assay (Invitrogen). Furthermore, the total protein amounts were analyzed by Pierce® BCA Protein Assay Kit (Thermo Scientific). The viability of hASCs was assessed by Live/dead staining (Invitrogen) after 14 days of culturing in the various conditions. Cells stayed viable for the whole culturing period and thus no dead cells at 14d could be detected. At 7d and 11d time points none of the conditions studied induced osteogenic differentiation of hASCs as determined by qALP activity and mineralization. The situation, however, was dramatically changed at 14d: even though none of the extracts induced higher total qALP activities compared to the controls, the mineralization in OM extracts was excessive (one order of magnitude higher than in the OM control). Parallel to the increased mineral formation, the cell amount in OM extracts was decreased. In conclusion, these results imply that BaG ions are able to stimulate the osteogenic differentiation of hASCs, even though osteogenic supplements, such as ascorbic acid, β-glycerophosphate and dexamethasone, are still required. The OM extracts could potentially provide a fast and effective way to differentiate hASCs *in vitro* prior to their utilization in clinical bone tissue engineering applications.

T-3112
THE EFFECT OF H2S ON PANCREATIC REGENERATION FROM HUMAN TOOTH PULP

Okada, Mio, Yaegaki, Ken, Ishkitiev, Nikolay, Imai, Toshio
Nippon Dental University, Tokyo, Japan

Objectives: Adult stem cells are more appropriate for the practice of regenerative medicine than ES cells or iPS cells. They are ethically acceptable, easily sourced from many tissues. Hydrogen sulfide (H2S) which works in several physiological processes increases hepatic differentiation from human deciduous tooth pulp stem cells (hDTPSC). The present study assessed differentiation of hDTPSC towards pancreatic lineages, and the effect of H2S on the differentiation. Methods: The cells were isolated from deciduous tooth pulp and were grown in DMEM supplemented with 10 % FBS. Cells between 3-5 passages, grown in serum-free medium and were characterized for a panel of stem cell markers by RT-PCR. CD117+ cells were magnetically separated. The cells were also subjected to pancreatic differentiation protocol. Expression of a panel of pancreatically related transcription factors and WNT/ beta catenin signaling pathway were determined with RT-PCR. Immunocytochemistry and flow cytometry of pancreatically related marker were carried out after the differentiation. Concentration of secreted insulin and c-peptide in the medium was determined with ELISA. During the differentiation the cultures were exposed to 0.1ng/mL air of hydrogen sulfide. The cells non-exposed to hydrogen sulfide were used as control. Results: Insulin, glucagon, somatostatin, and pancreatic polypeptide were positive by immunofluorescence and flow-cytometry after differentiation. Hydrogen sulfide increased the expression of insulin. WNT signaling pathways were activated in the differentiation, especially beta-catenin pathway, Ca2+ pathway, and cell development/proliferation/migration factor were highly expressed. Moreover H2S enhance these expressions. The concentration of insulin and c-peptide in the medium in H2S sample increased compared with control. Conclusions: Our results showed that CD117+ fractions

of hDTPSC are capable of differentiate toward pancreatic endocrine and exocrine cells, and the differentiation of pancreatic cells was increased by hydrogen sulfide. Low concentration of hydrogen sulfide may enhance the differentiation property. Our method of pancreatic differentiation may therefore have great potential for future cell therapy of pancreatic disorders.

T-3113
DIRECT DIFFERENTIATION OF DOPAMINERGIC NEURONAL CELLS FROM HUMAN ADIPOSE-DERIVED MULTILINEAGE PROGENITOR CELLS

Omori, Shigenari[1], Moriyama, Mariko[1], Taniguchi, Yuuki[1], Matsuyama, Akifumi[2], Moriyama, Hiroyuki[1], Hayakawa, Takao[1]
[1]Kinki University, Higashi-Osaka, Japan, [2]Foundation for Biomedical Research and Innovation, Kobe, Japan

Recent progress in stem cell research has raised hope for the development of stem cell therapies in neurodegenerative disorders, such as Parkinson's disease. Human adipose tissue-derived mesenchymal stem cells (ADSCs), also referred to as human adipose tissue-derived multilineage progenitor cells (hADMPCs), are multipotent stem cells that can differentiate into various types of cells, including neuronal cells. They can be easily and safely obtained from human adipose tissue without posing serious ethical issues and can also be expanded ex vivo under appropriate culture conditions. Moreover, because of their reduced risk of tumorigenesis, and their hypoimmunogenicity and immunomodulatory effects, hADMPCs are an attractive material for cell therapy and tissue engineering. In this study, we investigated a dopaminergic neuronal differentiation strategy in vitro by introducing four transcription factors, Achaete-scute homolog 1(ASCL1), forkhead box protein A2 (Foxa2), LIM homeobox transcription factor 1 alpha (Lmx1a), and Nuclear receptor related 1 (Nurr1). We transferred each of these genes to hADMPCs by recombinant lentiviral vectors, followed by neuronal induction. Intriguingly, addition of basic fibroblast growth factor (bFGF) caused a formation of spheroid like cells when introducing Foxa2 or Lmx1a into hADMPCs. Nestin expression was observed to be upregulated in these cells, suggesting that these spheroid like cells were neuronal stem cells. Further induction of neuronal differentiation resulted in the increased expression of Tyrosine hydroxylase (TH), Neurofilament-M (NF-M), and Microtuble-associated protein 2 (MAP2) in these cells. These data demonstrate that hADMPCs introduced by Foxa2 or Lmx1a have the ability to differentiate into dopaminergic neuron-like cells via a neuronal stem cell stage. Our study thus may help developing effective therapy for Parkinson's disease.

T-3114
USE OF FAT DEPOT-SPECIFIC ADIPOSE-DERIVED STEM CELL BIOMARKERS FOR HIGH THROUGHPUT SCREENING AND DIAGNOSIS

Ong, Wee Kiat[1], Toh, Sue-Anne[2], Han, Weiping[3], Sugii, Shigeki[3]
[1]Singapore Bioimaging Consortium, Singapore, [2]Department of Medicine, National University of Singapore, Singapore, [3]Singapore Bioimaging Consortium / Duke-NUS Graduate Medical School, Singapore

White adipose tissue (WAT) is widely regarded as clinically practical source of mesenchymal stem cells (MSCs) due to the abundance and ease of isolation of WAT and the adipose-derived stem cells (ASCs) that reside within the tissue. ASCs from the anatomically distinct subcutaneous and visceral depots of WAT differ in their inherent properties. Notably, ASCs from subcutaneous fat (SC-ASCs) differentiate better into mature adipocytes than ASCs from visceral fat (VS-ASCs). This has been proposed to be part of the underlying mechanism that contributes to the different pathophysiological functions of subcutaneous and visceral fat. The subcutaneous fat depot physiologically stores excess lipids thus preventing their deposition into other organs. Visceral fat accumulation, on the other hand, leads to pathological metabolic profile due to dysfunction in lipid storage. We previously performed a high content screening of over 240 cell surface markers on cultured SC- and VS- (omental region) ASCs in order to identify depot-specific cell surface markers of these ASCs. Subsequent confirmatory analyses identified depot-dependent expression of CD10 in SC-ASCs and CD200 in VS-ASCs across human subjects and in mice. In addition, CD10 showed positive and CD200 showed negative correlation with adipogenic capacity, as evident from their expression level during adipogenesis and better differentiation of CD10hi versus CD10lo SC-ASCs and CD200lo versus CD200hi VS-ASCs. Together these markers offer a valuable tool for tracking in imaging studies, diagnosis and easy high throughput screening methods of depot-phenotypic switch and adipogenic capability. A proof of concept pilot study was performed by screening a compound library for nuclear receptor ligands that altered CD10 and CD200 expression. Among the positive hits, dexamethasone markedly increased CD10 expression in SC-ASCs and moderately decreased CD200 expression in VS-ASCs. Remarkably, pre-treatment of ASCs with dexamethasone prior to adipogenic stimuli significantly increased adipogenesis. The data support potential use of these markers in drug screening for adipogenic compounds in a relatively quick (2-3 days versus 10-14 days of conventional methods) and high throughput manner by using human or animal-derived ASCs. The expression profile of these markers at the ASC level can thus be used as potential biomarkers to indicate quality of fat from patients' samples and predict their susceptibility to develop obesity and diabetes.

T-3115
EFFECT OF HYPOXIC CONDITION ON THE CELLULAR ACTIVITIES AND MRNA/MICRORNA EXPRESSION OF HUMAN BONE MARROW STEM CELLS

Park, Jung-Chul[1], Lee, Jung-Seok[1], Shim, Eun-Kyung[2], Kim, Tae-Wan[1], Cho, Kyoo-Sung[1], Kim, Chang-Sung[1]
[1]Periodontology, Research Institute for Periodontal Regeneration, Yonsei University College of Dentistry, Seoul, Republic of Korea, [2]iBMT, Anyang, Republic of Korea

It is well established that human bone marrow stem cells (hBMSCs) are involved in the healing of damaged tissues. Since hypoxic microenvironment is one of the very specific characteristics of damaged sites, the altered characteristics of hBMSCs under the hypoxic condition should be understood. In the present study, the effect of hypoxia on global gene expression in hBMSCs has been examined using DNA and RNA microarrays. hBMSCs were obtained during the vertebral surgery from five donors, and were exposed to hypoxia (1% O2) or 'normoxia' (21% O2). Biological activities including proliferation, cell surface marker expression, osteogenic/adipogenic/chondrogenic differentiation, and collagen regeneration potentials were examined. Microarray for DNA and micro RNA were performed and 124 genes were up-regulated and 37 down-regulated in response to hypoxia (>1.5-fold change, $P < 0.01$). 60 microRNA were significantly expressed. Collectively, it was shown that hypoxia has extensive effects on cellular activities of hBMSCs and their gene expression. This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIP) (No. 2012R1A2A4A01007124) and by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) Funded by the Ministry of Science, ICT and Future Planning (No. 2012M3A9B2052273).

T-3116

STRATEGY ENSURING RAPID AND EFFICIENT PROTOCOL FOR OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS USING SYNTHETIC GELS

Park, Hyun-Sook[1], Lee, Sunray[1], Yang, Ji Won[1], Chu, Seol[1], Ulijn, Rein V[2], Lee, Jongmin[3], Lim, Goon il[3]
[1]CEFO Research Center, Seoul, Republic of Korea, [2]Department of Pure and Applied Chemistry, Thomas Graham Building, 295 Cathedral Street, Strathclyde University., Glasgow G1 1XL, United Kingdom, [3]Department of Orthopaedics, Dongguk University, Seoul, Republic of Korea

Currently the importance of physical properties has been paid attention in stem cell differentiation. Here we propose a strategy ensuing rapid and efficient protocol for osteogenic differentiation of human mesenchymal stem cells (MSC) using synthetic gels with optimal stiffness. We suggest that optimizing stiffness of the synthetic gel could allow osteogenic differentiation of MSC as early as 5 days (determine by alizarin red staining) after differentiation and reproducible regardless of batch-variation and tissue sources such as wharton's jelly, bone marrow, adipose tissue etc. Either bone morphogenic protein (BMP) or Wnt3a did no additive effect on osteogenesis, implying that the optimal stiffness of synthetic gel plays a preferential role in fate determination of stem cells into osteogenesis. This study was supported by a grant (N01090009) form Global R and D Project of the Korea Institute for Advancement of Technology, Ministry of Knowledge Economy and a grant of the Korea Healthcare technology R and D Project, Ministry for Health, Welfare and Family Affairs, Republic of Korea. (A121962).

T-3117

DYSTROPHIC SKELETAL MUSCLE REGENERATION BY HUMAN DENTAL PULP AND AMNIOTIC FLUID DERIVED STEM CELLS

Pisciotta, Alessandra[1], Riccio, Massimo[1], Lu, Aiping[2], Gharaibeh, Burhan[2], Carnevale, Gianluca[1], Bruzzesi, Giacomo[3], Ferrari, Adriano[4], La Sala, Giovanni B.[5], Huard, Johnny[2], De Pol, Anto[1]
[1]Department of Surgical, Medical, Dental and Morphological Sciences, University of Modena and Reggio Emilia, Modena, Italy, [2]Stem Cell Research Center, Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA, USA, [3]Oro-Maxillo-Facial Department, AUSL Baggiovara, Modena, Italy, [4]Department of Biomedical Science, Metabolic and Neuroscience, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy, [5]Department of Obstetrics and Gynecology, Arcispedale Santa Maria Nuova, Reggio Emilia, Italy

Duchenne muscular dystrophy (DMD) is a genetic mutation resulting in muscle degeneration that leads to death by the mid-twenties. Cell therapy can be used to reintroduce dystrophin to repair damaged skeletal muscle fibers. Human dental pulp (hDPSCs) and amniotic fluid stem cells (hAFSCs) may represent an alternative, less controversial source to embryonic stem cells. Human DPSCs can be isolated from human dental pulp of enclosed third molars and deciduous teeth during routine extraction. Human AFSCs, which represent 1% of the cells in human amniocentesis specimens, can be obtained by immunoselecting the antigen c-Kit (CD117) positive population, via magnetic cell sorting. Human DPSCs and AFSCs have been shown to be self-renewing and multipotent, therefore they may represent promising tools for muscular dystrophy therapies. This study evaluated the myogenic potential of hDPSCs and hAFSCs using different conditions, to develop the most effective protocol to differentiate the cells towards a myogenic lineage. The first protocol consisted in a direct co-culture of hDPSCs and hAFSCs with C2C12 mouse myoblasts, while the second one was based on differentiation in myogenic medium - with or without the addition of conditioned medium from differentiated C2C12 - following a DNA-demethylating treatment by means of 5-Aza-2'-deoxycytidine. The direct co-culture resulted in new multinucleated myotubes formation, deriving from the fusion of both, murine and human, cell populations together, as shown by immunofluorescence staining specific for human nuclei. Also, the demethylation treatment was able to trigger the myogenic commitment of hDPSCs and hAFSCs, which showed the expression of muscle specific markers, such as myogenin and myosin. Human stem cells pre-differentiated after demethylation treatment, with and without the addition of conditioned media, were then injected into gastrocnemia muscles of 8-10 weeks old SCID/mdx mice. The animals were sacrificed at different time points and the muscles were harvested, snap frozen in liquid nitrogen and processed for immunofluorescence analysis. As early as 7 days after injection, an appreciable engraftment of hDPSCs and hAFSCs was detected in the host muscle, moreover, the double immunostaining by an antibody specific for human mitochondria and anti-von Willebrand factor antibody revealed the presence of both the human stem cell populations within the endothelium of new formed vasa, thus showing the angiogenesis promoting ability of hDPSCs and hAFSCs. Moreover, 14 days after the injection, hDPSCs and hAFSCs demonstrated their effective regenerative capability by restoring the expression of dystrophin, otherwise lacking in the dystrophic muscle: interestingly, the muscle fibers with restored dystrophin expression were positively stained by the anti-human mitochondria antibody. Dystrophin expression was detectable still 28 days after the treatment, therefore demonstrating that hDPSCs and hAFSCs are able to survive and sustain the muscle regenerating process for a relatively long time. The regeneration process was also confirmed by the histological analysis: the ongoing skeletal muscle regeneration was accompanied by a reduction of both fibrosis and necrosis, peculiar processes of DMD. These results suggest that hDPSCs and hAFSCs are suitable, non-controversial sources of stem cells that could be very useful for translational strategies aimed to enhance the repair of injured skeletal muscle in DMD patients.

T-3118

MITOCHONDRIAL DYNAMICS REGULATES MOUSE SKIN MESENCHYMAL STEM CELL (MSMSC) FATE

Forni, Maria Fernanda, Peloggia, Julia, Kowaltowski, Alicia Juliana
Universidade de São Paulo, São Paulo, Brazil

msMSCs are mesodermal precursors located at the dermis. They are CD90+, CD105+ and CD34- and, after induction, display the capacity to undergo chondro, adipo and osteogenesis in vitro. Recent evidence suggests that an extensive metabolic reconfiguration occurs during differentiation and much attention has been drawn to the fact that metabolic pathways may be controlled by the same signals that control the decision between cell proliferation/differentiation. It is known that the bioenergetic status of a cell is dependent of the overall quality and relative abundance of the mitochondria population, regulated through the processes of fusion and fission, but little is known about the impact of mitochondrial dynamics during the differentiation process. In order to examine this process we isolated MSCs from dermis of Swiss female mice (2-4 months) and performed a full flow-cytometry-based characterization of these cells, validating the expression of the above cited CD list. We also induced these cells to differentiate and validated the process through histochemistry and qPCR. Mitochondrial biogenesis was evaluated mainly through confocal microscopy, citrate synthase (CS) activity and mtDNA copy number estimation. The pattern of several intermediates of the fission (fis1 and 2, drp-1) and fusion (mfn1 and 2, opa-1) process was evaluated through qPCR and validated with WB. Using realtime measurements of O2 consumption (OCR) and acidification (ECAR) in cell cultures, the bioenergetic

profile during early (48h) and terminal (7d) differentiation was observed. In early chondrogenesis it was observed a profound change in basal respiration followed by a decrease in maximal respiratory capacity at 7d, also the levels of fission proteins were upregulated and CS activity levels decreased, leading us to suggest that the content of mitochondria is diminished very early. In osteogenesis, a increase in maximal respiration and a decrease of leakiness were observed followed by a increase in fusion and slightly increased levels of CS activity. In a completely different way, adipogenesis presented the highest CS levels and mitochondria was more fused and abundant (biogenesis), what led to higher basal and maximal respiration rates followed by an increase in leakiness. In brief, mitochondrial morphology and its regulating processes fission/fusion seen to be modulated early on during commitment leading to alterations in the bioenergetic profile during differentiation, leading us to propose a central role for mitochondria dynamics in the maintenance/commitment of mesenchymal stem cells.

PRE-CLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL CELLS

T-3121
ADIPOSE-DERIVED STEM CELLS ISOLATED FROM THE MEDIASTINAL FAT OF CARDIAC PATIENTS IMPROVE CARDIAC FUNCTION OF INFARCTED RAT HEARTS

Chi, Chao[1,2,3], Xiang, Bo[2,7], Deng, Jixian[1,2], Wang, Fei[1,2,3], Natarajan, Kanmani[4], Lin, Hung-Yu[1], Liu, Hongyu[3], Lin, Francis[4], Freed, Darren H.[6], Arora, Rakesh C.[1,2,5], Tian, Ganghong[1,2]

[1]National Research Council Canada, Winnipeg, MB, Canada, [2]Department of Physiology and Pathophysiology, Faculty of Medicine, University of Manitoba, Winnipeg, MB, Canada, [3]Department of Cardiac Surgery, The First Affiliated Hospital of Harbin Medical University, Harbin, People's Republic of China; Department of Physics and Astronomy, University of Manitoba, Winnipeg, MB, Canada, [4]Department of Physics and Astronomy, University of Manitoba, Winnipeg, MB, [5]St. Bonifcae Research Centre, Univeristy of Manitoba, Winnipeg, MB, Canada; [6]University of Alberta, Edmonton, AB, Canada, [7]Department of Pharmacology and Therepeutics, Faculty of Medicine, University of Manitoba , Winnipeg, MB, Canada

Background: Mediastinal fat may be excised during mediastinal and open-heart surgeries for various reasons. It would be clinically valuable if a potent stem cell population could be quickly isolated from the "to-be-discarded" fat tissue for autologous cardiac application. The present study was therefore designed to determine whether the mediastinal fat tissue of human cardiac patients contained potent stem cells and whether the mediastinal adipose-derived stem cells (ASC) could improve cardiac contractile function of failing hearts. Methods: ASC were isolated form the mediastinal fat tissue that was collected from 24 patients (age 61.3±9.8 years) during cardiac surgery following a standard median sternotomy. The ASC were then transfected with a vector encoding a gene for GFP. Morphology, cell surface markers, and differentiation capacity of the ASC were analyzed. To evaluate their cardioprotective capacity, a rat model of congestive heart failure was established by a complete occlusion of the left anterior descending coronary artery (LAD). One week after the LAD occlusion, ASC (~1.5x10^6 in 80uL) and cell-culture medium (CCM, 80uL) were injected into infarct rim in the group 1 (n = 13) and group 2 (n =5), respectively. Six weeks after the injections, heart function was assessed with MRI and pressure-volume (P-V) loop technique. The hearts were then sectioned for immunohistological assessments. Results: Mediastinal ASC exhibited a fibroblast-like morphology. They expressed some of the mesenchymal stromal cell markers (CD29, CD73, and CD90) and lacked expression of the hematopoietic markers (CD11b, CD34, CD45, and CD106). Moreover, the ASC expressed a significant level of key pluripotent genes, such as SOX-2 and Nanog. Following adipogenic, osteogenic, and cardiomyogenic inductions, the mediastinal ASC were stained positive for lipid drops, alkaline phosphatase, and myosin light chain 2C, respectively. Reverse transcription PCR results confirmed the immunohistological findings. They also expressed growth factors, TGF-β and VEGF. Moreover, MRI showed a significant decline in left ventricular ejection fraction (LVEF) 6 weeks after CCM injection relative to the pre-injection value (41.6 ± 4.7% vs. 48.6 ± 4.1, P<0.05). In the ASC-treated rat hearts, in contrast, MRI did not show any decrease in LVEF 6 weeks after the cell transplantation compared to the pre-transplantation (43.1±9.8% vs.42.9±7.5%, P>0.05). Furthermore, the maximal rate of rise of left ventricular pressure (dp/dt) was also significantly (P<0.05) greater in the ASC-treated hearts (7340 ± 971 mmHg/s) than in the CCM-treated hearts (5121 ± 2745 mmHg/s). A number of GFP-positive cells were detected on the tissue sections of the ASC-treated hearts. The GFP-positive cells were also stained positive for a human nuclear membrane protein (LAMIN). Conclusion: Human mediastinal fat tissue contains a population of multipotent stem cells. Under appropriate induction conditions, the mediastinal stem cells can differentiate into adipocyte-, osteocyte-, and cardiomyocyte-like cells. More importantly, the mediastinal stem cells are able to improve cardiac function of infarct hearts. We conclude that the mediastinal fat tissue could be a source of stem cells for treatment of ischemic heart disease.

T-3122
MICRORNA-152 ACCELERATES CELL CYCLE PROGRESSION AND INHIBITS THE APOPTOSIS OF IMPLANTED MESENCHYMAL STEM CELLS BY TARGETING P27/KIP1 IN ISCHEMIC MYOCARDIUM

Seo, Hyang-Hee[1], Ham, Onju[1], Lee, Se-Yeon[1], Lee, Chang Youn[2], Park, Jun-Hee[2], Lee, Jiyun[1], Seung, Minji[1], Yun, INa[1], Han, Sun M.[1], **Choi, Eunhyun**[1], Hwang, Ki Chul[3]

[1]Yonsei University College of Medicine, Brain Korea 21 Plus Project for Medical Science, Seoul, Republic of Korea, [2]Yonsei University, Department of Integrated Omics for Biomedical Sciences, Seoul, Republic of Korea, [3]Yonsei University College of Medicine, Severance Biomedical Science Institute, Seoul, Republic of Korea

Oxidative damage from the overproduction of reactive oxygen species (ROS) is the main cause of ischemia-reperfusion(I/R) injury. We previously reported that poor viability of transplanted mesenchymal stem cells (MSCs) in the infarcted myocardium is caused from enhancement of ROS production; many studies have revealed that elevated production of ROS arrests the cell cycle by regulating cell cycle related proteins such as P27$^{/kip1}$ and cyclinD1. Among them, P27$^{/kip1}$ is well-known to arrest cell cycle by inhibiting the Cdk-cyclin complex during the G1 phase. Growing evidences suggest that apoptosis is principally induced during the G1 phase. Therefore, we hypothesized that the down-regulation of P27$^{/kip1}$ levels in transplanted MSCs using microRNA(miR)-152, which is predicted to target P27$^{/kip1}$, would promote cell cycle re-entry and could inhibit apoptosis. Here, we found that P27$^{/kip1}$ expression increased in MSCs transplanted into the infarcted myocardium by using immunohistochemistry methods. Simultaneously, we confirmed that intracellular ROS and P27 levels increased under hypoxic conditions. We used hydrogen peroxide (H_2O_2) to elucidate the effect of ROS on P27$^{/kip1}$ expression. The results indicated that H_2O_2 treatment caused elevated P27$^{/kip1}$ levels in untransfected MSCs, but not in MSCs transfected with miR-

152(MSC$^{mir-152}$). In MSC$^{mir-152}$, expression of cell cycle-related proteins including cyclinD1, P-pRb and PCNA increased while expression of pro-apoptotic proteins such as caspase-3 and Bax diminished. Additionally, using an acute ischemia-reperfusion rat model, we found that transplantation of MSC$^{mir-152}$ markedly enhanced cardiac function compared to transplantation of MSC. Taken together, miR-152 inhibits cell cycle arrest and apoptosis of transplanted MSCs by targeting P27^{kip1} and subsequently enhances cardiac functions.

T-3123
MESENCHYMAL STEM CELLS PRIMED WITH ENDOTHELIAL BASAL MEDIUM REDUCE ARTERIAL BLOOD PRESSURE IN A RAT MODEL OF SYSTEMIC ARTERIAL HYPERTENSION

Oliveira, Lucas Felipe, Almeida, Thalles Ramos, Ribeiro Machado, Marcus Paulo, Cuba, Marília Beatriz, **Dias da Silva, Valdo Jose**
Dept. of Physiology, Triangulo Mineiro Federal University, Uberaba, Brazil

Previous data from our laboratory have demonstrated that syngenic transplantation of bone marrow mononuclear or mesenchymal stem cells (MSC) into spontaneously hypertensive rats (SHR) has reduced arterial blood pressure (ABP) around 15-25 mmHg for two consecutive weeks. The effect of these stem cell therapies seems to be related to endothelial dysfunction improvement. The central aim of the present study was to evaluate whether priming of MSC for 72 hours with growth factors present in endothelium basal medium (EBM-2) was able to increase their therapeutic effects on spontaneous arterial hypertension in SHR. Adult female SHR (200 - 230g) were treated with vehicle solution (n = 6), MSC cultured on conventional medium consisting of DMEM plus 10% FBS (n = 11) or MSC cultured on conventional medium followed by 72 hours in EBM-2 medium (primed MSC, n = 10). The animals of both MSC groups have received an intra-peritoneal injection (i.p.) of 5 x 10^6 MSCs (expanded until 5th passage, primed or not) while control group have received physiological saline, all in a volume of 1mL. Systolic arterial pressure (SAP) was monitored by means of tail occlusion method 5 days before and during 10 days after treatment. Following, for direct recordings of hemodynamic parameters, all animals were anesthetized with tribromoethanol (250 mg/kg, i.p.) and had their right femoral artery catheterized. After 24-48 hours of surgical recovering, ABP were directly recorded during 60 minutes, and at the end, after a new anesthesia session (sodium thiopental, 40 mg/Kg, i.p.), a catheter was inserted into left common carotid artery for injection of doses of acetylcholine (3-25ng/Kg) or sodium nitroprusside (0,5-4µg/Kg), in order to indirectly evaluate systemic endothelial function by means of depressor responses to endothelial-dependent and endothelial-independent vasodilators, respectively. At the end of the experimental protocol, heart weight was measured. Indirect measurements of SAP have shown long lasting reduction (10 days) of tensional levels (around 10 - 15 mmHg), after i.p. administration of MSC cultured for 72 hours in EBM-2 medium. In addition, MSC cultured in EBM-2 transplantation have also provoked a decrease in direct measurement of mean arterial pressure compared to vehicle or MSC cultured in DMEM (147±14mmHg vs 165±12mmHg and 161±7mmHg, respectively, $p<0.05$), although the cardiac hypertrophy was not significantly reduced. The endothelial function tests showed an improvement in vasodilation response in animals treated with MSC primed with EBM-2 medium compared to vehicle or MSC cultured in DMEM injections (19.0±7.0% vs 12.6±2.4% and 12.1±5.0% for Ach 3.125ng/Kg, $p<0.05$; 22.1±5.3% vs 16.0±2.6% and 13.6±4.9% for Ach 6.25ng/Kg, $p<0.05$; 26.6±5.4% vs 19.4±3.5% and 20.1±3.9% for Ach 12.5ng/Kg, $p<0.05$; 29.6±6.0% vs 27.6±5.3% and 24.5±2.3% for Ach 25ng/Kg). Taking all together, our findings seem to indicate that priming MSCs with endothelial basal medium boosts stem cell therapy to treat systemic arterial hypertension. This functional improvement of MSC could be relevant and desirable in a context of autologous transplantation since previous data from our laboratory have indicated that mesenchymal stem cells harvested from SHR donors present some biological dysfunctions.

T-3124
MESENCHYMAL STEM CELLS INDUCE NON-CLASSICAL IL-10 PRODUCING TH17 CELLS IN ARTHRITIS EFFECT OF GILZ

Luz-Crawford, Patricia Alejandra[1], Tejedor, Gautier[1], Mausset-Bonnefont, Anne-Laure[1], Beaulieu, Elaine[2], Morand, Eric[2], Jorgensen, Christian[1], Noel, Daniele[1], Djouad, Farida[1]
[1]INSERM U844, Montpellier, France, [2]Monash University, Melbourne, Australia

Regulatory T (Treg) cells have a critical inhibitory effect on inflammation in rheumatoid arthritis (RA) and are a potential target for therapies that suppress inflammation in an antigen-specific manner. Induction of Treg cells by mesenchymal stem cells (MSC) is proposed as one mechanism through which they exert immunosuppressive effects. The aim of this study was to evaluate the molecular mechanisms involved in the therapeutic effects of MSC in a murine model of RA to better understand how the immune balance between Th1/Th17 and Treg cells is regulated. We focused our study on the role of Gilz, which was recently described to act as an inhibitor of inflammatory responses in RA. MSC deficient for Gilz (Gilz-/- MSC) were isolated from the bone marrow of knockout mice and their therapeutic effects was compared to MSCs isolated from control mice (WT MSC), in a collagen-induced arthritis (CIA) murine model. The mechanisms of in vivo suppressive effects on effector T cell proliferation and the generation of regulatory T cells were also investigated. Analysis of clinical and immunological parameters revealed that Gilz expression is crucial for the ability of MSC to control the progression of experimental arthritis. Moreover, we showed that while treatment with WT MSC reduced the frequency of Th1 and Th17 cells in the draining lymph nodes during arthritis development, Gilz-/- MSC did not. The reduction of Th1 and Th17 frequency in response to MSC treatment was associated with the generation of IL-10-producing regulatory Th17 cells, an effect absent in Gilz-/- MSC-treated mice. Our results demonstrate the requirement for Gilz in the therapeutic effect of MSC in a model of RA, and that MSC induction of functional regulatory T cells bearing the CD4+RORγT+IL17LowIL10+ signature is also Gilz-dependent.

T-3125
THE ROLE OF CELL DELIVERY ROUTE IN CELL THERAPY FOR LIVER CIRRHOSIS

Bica, Rafael[1], Torres, André[1], Paula, Tatiana Pereira de[1], Souza, Sérgio[2], Dias, Juliana Vieira[3], Célia, Resende[2], Gutfilen, Bianca[2], Coelho, Henrique Sérgio Moraes[1], Campos de Carvalho, Antonio C.[3], Fonseca, Léa Mirian Barbosa[1], Goldenberg, Regina[3], **Rezende, Guilherme Ferreira da Motta**[1]
[1]Internal Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [2]Radiology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [3]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Introduction: Preclinical and clinical studies have suggested that therapy with bone marrow mononuclear cells (BMMC) may be useful in liver cirrhosis. However, the ideal route of stem cell delivery has not been established. Aim: To compare feasibility and safety of the use of peripheral vs. central vessels as infusion routes for BMMC in cirrhotic patients. Methods: BMMCs were isolated from autologous bone marrow (BM). Ten percent of cells were labeled with 99mTc-

SnCl2 and all were injected via the cephalic vein. Hepatic retention was measured by whole body scintigraphy (WBS), performed 3h and 24h after infusion. Liver function tests and abdominal ultrasound with color Doppler fluxometry (DUS) were regularly performed for 1 year. Results were compared to our previous study, where the same methodology was applied but BMMC were delivered by hepatic artery infusion (HAI). Results: Peripheral vein infusion (PVI) yielded a smaller liver retention of cells than HAI (15% after 3h and 10% after 24h vs. 41% and 32%, respectively). Distinctly from the previous trial, no improvement in liver function was observed based on MELD and Child-Pugh scores. Although HAI of BMMC had previously shown a significant reduction in hepatic artery resistance to blood flow, impedance indexes remained stable in this trial. After PVI, few and nonsevere adverse events were noted, as opposed to the relatively high incidence after HAI. Conclusions: PVI as a delivery route seems to be safer, less expensive and technically easier than HAI. However, it may be less effective in terms of cell engraftment in the target organ and of the resulting functional improvement. Phase II trials comparing distinct routes for cell delivery need to be conducted to clarify this point.

T-3126
THERAPEUTIC POTENTIAL OF AMNIOTIC FLUID-DERIVED STEM CELLS ON LIVER FIBROSIS MODEL IN MICE

Shaw, S.W. Steven[1], Peng, Shao-Yu[2], Cheng, Po-Jen[1], Cheng, Winston TK[3], Wu, Shinn-Chih[2]
[1]Obstetrics and Gynecology, Chang Gung Memorial Hospital, Taoyuan, Taiwan, [2]Institute of Biotechnology, National Taiwan University, Taipei, Taiwan, [3]Department of Animal Science and Technology, National Taiwan University, Taipei, Taiwan

Liver fibrosis results from the wound healing response to chronic liver damage. Advanced liver fibrosis results in cirrhosis and liver failure, and liver transplantation is often the only option for effective therapy; however, the shortage of available donor livers limits this treatment. Thus, new therapies for advanced liver fibrosis are essential. Amniotic fluid contains an abundance of stem cells, which are derived from all three germ layers of the developing fetus. These cells do not induce teratomas in vivo and do not pose any ethical concerns. To generate liver fibrosis models, male ICR mice were treated with CCl4 via oral gavage for 4 weeks, and the serum levels of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and albumin (ALB) were higher than the control group following chemical induction. To assess the potential of amniotic fluid-derived stem cells (mAFSCs) to ameliorate liver fibrosis in vivo, mAFSCs were isolated from amniotic fluid of 13.5-day transgenic mice, which globally express the fluorescent protein, EGFP, for tracing purposes (EGFP-mAFSCs). Single cells were injected via the mesentery (1x106 cells/mouse) of transplanted mice with liver fibrosis. Four weeks after EGFP-mAFSC transplantation, the serum GOT, GPT, and ALB levels of recipient mice in the EGFP-mAFSC-injected group was significantly decreased when compared with mice in the saline-injected group. Additionally, fibrotic tissues were evaluated using Masson's trichrome staining 4 weeks after cell transplantation. Shrinkage of the fibrotic area was observed in the EGFP-mAFSC-injected group. The tissue-repairing effects were also confirmed by hydroxyproline content analysis. The possible repair mechanism from our data revealed that EGFP-mAFSCs may fuse with the recipient liver cells. Overall, EGFP-mAFSCs can ameliorate liver fibrosis in mice, thus providing insight into the future development of regenerative medicine.

T-3127
CHEMICAL ENRICHMENT OF SOMATIC TISSUE STROMAL CD34+ MSCS FOR REGENERATIVE MEDICINE

Shih, Daniel Tzu-Bi[1], Hwang, Shiaw-Min[2], **Tsai, Ming-Song**[3]
[1]Taipei Medical University, Taipei, Taiwan, [2]Bioresource Collection and Research Center, Hsinchu, Taiwan, [3]OBS and GYN, Cathay General Hospital, Taipei, Taiwan

Evidences have shown that microenvironment may lead to changes the stem/progenitor cell phenotype and fate. By Chemical defined culture condition, a rare population of CD34+ Mesenchymal type of human tissue stromal stem cells (C34+ MSCs) with embryo stem cell gene expression has been enriched and stably expanded *in vitro*. This type of CD34+ MSCs can be isolated from neonatal placenta as well the adult gingival and endometic tissues. These cells possess similar tissue differentiation potentials, as ESCs and iPCs do. A comparative c-DNA micro array analysis of their tissue specificity and function has been conducted using human ESCs as the control. The overall characteristics of these CD34+ MSCs potential studied were shown as an ideal alterative of ESC and iPCs for regenerative medicine. A hypothetic molecular mechanism of CD34+ MSC function will be proposed.

T-3128
OVERCOMING BIOMATERIAL DRAWBACKS IN TREATMENT OF LARGE CRANIOFACIAL BONE DEFECTS WITH MESENCHYMAL STEM CELLS

Adamzyk, Carina[1], Michael, Woeltje[2], Rheinnecker, Michael[2], Boebel, Melanie[2], Jahnen-Dechent, Wilhelm[3], Tolba, Rene[4], Lethaus, Bernd[5], Neuss, Sabine[1]
[1]Pathology, RWTH Aachen University, Aachen, Germany, [2]Spintec Engineering, Aachen, Germany, [3]Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany, [4]Institute for Laboratory Animal Research, RWTH Aachen University, Aachen, Germany, [5]Cranio-Maxillofacial Surgery, RWTH Aachen University, Aachen, Germany

So far, there is no scaffold biomaterial available that incorporates all essential needs for large bone defects in craniofacial surgery, such as mechanical stability, osseointegration, permeability for radiation, and degradability. Our study investigates the in vitro and in vivo performance of biomaterials (PEKK, PDLLA), which are already used for craniofacial surgery because they offer essential advantages. In addition, we included investigation of a novel silk type from Bombyx mori, modified with hydroxyapatite (HA) as a potential new bone graft. We aimed to overcome drawbacks, e.g. lacking osseointegration, of the biomaterials by covering them with autologous mesenchymal stem cells (MSC). The cell seeded biomaterials were investigated in vitro for cytocompatibility and are currently tested in a sheep model for critical-size cranial defects. Therefore, all in vitro experiments were performed with human and sheep MSC, in parallel. All biomaterials were non-cytotoxic. Differences in the initial number of adherent MSC were observed, revealing lower cell numbers on PEKK and PDLLA compared to silk. The best results for osteogenic differentiation were achieved following a three week culture on PEKK with addition of osteogenic induction medium. According to the in vitro results, PEKK assisted both MSC viability and osteogenic differentiation. This result supports our theory that MSC seeding might improve insufficient osseointegration of PEKK. Silk also revealed a suitable cytocompatibility and supported differentiation. Therefore, our MSC seeded silk constructs might be suitable candidates for treating bone defects in craniofacial surgery.

T-3129

MSCS ISOLATED FROM MENSTRUAL BLOOD SHOW AN UNMATCHED ANTI-TUMORAL EFFECT ON A HUMAN PANCREATIC CARCINOMA CELL LINE

Alcayaga-Miranda, Francisca[1], Fernandez, Ainoa[2], Rosati, Antonella[3], Martin, Aldo[4], Khoury, Maroun[1]
[1]Faculty of Medicine, Universidad de Los Andes, Santiago, Chile, [2]Universidad de Andres Bello, Santiago, Chile, [3]Universidad de Viña del Mar, Viña del Mar, Chile, [4]Cells for Cells-REGENERO, Santiago, Chile

Introduction Mesenchymal stem cells (MSC) therapeutic effect on cancer cells remains controversial due to discrepancies in results. This controversy is thought to be due to the fact that tumor growth can both be stimulated and inhibited by various MSC secreted factors. Menstrual derived stem cells (MenSC) are a recently identified stem cell population, isolated from the menstrual fluids. We have showed that MenSC possess weaker immunomodulatory properties when compared to bone marrow MSCs (BM-MSC). While the lack of important immunosuppressive properties put the MensCs in a disadvantage for their therapeutical uses for inflammatory diseases, we investigate the direct effect of the lower immunosuppressive effect of MenSC on tumor growth. Methods: The tumor suppressive activity of MenSC in comparison to BM-MSC was tested in co-culture at different ratio with various cancer cell lines (PC3, A549, MiaPaca-2, SkMel28, MBMD231, and PANC-1) through cell proliferation , tumor sphere and colony formation (CFU) assays. The effect of MSCs on cancer cell migration was evaluated in a transwell assay. The expression levels of TRAIL and DKK3 were determined by Q-PCR and Elisa. Immunodeficient NSG mice were then injected with pancreatic human cancer cell line alone or co-injected with MSCs at different time points. Tumor growth was then monitored and samples were collected, weighed and analyzed for histology and IHC. Results: The anti-tumoral effect of MSCs was variable and depended largely on the cell ratio and type of cell line tested. The most significant effect was observed on MiaPaca-2, a human pancreatic carcinoma, at a ratio of 2:1 (MiaPaca-2:MSCs). A 60% inhibition of the cancer cell CFU was observed in the presence of MenSCs , in comparison to a 40% inhibition when BM-MSCs were used. A significant reduction of the proliferation and the size of the tumor spheres was also noted. While the BM-MSCs conditioned media (CM) enhanced the migration of the cancer cells relative to a control media, MenSC-CM showed the opposite effect. Furthermore, the basal expression levels of TRAIL and DKK3 were 3 and 4-folds higher in MensCs. When MiaPaca-2 and MSCs were co-injected in NSG mice, a statistically significant delay of the incidence of the tumor apparition and of the tumor growth was observed only in MenSCs injected groups. In addition, when MenSCs were injected intratumorally at 10 days post-tumor engraftment a 6-fold decrease of the tumor growth was noted. Conclusion: We have demonstrated an anti-tumoral effect of MenSCs on a pancreatic tumor cell line that was unmatched with the effect of BM-MSCs. Understanding the differential anti-tumoral effect of distinct sources of MSCs will greatly contribute in clearing the confusion surrounding the use of MSCs in cancer, and unraveling the mechanism behind it, will advance to new approaches for anti-cancer treatments.

T-3130

INJECTABLE STEM CELLS FOR THE INJURED ELITE ATHLETE. WHERE ARE WE AT?

Anderson, John, Tucker, Brad, Christopher, Dodson, Marchetto, Paul, Frederick, Rob, Freedman, Kevin, Ciccotti, Michael G.
Rothman Institute, Philadelphia, PA, USA

Musculoskeletal injuries commonly occur in the elite athlete. Many forms of treatment exist, but there has been a recent surge in interest by patients and physicians alike in the use of stem cell injections to delay or prevent surgery. However, the evidence for such treatment is currently not well known, nor are specific FDA guidelines for stem cell administration. The role of injectable stem cells for the treatment of musculoskeletal injury in elite athletes was reviewed. We also reviewed current FDA guidelines for stem cell administration. We searched for recent and ongoing clinical studies to determine outcomes of using injectable stem cells for musculoskeletal injury in the elite athlete. The FDA website was visited to determine guidelines for injectable stem cells. A PubMed search was then undertaken. The key phrases "stem cells athlete", "stem cells football" and "stem cells sports" were used. A search on current clinical trials in progress was then performed on the clinicaltrials.gov website and then on the Google search engine, using the same search terms. FDA Guidelines were outlined. Injectable stem cells for musculoskeletal injury in the elite athlete have not been well reported in the PubMed literature, nor on the clinicaltrials.gov website. A search of Google found various stem cell centers in different areas offering stem cell injections harvested from various sources. (Table 1). The use of injectable stem cells for the treatment of musculoskeletal injury in the elite athlete is an extremely topical subject, but not well reported in the scientific literature. Randomized controlled trials, performed within FDA guidelines, are required to further assess this treatment. The safety and well-being of the athlete must remain the most important goal of any medical management.

T-3131

UNDERSTANDING THE PERICYTE - MESENCHYMAL STROMAL CELL RELATIONSHIP: A COMPARISON OF HUMAN VASCULAR PERICYTE AND UMBILICAL CORD MATRIX MESENCHYMAL STROMAL CELL APPLICATION FOR TRAUMATIC SPINAL CORD INJURY

Badner, Anna[1], Vawda, Reaz[2], Mikhail, Mirriam[3], Laliberte, Alex[1], Fehlings, Michael G.[4]
[1]Institute of Medical Science, University of Toronto, Toronto, ON, Canada, [2]University Health Network, Toronto, ON, Canada, [3]University of Toronto, Toronto, ON, Canada, [4]Toronto Western Hospital, Toronto, ON, Canada

There is no regenerative therapy for spinal cord injury (SCI) and the damage is often permanent. Although current treatments are aimed at reducing injury progression, the limited self-repair of damaged nervous tissue highlights the need for alternative approaches. Cellular therapies are a potentially attractive option for central nervous system repair. As mesenchymal stromal cells (MSCs) have a pericytic association and have been shown to retain some pericyte function, they can potentially promote post-injury vascular repair and restoration of the blood spinal cord barrier (BSCB). Consequently, MSCs isolated from the human umbilical cord's gelatinous matrix, known as Human Umbilical Cord Matrix Cells (HUCMCs), may be an effective cell therapy option for addressing the primary insult of SCI. Systemic infusion of HUCMCs and a brain-derived pericyte control (HBVPs) was completed 1hour post-SCI (35g, C7) in Wistar rats. Cell localization and multi-organ distribution was determined via human sequence specific PCR (n=3). Evans Blue (EB) and Drabkin's spectrophotometric assays, as well as ultrasound imaging were used to evaluate vascular disruption in the acute injury. BBB, inclined plane, grip strength and Catwalk were applied to test animal functional recovery after treatment. 3D ultrasound imaging was also used to measure the chronic cavity size and extent of lesional tissue at 10 weeks post-SCI. PCR analysis showed that a majority of human DNA (cells) were located in the lung (0.305% human DNA/rat DNA), liver (0.115% human DNA/rat DNA), kidneys (0.101% human DNA/rat DNA) and 5mm segment of the spinal cord

lesion (0.085% human DNA/rat DNA) 24hours following infusion. The heart (0.002%) and spleen (0.006%) only showed traces of human DNA. At 24 and 72hours post-SCI, the HBVP and HUCMC-infused animals (n=5) had substantially less vascular permeability and hemorrhage than HE (HBSS + 2mM EDTA) (n=5) and fibroblast-infused (n=5) controls. Cell treated animals also had improved kidney function, as measured by reduced proteinuria, hematuria and Evans Blue vascular permeability ($p<0.05$). However, only HBVP treated animals showed a significant improvement in motor function recovery (BBB and grip strength) and reduced lesional tissue (via ultrasound). Overall, these results illustrate a novel application of MSCs for SCI and suggest that there is an underlying similarity between HUCMCs and HBVPs (based on acute outcome measures). It is also of interest to explore the varying effects of these cells in the chronic phase of injury, which may be a result of tissue source differences (fetal brain vasculature vs. neonatal umbilical cord matrix).

T-3132
MESENCHYMAL STROMAL CELLS PRIMING WITH AUTOCRINE MOTILITY FACTOR INCREASE THEIR RECRUITMENT TOWARDS HEPATOCELLULAR CARCINOMA

Bayo Fina, Juan Miguel[1], Fiore, Esteban Juan[1], Piccioni, Flavia[1], Bolontrade, Marcela F.[2], Sganga, Leonardo[3], Malvicini, Mariana[1], Peixoto, Estanislao[1], Rizzo, Manglio Miguel[1], Alaniz, Laura[1], Aquino, Jorge B.[4], Andriani, Oscar[1], Podhajcer, Osvaldo[1], Garcia, Mariana[5], Mazzolini, Guillermo[1]

[1]Gene Therapy Lab, Universidad Austral, Pilar, Argentina, [2]Fundacion Instituto Leloir, Buenos Aires, Argentina, [3]Fundación Instituto Leloir, Buenos Aires City, Argentina, [4]School of Medicine, Austral University, Derqui (Pilar), Buenos Aires, Argentina, [5]Austral University, Pilar, Argentina

Hepatocellular carcinoma (HCC) is the 3rd cause of cancer-related death worldwide. Unifortunately, its incidence and mortality are increasing steadily. Curative therapies can only be applied to a minority of patients. Particularly, several proinflammatory cytokines, chemokines and growth factors produced by tumor stroma have the ability to recruit the mesenchymal stromal cells (MSCs). However, the mechanisms involved in human MSC migration and anchorage to HCC are not fully elucidated. Moreover, MSCs were used as carriers of antitumoral genes towards HCC exploiting their capability to home into HCC; however enhancement of their recruitment into tumor is needed. Autocrine Motility Factor (AMF), a cytokine released by HCC cells, has been previously described to stimulate tumor cell motility, metalloproteinase (MMP) secretion, and enhancement of integrin β1 activity. Particularly, the AMF/AMFR pathway could be regulated by several proteins including Caveolin-1, Caveolin-2 and GDI-2. The aim of this study was analyze the role of AMF in MSC migration towards HCC. For that purpose, in vitro migration was studied by modified Boyden chamber, observing that MSCs not only migrated to recombinant AMF (rAMF) but also AMF blockage with an specific antibody reduced their migration toward conditioned medium (CM) derived from HCC tumors developed in nude mice (HuH7 y HC-PT-5). Similarly, zymography assays showed that MMP-2 activity was induced by the stimulation of MSCs with rAMF or HuH7-CM. Moreover, the specific blockage of AMF in HuH7-CM reduced the MMP-2 activity increase. Finally, MSCs were primed with rAMF (MSC-rAMF) and their migration capability was compared with unstimulated MSC. MSC-rAMF showed increased in vitro migration towards CM derived from both HCC tumors, and increased adhesion to endothelial cells. MSC-rAMF also had an induction in the mRNA levels of AMF receptor, MMP-3, caveolin-1, caveolin-2 and the inhibition of GDI-2. Western blot showed an increased level of AMFR, JNK, p-JNK, c-Fos, p-c-Fos and p-CREB in MSC-rAMF. In order to evaluate MSC in vivo migration, HCC cell line HuH7 was subcutaneously inoculated in nude mice and 10 days later CMDiI-DiR-labeled MSCs were intravenously injected. Three days later mice were sacrificed, tumor, liver, spleen and lung dissected and analyzed by Xenogen In Vivo Imaging System. In vivo migration showed that tumors from animals with MSC-rAMF have an increased signal in comparison with mice injected with unstimulated MSCs. Similar signal was observed in liver, lung and spleen from both groups. Moreover, the presence of CM-DiI (+) MSC in tumors was confirmed by fluorescent microscopy. Our results demonstrate that AMF plays a critical role in MSC recruitment to HCC, and itspriming with rAMF enhances MSC migration to hepatocellular carcinoma, becoming a promising strategy to improve their therapeutic efficacy.

T-3133
TRANSFECTED MESENCHYMAL CELLS AS A PROPHYLACTIC BIOMEDICAL COUNTERMEASURE TO VENEZUELAN EQUINE ENCEPHALITIS VIRUS EXPOSURE

Braid, Lorena Ruth[1], Hu, Weigang[1], Davies, John E.[2], Nagata, Les[1]

[1]BioThreat Defence Section, Defence R and D Canada, Suffield Research Centre, Medicine Hat, AB, Canada, [2]University of Toronto/Tissue Regeneration Therapeutics, Toronto, ON, Canada

Direct administration of neutralizing antibodies is an effective, but short-lived, approach for immediate protection against a biological threat. This study is the first demonstration of extended, single-dose passive immunity using mesenchymal stromal cell (MSC)-mediated gene therapy. Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne pathogen affecting humans and equines. VEEV is readily aerosolized, easily propagated, and classified as a potential biological weapon. In humans VEEV causes a pathologic spectrum including acute neurological encephalitis, which may culminate in death. No licensed vaccine or antiviral currently exists to combat VEEV infection in humans. We previously developed a humanized, VEEV-neutralizing antibody (anti-VEEV) that fully protects mice when administered 24 hours before, or after, exposure to a lethal dose of VEEV. To expand the protective window of this antibody, we examined whether human MSCs, engineered with a transgene encoding the experimental VEEV-neutralizing antibody, could provide a renewable source of antibody protection *in vivo*. Human umbilical cord perivascular cells (HUCPVCs) were used as the MSC source. The antibody gene was cloned into an adenovirus vector to facilitate high-efficiency gene transfer to HUCPVCs. Modified HUCPVCs expressing the anti-VEEV antibody transgene were phenotypically unaltered. Functional ELISAs, using live VEEV antigen, were used to generate *in vitro* and serological profiles of anti-VEEV expression. *In vitro*, modified HUCPVCs secreted clinically relevant quantities of anti-VEEV antibody. Within 24 hours, mice receiving an intra-muscular dose of 2.5 million modified HUCPVCs exceeded serum titers of mice receiving a protective dose of purified anti-VEEV antibody. The anti-VEEV antibody has a half-life of 3.7 days in mice, limiting protection to 2 or 3 days after administration. In contrast, modified HUCPVCs generated protective anti-VEEV serum titers in mice for 21 to 38 days. A substantial number of HUCPVCs persisted post-transplant in 40% of mice, producing peak serum titers at days 7 or 10, when the purified antibody was depleted, and extended putative protective titers until at least day 21. At 109 days post-transplant, 10% of mice still had circulating anti-VEEV antibody. Indeed, *in vivo* optical imaging confirmed survival of bioluminescent HUCPVCs at least 123 days after intra-muscular administration. Strikingly, a single dose of HUCPVCs modified with the anti-VEEV antibody transgene conferred both rapid and prolonged immune protection against VEEV.

Pre-treating mice with modified HUCPVCs 24 hours or 10 days before infection protected against 20 times the lethal dose of VEEV, but such pre-treatment with the purified antibody was ineffective. This proof-of-concept study identifies the HUCPVC gene therapy system as a novel, improved technology for immediate and sustained passive immunity against a known bio-threat. Given the success of this pilot study, we expect that HUCPVC-mediated gene therapy will provide a broad-spectrum solution for stealth delivery of therapies for a range of applications, including delivery of additional medical countermeasures for bio-defence purposes.

T-3134
COUPLING MESENCHYMAL STEM CELLS AND PULSED FOCUSED ULTRASOUND BETTER PREVENTS ACUTE KIDNEY INJURY AND RESCUES FUNCTION DURING ONGOING RENAL INSUFFICIENCY

Burks, Scott R.[1], Nguyen, Ben A.[1], Tebebi, Pamela[2], Kim, Saejeong[1], Street, Jonathan[1], Yuen, Peter[1], Star, Robert[1], Frank, Joseph[1]
[1]National Institutes of Health, Bethesda, MD, USA, [2]Catholic University of America, Washington, DC, USA

Objective: Pulsed focused ultrasound (pFUS) can noninvasively enhance homing of i.v. infused mesenchymal stem cells (MSC) to healthy pFUS-treated kidneys. This study examined pFUS as a tool to enhance homing to murine kidneys during cisplatinum (CIS)-induced acute kidney injury (AKI). Experimental MSC models and clinical MSC trials administer cells early in disease and attempt to prevent subsequent renal failure. However, most clinical AKI requires treatment as an established disease--an arena where therapeutic development has lagged. Thus, dialysis is the only clinical option and mortality rates remain high. Therefore, we investigated pFUS-enhance MSC homing to kidneys during early AKI (to further improve current MSC approaches) and we also investigated delayed administration of pFUS and MSC until injury was clinically obvious and attempted to rescue renal function. METHODS: C3H mice received CIS i.p. (15 mg/kg) and pFUS/MSC as described previously (Ziadloo et al. Stem Cells 30:1216). Five groups of mice were used: AKI, AKI+pFUS, AKI+MSC, AKI+pFUS+MSC, and healthy controls. For early treatment (like previous studies), CIS was given on Day 0 with pFUS/MSC on Day 1. Disease developed until Day 4 when renal function (blood urea nitrogen [BUN] and serum creatinine [SC]), morphology, apoptosis, and Ki67 expression were measured. To treat established renal failure, mice received CIS on Day 0 and MSC/pFUS on Day 3. Renal function was measured on Days 4 and 6, and survival was recorded through Day 7. ANOVAs were used for multiple comparisons and log-rank tests compared survival data. Results: pFUS treatment at Days 1 (early) or 4 (late) post-CIS enhanced homing of i.v.-injected MSC by 2-3 fold at either day. To examine improvements over previous MSC therapies for AKI, MSC and/or pFUS were given 1 day post-CIS. MSC alone provided modest protection against AKI (improved BUN and tubular necrosis). While pFUS alone had no effects on AKI, enhanced MSC homing through pFUS provided additional protection against injury. pFUS+MSC further improved BUN and SCr clearance, as well as tubular necrosis/apoptosis and Ki67 expression. To examine the more clinically-relevant scenario of intervention during established renal failure, pFUS/MSC treatment was delayed until Day 3 post-CIS. Renal function was measured at Days 4 (24h post-treatment) and 6 (72h post-treatment) and only MSC with pFUS improved BUN clearance. MSC alone (no pFUS) significantly improved survival through Day 7, but pFUS-enhanced MSC homing yielded additional significant increases in survival. Conclusions: 1) These pFUS exposures have previously been shown to be benign in kidney tissue and they alter the AKI microenvironment to create a "molecular zip code" where MSC preferentially home. 2) Enhanced MSC homing during early AKI further improves MSC protection against AKI and may be the preferred therapeutic approach when AKI is foreseeable (e.g. surgery or chemotherapy). 3) MSC alone improve survival when administered during ongoing renal failure and even greater survival rates are achieved with enhanced MSC homing through pFUS. Therapies have largely failed for AKI that is unforeseeable (e.g. from trauma, sepsis, inadvertent toxicity) and requires treatment after renal function declines. Therefore, MSC with pFUS represent a novel and lone potential therapeutic options to treat established AKI.

T-3135
NEUROPROTECTIVE EFFECT OF MICROVESICLES DERIVED FROM HUMAN MESENCHYMAL STEM CELLS TREATED WITH BRAIN EXTRACT IN A RAT STROKE MODEL

Choi, Seong-Mi, Kim, Han-Soo
Innovative Cell and Gene Therapy Center, International St. Mary's Hospital, Incheon, Republic of Korea

Transplantation of human mesenchymal stromal/stem cells (hMSCs) has been shown to improve functional outcome in rat model of ischemic stroke and paracrine action of hMSCs is primarily responsible for the beneficial effects. Subsequent studies suggested that microvesicles/exosomes of hMSCs can replace the beneficial effects of hMSCs in these models. This study focused on the contribution of microvesicular components derived from hMSCs pre-treated with normal or ischemic brain extract to therapeutic benefits in rats with permanent middle cerebral artery occlusion (pMCAo). For generation of microvesicles (MVs) from hMSCs, MV-free, cell-free fraction of normal and ischemic brain extracts were collected and added to the culture of hMSCs. After 24hr of culture, cell-free MVs were purified by a combination of ultra-filtration, sucrose gradient and Optiprep density gradient centrifugation were analyzed by 1-D SDS PAGE, nano-LC-MS/MS and proteomic analysis were performed. To test the hypothesis *in vivo*, single intra-arterial injection of MVs was done into rats 48hr after pMCAo. Injection of MVs showed reduced the brain ischemia significantly with greater improved functional recovery than did control rats. Of 201 proteins identified, 93.0% of them were shared by MVs of hMSCs treated with normal brain extract (MV-NB) and MVs of hMSCs treated with stroke brain extract (MV-SB). These results indicate the intra-arterial administration of MVs from hMSCs exhibited a similar therapeutic effect as hMSCs at early stage of pMCAo and the feasible application of stem cell-based, noninvasive therapy for treating stroke. This study was supported by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIP) (No. 2012M3A9B4028639).

T-3136
MESENCHYMAL STEM CELL-BASED COMBINED CARBOXYLESTERASE AND TRAIL GENE THERAPY IN A MOUSE DIFFUSE PONTINE GLIOMA MODEL

Choi, Seung Ah[1], Lee, Young Eun[2], Lee, Ji Yeoun[1], kwack, Phil Ae[1], Kyeung Min, Joo[3], Phi, Ji Hoon[1], Moon, Yeon Joo[1], Wang, Kyu-Chang[1], Kim, Seung-Ki[1]
[1]Seoul National University Children's Hospital, Seoul, Republic of Korea, [2]Samsung Advanced Institute for Health Sciences and Technology (SAIHST) Sungkyunkwan University, Seoul, Republic of Korea, [3]Samsung Medical Centrr, Seoul, Republic of Korea

Human adipose-derived mesenchymal stem cells (hAT-MSCs) have been one of the most attractive vehicles for gene therapy because of their extensive migratory ability toward cancer and potential of the

autologous transplantation. We investigated the therapeutic efficacy and safety of carboxylesterase (CE) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) producing hAT-MSCs against diffuse pontine gliomas mouse model. We isolated hAT-MSCs from the axillary fat tissues and characterized of morphology, expression of surface markers and the ability of differentiation. We transfected hAT-MSCs with CE using lentiviral transduction and then transduced cells with TRAIL using nucleofector. The therapeutic potential of CE-TRAIL producing hAT-MSCs (hAT-MSCs.CE.TRAIL) was evaluated *in vitro* and *in vivo* in an orthotopic brainstem gliomas mouse model. hAT-MSCs were successfully isolated from axillary fat and satisfied the criteria of true MSCs. The hAT-MSCs.CE-TRAIL converted CPT-11 to SN38 and secreted TRAIL, inducing potent apoptotic activity *in vitro* and *in vivo.* Brainstem gliomas-bearing mouse treated with hAT-MSCs.CE-TRAIL and CPT-11 decreased the tumor volume and prolonged survival significantly compared to normal control, hAT-MSCs.CE treated, hAT-MSC.TRAIL treated group. Our study demonstrated that the synergistic effect and safety of hAT-MSC.CE.TRAIL, suggesting possibility of clinical application

T-3137
USE OF CONDITIONED MEDIUM FROM HUMAN AMNIOTIC MEMBRANE-DERIVED MESENCHYMAL STEM CELLS FOR THE TREATMENT OF ACUTE MYOCARDIAL INFARCTION

Ciuffreda, Maria Chiara[1], Malpasso, Giuseppe[1], Danieli, Patrizia[1], Borruso, Maria Giuliana[1], Copes, Francesco[1], Pisano, Federica[1], Mura, Manuela[1], Gnecchi, Massimiliano[2]
[1]Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, [2]University of Pavia, Pavia, Italy

Mesenchymal stem cells of fetal origin (hAMC) can be isolated from the amniotic membrane of human placenta. I*n vitro* (CM) from hAMC (hAMC-CM) exert remarkable cytoprotective and pro-angiogenic effects and stimulate the proliferation and migration of cardiac progenitor cells. Here, our goal was to study the functional and structural effects of intramyocardial injection of concentrated hAMC-CM using a well-established model of ischemia/reperfusion injury in rats. Ischemic injury was induced in Sprague Dawley rats by temporary ligation of the coronary artery. Three groups (n=20 animals for each group) were considered: control animals received saline injection and CM-treated animals received either hAMC-CM or CM from human dermal fibroblasts (fib-CM). Injections were performed at the infarct border zone. Cardiac function was assessed at baseline, 48 hrs and 30 days after surgery. hAMC determined a sustained improvement in cardiac function. In particular, hAMC-CM significantly limited LV fractional shortening (FS) impairment at 48 hrs compared with both saline (+36%; $p<0.05$) and fib-CM (+37%; $p<0.01$). Differences in FS persisted also at 30 days. hAMC-CM improved LV ejection fraction (EF) over time (+15% at 30 days *vs* 48 hrs; $p<0.05$). Functional data were matched by reduced infarct size (IS) in hAMC-CM-treated hearts (-28.5% vs saline, $p<0.01$; -28.3% vs fib-CM, $p<0.01$). Consistently, in the hearts treated with hAMC-CM, the number of TUNEL$^+$ cardiomyocytes (CMC) at the infarct border zone was reduced compared with both saline (-43.4%; $p<0.001$) and fib-CM (-39.4%; $p<0.001$). Morphometric analysis demonstrated that administration of hAMC-CM prevented the thinning of the anterior wall compared with both saline (+27%; $p<0.01$) and fib-CM (+30%; $p<0.01$) and also the scar area was smaller compared with controls. Consistently, Picrosirius Red staining demonstrated that hAMC-CM reduces collagen deposition compared with both saline (-37.2%; $p<0.05$) and fib-CM (-39.4%; $p<0.05$). Importantly, hAMC-CM also prevented LV dilatation (LV expansion index -40.8% *vs* saline and -43% *vs* fib-CM; both $p<0.05$). Furthermore, isolectin immunostaining showed that hAMC remarkably promotes neoangiogenesis: after 30 days, the number of capillaries at the infarct border zone was 100% ($p<0.001$) and 36% ($p<0.05$) higher in the hAMC-CM group compared with saline and fib-CM, respectively. Finally, our data show that hAMC may trigger endogenous cardiac regeneration. Indeed, only in the hearts treated with hAMC-CM we observed CMC positive for both Ki67 and BrdU. Furthermore, we detected Ki67$^+$ and BrdU$^+$ cells that co-stained positive for the cardiac protein α-sarcomeric actin and had high nucleus-to-cytoplasm ratios, which is typical of cardiac progenitor cells. hAMC-CM exerts powerful paracrine effects that positively affect several aspects involved in heart repair following myocardial infarction. In particular, a single intramyocardial injection of concentrated hAMC-CM leads to improvement in cardiac function by limiting infarct size and negative ventricular remodelling and by increasing angiogenesis and endogenous regeneration.

T-3138
THE SECRETOME OF RAT MESENCHYMAL STEM CELLS MAINTAINS FUNCTION IN A RAT MODEL OF STRESS URINARY INCONTINENCE

Damaser, Margot S.[1], Deng, Kangli[1], Lin, Dan Li[2], Hanzlicek, Brett[2], Balog, Brian M.[1], Penn, Marc S.[3], Kiedrowski, Matthew[3], Zhu, Hui[4]
[1]Department of Biomedical Engineering, The Cleveland Clinic, Cleveland, OH, USA, [2]Advanced Platform Technology Center of Excellence, Louis Stokes Cleveland VA Medical Center, Cleveland, OH, USA, [3]Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH, USA, [4]Surgery Service, Louis Stokes Cleveland VA Medical Center, Cleveland, OH, USA

Introduction and Objective: Current treatments for stress urinary incontinence (SUI), the most common form of urinary incontinence, either have poor long-term efficacy or have a high revision rate. Stem cells are currently in clinical trials for SUI, which is thought to result in part from the maternal injuries of childbirth; however, their mechanism of action remains unknown. A growing body of evidence suggests that stem cells may secrete factors that act locally or systemically to facilitate recovery. The objective of this study was to determine if intraperitoneal (ip) administration of the secretions of MSCs, as in concentrated conditioned media (CCM), could be as effective as intravenous (iv) administration of mesenchymal stem cells (MSCs) in a rat model of simulated childbirth injury that produces symptoms of SUI. Methods: Forty-four adult female Sprague-Dawley rats (250-300g) were randomly allocated into 3 groups: sham injured treated iv with saline (n=15), pudendal nerve crush (PNC) + vaginal distension (VD) as a simulated childbirth injury treated iv with saline (n=13), and PNC+VD treated iv with 2 million rat MSCs (n=16). An additional 54 female rats were allocated into 3 experiment groups: sham injured treated ip with concentrated control media (CM; n=18), PNC + VD treated ip with CM (n=19), and PNC+VD treated ip with CCM (n=17). Three weeks after injury, 5 rats in each group were euthanized for blinded qualitative histological and immunofluorescence assessment of the urethra. The remaining rats underwent leak point pressure (LPP) testing with simultaneous external urethral sphincter (EUS) electromyography (EMG), as well as pudendal nerve sensory branch potential (PNSBP) recording 3 weeks after injury. Results: LPP was significantly decreased 3 weeks after PNC+VD with saline or CM treatment compared to sham injured rats, but not with MSC or CCM treatment. EUS EMG showed no improvement with MSC or CCM treatment. PNSBP amplitude and firing rate were significantly decreased 3 weeks after PNC+VD treated with saline or CM compared to sham injured rats, but not with MSC or CCM treatment. After PNC+VD, elastic fibers near the EUS were disrupted and twisted.

In addition, their density increased and their orientation changed from circumferential to radial. A greater increase in elastic fibers was observed in animals treated with MSC or CCM, indicating that both MSC and CCM stimulate elastogenesis. Neuromuscular junctions in the EUS were diffuse and not well innervated after PNC+VD. Axons innervating the EUS had a torturous course and multiple collaterals with MSC or CCM, indicative of ongoing neuroregeneration. Three weeks after PNC+VD and treatment with saline or CM, nerve fascicles in the pudendal nerve sensory branch were less dense and axons had irregular shapes, indicating injury and the beginning of neuroregeneration. In contrast, the pudendal nerve demonstrated both normal and abnormal nerve fascicles with a greater number of normal fascicles with MSC or CCM treatment, indicating facilitated neuroregeneration or neuropreservation. Conclusions: Both MSC and CCM demonstrate protective effects compared with controls, likely due to elastogenesis and/or neuromuscular preservation. Therefore, independent of MSC, CCM has the potential to facilitate recovery from the maternal injuries of childbirth and prevent or ameliorate SUI via the MSC secretome in an endocrinological fashion.

T-3139

HIGH DOSES OF PORCINE ADIPOSE-TISSUE DERIVED MESENCHYMAL STEM CELLS INJECTED DIRECTLY INTO CARDIAC MUSCLE IMPROVES MYOCARDIAL PERFUSION OF LEFT VENTRICLE REDUCING SCAR AREAS AND ATTENUATING CARDIAC REMODELING IN PIGS TREATED WITH IACE AND BETA-BLOCKER

Dariolli, Rafael, Marques, Euclydes Fontegno, Takimura, Celso Kiyochi, Tsutsui, Jeane Mike, Naghetini, Marcus Vinicius, Zogbi, Camila, Mathias Jr, Wilson, Lemos Neto, Pedro Alves, Krieger, Jose Eduardo

Heart Institute (InCor), University of Sao Paulo, Sao Paulo, Brazil

Introduction: The beneficial effects associated with intramyocardial injection of adult stem cells in rodents have not been consistently reproduced in larger animals and humans. Aim: We evaluated the dose of porcine adipose-tissue derived mesenchymal stem cells (pASC) to increase cardiac tissue perfusion in pigs treated with ace-inhibitors and β-blockers to mimic human management post-MI. Methods: Animals were subjected to LCx occlusion and 4 weeks after MI blinded randomized in 4 groups to receive intramyocardial injection of pASC (1, 2 and 4x10^6 pASC/Kg bw) or placebo. Real time myocardial perfusion echocardiography (RTMPE) was conducted using commercial micro-bubbles before injection and 4 weeks after treatment with pASC. Anatomopathological assessments were performed to evaluated MI area, LV remodeling. Results: Eight weeks after MI, the pigs treated with the highest dose of pASC showed a significant increase of myocardial blood flow in both remote (3.9 times) and border zone (3.7 times) versus the other groups, which was also in agreement with the increase in vessel numbers (about 54 and 56%, respectively) compared to the other groups ($p>0.05$). Interestingly, the non-perfused area was reduced (up to 38%) and the thinning ratio was higher (25%) in the 4x10^6 pASC/Kg.bw group compared with placebo or the other cell groups. Conclusion: Altogether, we provide evidence that intramyocardial injection of pASC post-MI increased cardiac perfusion and vessel number with the highest dose, which may have contributed to attenuate the LV adverse remodeling 2 months after the MI.

T-3141

IMMUNOPRIVILEGED HUMAN PLURIPOTENT- AND TISSUE-DERIVED PERICYTES MODULATE ALLOIMMUNE RESPONSE BY INDUCTION OF SUPPRESSIVE REGULATORY T CELLS

Domev, Hagit, Milkov, Ira, Itskovitz-Eldor, Joseph, Dar, Ayelet

The Ruth and Bruce Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel

Despite growing interest in pericyte-based stem cell therapy, their immunogenicity is still poorly defined, in particular that of vasculogenic pericytes, generated from human pluripotent embryonic or induced stem cells (hPSC). We have previously shown that multipotent perivascular precursors develop during spontaneous differentiation of hPSC. Here we demonstrate that unstimulated hPSC-pericytes exhibited non-immunogenic phenotype similarly to tissue embedded and cultivated brain and placenta pericytes as well as BM and hPSC-mesenchymal stem cells, expressing MHC Class I but not MHC Class II or CD80/CD86. Pre-treatment with inflammatory mediators failed to induce antigen-presenting cell-like phenotype in stimulated pericytes. When co-cultured with naïve peripheral blood CD25-CD4+ T cells, pericytes did not stimulate activation and proliferation of allogeneic resting T cells and preferentially induced the formation of CD4+CD25highFoxP3+CD127-, functionally suppressive regulatory T cells. Constitutive expression of the inhibitory molecules PD-L1/2 and secretion of TGF-β by hPSC-pericytes directly regulated the generation of pericyte-iTregs. Human brain-, placenta- or PSC-pericytes co-transplanted with allogeneic CD25- T cells into immunodeficient NOD/SCID mice, maintained non-immunogenic phenotype and mediated the development of Tregs in vivo. Altogether these findings reveal a novel feature, shared by native tissue and hPSC-pericytes and support the notion that pericytes can be applied for allogeneic cell therapy.

T-3142

TSG-6 DOWN-REGULATES PROINFLAMMATORY CYTOKINE EXPRESSION IN SULFUR MUSTARD-INJURED KERATINOCYTES

Eaton, Erik[1], Nealley, Eric[1], Nipwoda, Theresa[1], Guignet, Michelle[1], Kaski, Shane[1], Varney, Timothy R.[2]

[1]United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, USA, [2]Unites States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, USA

Mesenchymal stem cells (MSCs) represent an injury repair cell type present in the bone marrow of adults. One aspect of the role that these cells play in wound healing is the secretion of anti-inflammatory proteins at sites of tissue damage. The MSC-secreted protein TNF-α Stimulated Gene 6 (TSG-6) is one example that has been shown to be effective in reducing inflammation in injuries to the lungs, eye, heart and gut. The ongoing *in vitro* studies described here are evaluating the effectiveness of TSG-6 in reducing inflammation in cultured keratinocytes and corneal epithelial cells after exposure to the chemical warfare agent sulfur mustard (HD). TSG-6 treatment results in a dose-dependent decrease in proinflammatory cytokine expression in human keratinocytes exposed to 75μM HD. At the highest treatment dose tested to date (500ng TSG-6/mL), TNF-α, IL-8 and IL-6 expression levels are reduced by 72.5%, 58.8% and 43.2%, respectively. Follow on studies will examine the effects of TSG-6 on cell growth and migration in the context of HD injury. This work will provide the basis for future studies to compare the anti-inflammatory activity of TSG-6 to the entire MSC secretome. The ultimate goal of this project is to characterize the

beneficial properties of proteins produced and secreted by tissue repair cells to support medical countermeasure development for use in the battlefield environment.

T-3143
MESENCHYMAL STROMAL CELLS ENGINEERED TO PRODUCE IGF-I AMELIORATE LIVER FIBROSIS WITH INDUCTION OF HEPATIC REGENERATION IN MICE

Fiore, Esteban Juan[1], **Bayo Fina, Juan Miguel[1],** Garcia, Mariana[1], Malvicini, Mariana[1], Lloyd, Rodrigo[1], Piccioni, Flavia[1], Rizzo, Manglio[1], Estanislao, Peixoto[1], Solá, Maria B.[1], Atorrasagasti, Catalina[1], Alaniz, Laura[1], Prieto, Jesús[2], Aquino, Jorge B.[1], Mazzolini, Guillermo D.[1]
[1]Gene Therapy Lab, Austral University, Pilar, Argentina, [2]University of Navarra, Pamplona, Spain

Liver cirrhosis involves chronic damage and wound healing processes. Mesenchymal stromal cells (MSCs) were previously shown to support tissue repair. Insulin Growth Factor like-I (IGF-I) is known to counteract fibrosis and to induce hepatocytes proliferation and survival. We aimed to evaluate the effects of applying MSCs engineered to produce IGF-I- in an experimental *in vivo* model of advanced liver fibrosis. Bone marrow MSCs from BALB/c mice were infected with an adenovirus codifying for IGF-I (AdIGF-I) or green fluorescence protein (AdGFP-MSCs). Fibrosis was induced in BALB/c mice by chronic administration of thioacetamide (TAA) during 8 weeks. On week 6, AdIGF-I-MSCs, AdGFP-MSCs or saline were intravenously administered in fibrotic animals which were sacrificed at 1, 3 or 14 days after treatment. The effect of a single cellular dose treatment was compared with that of repeated MSCs applications (3 doses) which were separated by 2 weeks in between. All animals were sacrificed at week 12. *In vitro* experiments were aimed to evaluate the effect of AdGFP-MSCs or AdIGF-I-MSCs supernatants on CFSC-2G hepatic stellate cells and mouse hepatocyte primary cultures. The application of AdIGF-I-MSCs resulted in a further amelioration of liver fibrosis when compared to AdGFP-MSCs, as shown by morphometric studies. Expression levels of pro-fibrogenic factors in liver samples and in hepatic stellate cells were consistent with AdIGF-I-MSCs exerting anti-fibrotic effects. In the liver, an induction in IGF-I and in hepatocyte growth factor (HGF) expression levels was observed at 1 day and a downregulation in TGF-beta1 and alpha-SMA at 3 days after MSCs application, with higher levels found in samples from AdIGF-I-treated animals. *In vitro* studies also showed a reduction in HSCs activation and an upregulation in IGF-I and HGF mRNA expression in hepatocytes after incubation with conditioned media from AdIGF-I-MSCs. Interestingly, an induction in PCNA mRNA and protein expression levels was found in the liver of AdIGF-I-treated animals at 1 day after transplantation suggesting the involvement of regenerative mechanisms. Finally, multiple doses of Ad-IGF-I-MSCs further reduced collagen deposition when compared to a single dose treatment.Our data support the experimental application of repeated doses of AdIGF-I-MSCs as therapeutic tools in the long-term treatment of liver fibrosis, and uncover early events likely involved in their anti-fibrotic effect.

T-3144
CYCLO-OXYGENASE OR TNF ALPHA INHIBITORS INTERFERES WITH THE MECHANOTRANSDUCTIVE EFFECTS OF PULSED FOCUSED ULTRASOUND BLUNTING THE ENHANCED HOMING OF MESENCHYMAL STEM CELL: IMPLICATION FOR REGENERATIVE MEDICINE

Frank, Joseph[1], Tebebi, Pamela[1], Kim, Sage[1], Nguyen, Ben[1], Burks, Scott R.[2]
[1]Frank Laboratory, Radiology and Imaging Sciences, National Institute of Health, Bethesda, MD, USA, [2]National Institute of Health, Bethesda, MD, USA

Recent studies have demonstrated that exposure to the mechanotransductive effects of pulsed focused ultrasound (pFUS) results in enhanced homing permeability and retention (EHPR) of stem cells to targeted tissues. pFUS targeted exposure to skeletal muscle (M) or kidney (K) results in micro-environmental changes associated with increased expression cyclo-oxygenase (COX2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) in tissues along with a cascade of cytokines, chemokines and trophic factors (CCTF) and cell adhesion molecules (CAM) initiating within 10 minutes and lasting up to 48 hours. pFUS induced a transient 4-6x increase in Tumor Necrosis Factor alpha (TNFa) within 10 minutes after exposure that returned to control levels of protein expression by 30 minutes. No evidence of heat shock protein -70 elevation was observed following pFUS indicating that thermal damage was not present in the tissues. Following TNFa increases there was increases in both pro-inflammatory and anti-inflammatory factors (e.g., Interleukins 1, IL2, IL6, IL10, IL13, IL15, IL12p40, MCP-1, RANTES, VEGF, M-CSF, MIP-2, PDGF and FGF, EGF, TGFb) and CAM expressed in pFUS treated M or K. Coupling pFUS with 106 IV of mesenchymal stem cells (MSC) after resulted in 5-8 times (ANOVA $p<0.01$) more of infused cells homing to M or K as compared to contralateral tissue. Coupling intravenous infusion of mesenchymal stem cells (MSC) either before or up to 16 hours after pFUS resulted in ~5 times (ANOVA $p<0.01$) more of infused cells homing to muscle as compared to contralateral limb. Pretreatment prior to pFUS with nonspecific COX inhibitor, ibuprofen (IB) or TNFa receptor binding protein, etanercept (ET), resulted in significant reduction in CCTF, COX2 and NFkB protein expression when compared results in pFUS only treated mice. Treatment with IB or ET prior to pFUS and IV MSCs resulted in significant (ANOVA $p<0.05$) reduction in homing to pFUS treated M or K similar to numbers of cells located in the contralateral tissue. pFUS exposure in COX2-/- knockout mice resulted in no differences in number of MSCs homing to treated tissue compared to contralateral control. pFUS induces a transient molecular zip code in treated tissue that can be used to target cell therapies and evaluate in vivo drug-host interactions through interference of pathways that elicit the transient molecular zip code and the impact on enhanced stem cell homing to tissue.

T-3145
COMBINING MESENCHYMAL STROMAL CELLS WITH HYALURONAN MAY AFFECT THE ANTI-INFLAMMATORY RESPONSE: IMPLICATIONS FOR CELL-BASED THERAPY OF OSTEOARTHRITIS.

Gomez-Aristizabal, Alejandro[1], Keating, Armand[2], Viswanathan, Sowmya[2]
[1]Arthritis Program, Toronto Western Hospital, Toronto, ON, Canada, [2]Cell Therapy Program, University Health Network, Toronto, ON, Canada

Osteoarthritis (OA) is a progressive degenerative joint disorder, in which chronic inflammation plays an important role. OA has a huge

health and economic impact, especially for an ageing demographic; around one third of persons older than 65 years have knee OA. Current treatment strategies include palliative approaches and eventual surgery, including joint replacement. Mesenchymal stromal cell (MSC)-based therapy represents a new opportunity, as it shows reparative effects in animal studies and in early clinical trials. Experimental therapy with MSCs is often delivered with hyaluronan (HA), a viscosupplement approved for pain management of OA joints. However, there is limited mechanistic understanding of the interaction of MSCs with HA in an inflammatory environment. In this study, we investigated different molecular weights (MW) of HA on the immunosuppressive properties of MSCs in the context of the specific toll-like receptors (TLR) implicated in modulating the anti-inflammatory properties of MSCs. MSCs from 3 healthy volunteers were left untreated or activated with IFN-γ (500 U/ml, 24 hours), Poly(I:C) (1 ug/ml, 24 hours), a TLR3 ligand or LPS (1 ng/ml, 1 hour), a TLR4 ligand and then washed twice with PBS before exposure to HAs and/or peripheral blood mononuclear cells (PBMC). HAs with an average MW of 1.6 MDa, 678 kDa and 150 kDa were used at a concentration of 500 ug/ml in culture. The 1.6 MDa and 150 kDa HA were also digested with hyaluronidase to verify that the observed differences among the various HAs were indeed due to differences in MW. The immunosuppressive effects of MSCs were tested by co-culture with PBMCs stimulated with anti-CD3 and anti-CD28, at a 1:20 ratio. T-test after logarithmic transformation was used for statistical analysis, with n=3 for all samples. MSCs from all donors suppressed the proliferation of stimulated PBMCs: with a proliferation of 37±6%, 25±3% and 54±3% for donors 1, 2 and 3 relative to stimulated PBMCs alone ($p<0.01$) in cultures without HA. Notably however, high MW HA, 1.6MDa, reduced significantly the suppressive effect of MSCs from all donors: with a PBMC proliferation of 51±4 %, 43±5% and 68±6%, respectively ($p<0.05$). In the presence of lower MW HA, 678 kDa, there was less reduction of PBMC proliferation, that appeared to be donor-specific: only MSCs from donor 2 significantly reduced MSC-mediated inhibition; PBMC proliferation was 41±5%. Digestion of 1.6 MDa HA resulted in 72kDa MW HA and significantly reduced the dampening effect of high MW HA on MSC-mediated suppression of PBMC proliferation: 35±3%, 29±2% and 47±2% ($p<0.05$). 150 kDa HA had no effect in cocultures with MSCs from donor 1 and minor effects with other donors: PBMC proliferation was 36±8% ($p=0.9$), 33±3% ($p<0.05$) and 59±1% ($p<0.05$) with MSCs from donors 1, 2 and 3, respectively. Co-culture with activated MSCs did not alter the extent of MSC-mediated suppression of PBMC proliferation, yet there was a significant increase of PBMC survival in co-cultures with LPS-activated MSCs: 60±4% vs 50±3% ($p<0.01$). Here we show that high MW HA reduces MSC-mediated suppression of PBMC proliferation but that MSC activation with TLR ligands has no significant effect. These preliminary data have implications for the co-administration of specific HA molecules and MSCs, and warrant further studies of effects on specific lymphocyte subsets and the use of more suitable pre-clinical models to determine the optimum role of HA and MSCs in the management of OA.

T-3146
THE ROLE OF SIRT1 IN ORGAN SPECIFIC STEM CELLS OF MAMMARY EPITHELIUM AND BONE MARROW

Gu, Yansong
Obenomics, Bellevue, WA, USA

SIRT1 is a NAD-dependent deacetylase that links metabolic activity to many aspects of life. Understanding the direct role of SIRT1 in organ physiology is often hindered by SIRT1 function in organ specific stem cells. To dissect those complicated roles, we have generated and characterized Sirt1 conditional knockout mice (JAX_B6;129-Sirt1tm1/yguJ). We found that Sirt1 modulates the estrogen-IGF1 signaling for epithelial stem cell differentiation during mammary gland development, which elucidates the role of Sirt1 in epithelial stem cell differentiation as well as breast cancer development. Knocking out Sirt1 in adipocytes bypasses this role of Sirt1 and yet alters the expression of a small group of genes implicated in several metabolic pathways. Interestingly, the mice are capable of resisting fat diet induced weight gain, indicating that SIRT1 may act as one of the missing links between fat diet-induced adiposity and the onset of estrogen-dependent breast cancer. In another example, understanding the role of Sirt1 in adult hematopoiesis has been complicated by high perinatal mortality of Sirt1-deficient mice. We perform a comprehensive in vivo study of the hematopoietic stem cell (HSC) compartment in adult Sirt1-deficient mice and show that, apart from anemia and leukocytosis in older mice, production of mature blood cells, lineage distribution within hematopoietic organs and frequencies of the most primitive HSC populations are comparable to those of wild type littermate controls. Furthermore, we show that Sirt1-deficient bone marrow cells confer stable long-term reconstitution in competitive repopulation and serial transplantation experiments. Taken together, while our results rule out an essential physiological role for cell-autonomous SIRT1 signaling in maintenance of adult HSC compartment in the mouse, we suggest that inhibiting SIRT1 activity in adipocytes may attenuate both the local effect of estrogen and IGF1 and the global effect on diet induced adiposity.

T-3147
EXPRESSION OF PD-L1 AS A POTENTIAL POTENCY ASSAY FOR MESENCHYMAL STEM/STROMAL CELLS USED FOR IMMUNOMODULATORY FUNCTION

Guan, Qingdong[1], Yang, Bin[1], Peng, Zhikang[1], Wall, Donna[2]
[1]University of Manitoba, Winnipeg, MB, Canada, [2]University of Manitoba, Winnipeg, MB, Canada

Mesenchymal stem/stromal cells (MSCs) are being studied as cell therapy for the treatment of inflammatory disorders (multiple sclerosis, inflammatory bowel disease, arthritis) because of their ability to suppress T cell response through direct cell contact or release of soluble mediators. Current Good Manufacturing Practice-compliant manufacturing of cellular therapeutic products requires reproducible, exportable, and relevant potency assays which correlate with clinical outcome. This has been a challenge in the case of MSC where the conventional assay of suppression of T cell response to mitogen has not been standardized across laboratories and is notoriously variable within laboratories. We postulated that expression of inhibitory receptors/ligands expressed on the MSC surface may correlate with immune modulatory function. PD-L1 expressed on MSC, has been shown to be involved in the suppression of T cell response by binding PD-1 expressed on T cells which inhibits TCR-mediated activation of IL-2 production and T cell proliferation. To test this MSC were generated by culturing human bone marrow cells with DMEM and 5% human platelet lysate. After 2-3 passages MSCs were >90% purity by flow cytometry (positive CD90, CD73, CD105, and negative CD14, CD34 and CD45). The standard assay-CFSE dilution assay demonstrated dose dependent suppression of proliferation of T lymphocytes, and MSC inhibited the differentiation of $CD4^+IFN\text{-}\gamma^+$Th1 and $CD8^+IFN\text{-}\gamma^+$ T cells dose-dependently. We examined the impact of fresh vs. frozen, passage number, IFN-γ exposure, and culture media on suppressive function of MSC - validating the reproducibility of assays - which required 5 day incubation and were dependent on the normal donor T cell responses to stimulus. Interestingly high expression level of PD-L1 on MSC correlated with stronger suppression in the CFSE assay, in which MSC with 36.4% expression of PD-L1 showed 89.21%

suppression rate of CFSE assay, but MSC with 33.5 or 18.2% expression of PD-L1 had 75.65% or 61.2% suppression rate of CFSE assay, respectively. Blocking PD-L1 using anti-PD-L1 antibodies reversed the suppressive functions of MSC. These results suggest that the expression levels of PD-L1 on MSC might correlate with suppressive functions of MSC - if so it may be a useful surrogate potency assay useful in the cGMP compliant manufacturing of MSC.

T-3148
TRANSPLANTATION OF WHARTON'S JELLY HUMAN MESENCHYMAL STEM CELLS IN A RABBIT MODEL OF INTERVERTEBRAL DISC DEGENERATION

Han, Inbo, Ahn, Jongchan, Park, Eunmi, Lee, Soohong
CHA University, Seongnam-Si, Republic of Korea

OBJECTIVE: The purpose of the present study is to assess the possibility of disc regeneration by treatment with mesenchymal stem cells (MSCs) from Wharton's Jelly in a rabbit model of degenerative disc disease, and to evaluate the efficacy of a percutaneous technique for constructing a model of degenerative disc disease in rabbits. METHODS: The study sample consisted of 20 mature male New Zealand white rabbits. Intervertebral discs were injured in each rabbit by a percutaneous technique at L2-3, L3-4, and L4-5 under C-arm guidance with a 19-gauge spinal needle. Magnetic resonance images (MRI) were checked at 3 weeks after injury to evaluate disc degeneration. Three weeks after injury, M SCs were injected into the L4-5 disc space, with saline injected into the L3-4 disc as a control, using a 21-gauge spinal needle. Histologic confirmations of degenerated discs were performed at 8 weeks after injury to evaluate regeneration of intervertebral disc. RESULTS: MRI revealed intervertebral disc degeneration from 3 weeks after injury, when compared with uninjured control discs. We confirmed the proliferation of MSCs at the L4-5 level in 8-week rabbits after cell injection. Histologically, the MSC-injected discs exhibited elevated extracellular matrix secretion compared with degenerative control discs. CONCLUSIONS: These results suggest that the injection of MSCs from Wharton's Jelly into injured lumbar discs could be an effective treatment for degenerative disc disease by promoting the cartilage regeneration.

T-3149
PHASE I CLINICAL TRIAL OF AUTOLOGOUS MESENCYMAL STEM CELL-DERIVED NEURAL PROGENITORS INJECTED INTRATHECALLY IN MULTIPLE SCLEROSIS

Harris, Violaine, Chirls, Sydney, Vyshkina, Tamara, Sadiq, Saud A.
Tisch MS Research Center of New York, New York, NY, USA

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system that is characterized by an early predominantly inflammatory demyelinating disease phase that over a variable period of time evolves into a later predominantly degenerative stage associated with axonal loss and scar formation, causing physical and cognitive disability. Currently available treatments for MS target the inflammatory disease response but are largely ineffective against the degenerative phase of the disease. The objective of this study is to investigate, as a reparative strategy, the feasibility of injecting autologous bone marrow mesenchymal stem cell-derived neural progenitor cells (MSC-NPs) directly into the cerebrospinal fluid (intrathecal) of MS patients with disability. MSC-NPs represent a neural subpopulation of bone marrow-derived MSCs with reduced pluripotency and minimized risk of ectopic differentiation. In preclinical studies in mouse experimental autoimmune encephalomyelitis (EAE), we established that intrathecal delivery of MSC-NPs was associated with cell migration to lesion areas, suppression of local inflammatory immune response, and trophic support for damaged cells at the lesion site. These pathological features were associated with improvement in clinical scores of EAE. Preliminary clinical experience with autologous MSC-NPs in a small number of patients further supports the safety and tolerability of this treatment, and warrants a clinical study. The study is a 20 patient, open-label, FDA-approved phase I clinical trial of autologous MSC-NPs administered intrathecally in three injections of up to 10 million cells per injection, spaced three months apart. Pre-administration quality testing of autologous MSC-NPs expanded from bone marrow aspirates included analysis of sterility, purity, identity, and chromosomal stability. Enrollment criteria included MS patients with established disability (EDSS disability score of 3.0 or greater) and relatively stable disease as evidenced by less than 1.0 point change in EDSS in the last year, and stable MRI disease burden with no gadolinium-enhancing lesions in the last six months. Primary safety and tolerability outcomes include adverse event assessments. Secondary outcomes to observe trends in efficacy include neurological exam, MRI, evoked potentials, and urodynamic testing. Participant demographics and preliminary safety outcomes will be presented. The MSC-NP trial is the first of its kind to test intrathecal administration of neural progenitors as a regenerative therapy for MS.

T-3150
CO-TRANSPLANTATION OF HUMAN MESENCHYMAL STEM CELLS WITH HUMAN OR NEONATAL PORCINE ISLET GRAFTS IN MICE

Hayward, Julie Amelia[1], Seeberger, Karen[1], Ellis, Cara[1], Korbutt, Gregory[2]
[1]Surgery, University of Alberta, Edmonton, AB, Canada, [2]University of Alberta, Edmonton, AB, Canada

Islet transplantation is a promising treatment for type I diabetes, although insulin independence rates significantly decline after 5 years post-transplantation. The success of transplantation is impeded by an inflammatory response and hypoxic conditions post-transplant. The inflammatory response is characterized by pro-inflammatory cytokines such as TNF-, IL-1, and IFN-. These factors have damaging effects on islets; they increase -cell apoptosis, degrade islet structure, and impair glucose responsiveness. Mesenchymal stem cells (MSCs) exhibit anti-inflammatory properties through secreted factors such as HGF, IL-10, IL-6, and TFG-. They also secrete VEGF, a factor which promotes vascularization. Co-culture experiments have demonstrated that MSCs preserve structure and glucose responsiveness of islets cultured with cytokines. Human (*n=6*) or neonatal porcine (*n=22*) islets were transplanted under the kidney capsule of streptozotocin-induced diabetic (≥15mmol/L) mice with or without 1 x 10^6 mesenchymal stem cells. Islets are aggregated with or without MSCs for 24-48 hours pre-transplant. Blood glucose and weight are monitored weekly until normalization. The mice then undergo an oral glucose tolerance test (OGTT) and blood glycemia is recorded at various time points. Survival nephrectomies are subsequently performed to ensure the mice return to hyperglycemia. Graft sections are stained for insulin and von Willibrand factor, and graft insulin content is measured. Mice with human islets were normalized within one week. There was no difference between the islets + MSC group in glycemia or OGTT. Mice with neonatal porcine islet transplants normalized after an average of 171 and 150 days (without and with MSCs, respectively). Blood glycemia declined with relatively no difference between groups. The OGTT resulted in the MSC group with a significantly smaller area under the curve (1117mmoll^{-1}h^{-1}) than the islets alone group (1305mmoll^{-1}h^{-1}) (p=0.045). The insulin content of the grafts was slightly higher in the islets + MSC grafts (15.17μg) compared to the islets alone grafts

(12.03µg), although not significant (p=0.38). Mice with human islet transplants showed no benefit to co-transplant with MSCs. This group was very small, however, so these results are not significant. Mice receiving newborn pig islet transplants were successfully normalized, with the islets + MSC group showing better glucose tolerance, less time to normalization, and greater insulin content.

T-3151
THE EFFECT OF MESENCHYMAL STEM CELLS ON CARDIAC MITOCHONDRIAL RESPIRATION IN A MURINE MODEL OF ACUTE MYOCARDIAL INFARCTION

Goldenberg, Regina[1], **Irion, Camila Iansen**[1], Beiral, Hellen Jannisy Vieira[1], Dias, Grazielle Suhett[2], Ferreira, Raphaela Pires[1], Pimentel, Cibele Ferreira[1], Campos de Carvalho, Antonio C.[1], Carvalho, Adriana B.[1], Vieyra, Adalberto Ramon[1]
[1]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [2]Hospital Israelita Albert Einstein, São Paulo, Brazil

Acute myocardial infarction (AMI) remains an important cause of death and heart failure worldwide. Myocardial ischemia is characterized by severe hypoxia, alterations in ion homeostasis and mitochondrial dysfunction. Inhibition of mitochondrial respiration results in changes in contractility, stimulates the production of reactive oxygen species and causes cell death. Cell therapy with mesenchymal stem cells (MSCs) have therapeutic potential in AMI due to their paracrine effects, secretion of vesicles and mitochondrial transference. This study aimed to evaluate the effect of MSCs from bone marrow (BM) on the mitochondrial respiration of infarcted cardiac tissue in a co-culture model. Method: Female Wistar rats between 8 to 10 weeks of age underwent permanent left anterior descending artery (LAD) ligation (n=4). Age matched sham procedures (n=5) were used as a control. Confirmation of AMI was performed by electrocardiogram (ECG). Tissue necrosis was measured by triphenyltetrazolium chloride staining. Isolated MSCs from Lewis rats (LEW-Tg (EGFP) F455.5/Rrrc) at 3-4th passage were used and characterized by flow cytometry to analyze cell surface antigens and by immunofluorescence to check GFP expression. In mitochondrial respiration assays, rats were euthanized 24 h after AMI; slices of heart tissue (about 6 mg in triplicate) from infarcted zone (IZ) and border zone (BZ) were obtained with a Tissue Chopper and maintained in co-culture with MSCs (3×10^5) for 24 h using a Transwell system. Slices (previously co-cultured or not with MSCs) or MSC alone (1×10^5) were placed into the chambers of an Oroboros Oxygraph-O_2k to quantify their respiration. Oxygen consumption (pmol O_2 s^{-} mg^{-1}) was calculated using DatLab 4 software. Results: Infarcted rats that showed pathologic Q wave and ST-elevation in ECG were included in the study. Infarction size was 41.4 ± 3.3%. MSCs showed a fibroblast like morphology at 3rd passage, expression of GFP protein and a very high expression (100%) of CD90+ and negligible expression of CD45 and CD34. After 24 h of culture BZ and IZ slices (2.2 ± 0.6 and 0.9 ± 0.5 pmol O_2 s^{-} mg^{-1} respectively) from infarcted rats presented a decrease in mitochondrial respiration when compared to sham group (3.5 ± 0.4 pmol O_2 s^{-} mg^{-1}). Treated MSCs-IZ slices from infarcted rats showed no difference in mitochondrial respiration when compared to non-treated slices (1.3 ± 0.3 *vs.* 0.9 ± 0.5 pmol O_2 s^{-} mg^{-1}; p>0.05). However, treated BZ slices presented an improved mitochondrial respiration when compared to non-treated BZ slices (3.3 ± 0.4 *vs.* 2.2 ± 0.6 pmol O_2 s^{-} mg^{-1}; p<0.05). MSCs alone, co-cultured with sham or with BZ slices showed an O_2 consumption of 129.6 ± 6.4, 91.9 ± 12.4 and 83.4 ± 16.6 pmol O_2 s^{-} mg^{-1} respectively with significant difference between MSCs alone and MSCs in co-culture with BZ slices and with sham group (p<0.05). Interestingly, MSCs showed barely detectable mitochondrial respiration when co-cultured with IZ slices. Conclusion: Co-culture with MSCs improved basal mitochondrial respiration in BZ but not in IZ infarcted heart, suggesting that the MSCs mechanism of action involves a paracrine effect, which is able to preserve only the less affected region. The conditioned medium recovered after co-culture of MSCs with IZ affected MSCs respiration, suggesting the existence of a cross-talk between the infarcted tissue and MSCs.

T-3152
INTRACORONARY CARDIAC PROGENITOR CELL INFUSION IN PATIENTS WITH SINGLE VENTRICULAR PHYSIOLOGY INTERIM RESULTS OF PHASE I-II CLINICAL TRIALS

Ishigami, Shuta, Okuyama, Michihiro, Tarui, Suguru, Ohtsuki, Shinichi, Ousaka, Daiki, Eitoku, Takahiro, Kobayashi, Junko, Kasahara, Shingo, Sano, Shunji, Oh, Hidemasa
Okayama University Hospital, Okayama, Japan

Backgrounds- Single ventricle physiology includes hypoplastic left heart syndrome (HLHS) is a fatal congenital heart malformation requiring active intervention within the first few days of life. Objective- The aim of this study is to determine whether intracoronary infusion of cardiac progenitor cells (CDCs) is feasible and safe to treat the children and that improves global cardiac function with HLHS after staged palliations. Methods and Design- This clinical study was conducted by two staged trials. In stage I prospective controlled study, 7 patients were consecutively enrolled to receive CDC infusion followed by 7 patients treated by standard care alone as control group (TICAP trial: NCT01273857). In stage II, 34 patients were randomly allocated in 1:1 ratio to received CDC treatment or standard palliation alone (PERSEUS trial: NCT01829750). All eligible patients must undergo staged-2 or -3 shunt procedures in a diagnosis of HLHS or univentricular heart diseases during the initial enrollment. In patients assigned to receive CDC treatment, cardiac tissue specimens were harvested from right atria during cardiac surgery to isolated autologous CDCs. One month after shunt procedures, 0.3 million cells/kg CDCs were infused into coronary arteries. The primary endpoint was to assess the safety and the secondary endpoint was the preliminary efficacy to verify the improvements of the right ventricular function and the clinical symptoms of heart failure status from baseline (1 month after palliations) to 3, 12, and 24 months of follow-up. The heart failure status (Ross classification and New York University Pediatric Heart Failure Index; NYU PHFI) and Z scores for weight-for-age at each follow-up were also evaluated. Results- No complications, including cardiac death, myocardial ischemia, arrhythmia, hospitalization for heart failure, and tumor formation, were reported within 24 months of CDC infusion. Echocardiogram showed that at 3 months after CDC infusion, improvement of right ventricular ejection fraction (RVEF) was greater in the CDC-treated group (n=10: +6.0±3.9%) than in controls (n=10: +0.6±3.4%, P=0.01). This cardiac function enhancement was manifested even 1 year and 2 years after CDC infusion (n=7: +7.8±4.9% vs. +2.2±3.1% at 1 year, P=0.03; n=7: +8.8±3.7% vs. +3.4±6.4% at 2 years, P=0.04). The absolute improvements in RVEF between 2 groups was confirmed by using right ventriculogram (+8.9±7.6% vs. +2.0±2.8% at 1 year, P=0.045; +7.8±5.9% vs. +2.9±3.9% at 2 years, P=0.047). In addition, RVEF measured by cMRI was also markedly improved in CDC-treated patients from 36.1±7.5% at baseline to 42.7±8.7% at 1 year (P=0.04) and to 42.4±7.7% at 2 years (P=0.047). Notably, heart failure status was reduced in CDC-treated group as shown by significant decrease in both Ross Heart Failure Class (2.6±0.8 at baseline vs. 1.4±0.5 at 2 years, P=0.01) and NYU PHFI (10.3±4.4 at baseline vs. 6.1±0.9 at 2 years, P=0.04), and Z scores for weight-for-age was significantly increased from -4.0±2.7 at baseline to -2.2±1.4 at 2 year (P=0.02), whereas all

of these parameters did not change in control subjects. Conclusion- These interim results of 2-year follow-up of our trials suggest that intracoronary infusion of autologous CDCs is feasible and safe to treat the children with HLHS. This novel therapeutic strategy may impact on cardiac function as well as clinical symptom of heart failure status and somatic growth after staged palliations in long-term outcome.

T-3153
AN EXPLORATORY CLINICAL TRIAL FOR IDIOPATHIC OSTEONECROSIS OF FEMORAL HEAD BY CULTURED AUTOLOGOUS MULTIPOTENT MESENCHYMAL STROMAL CELLS AUGMENTED WITH VASCULARIZED BONE GRAFTS

Toguchida, Junya[1], Aoyama, Tomoki[2], Goto, Koji[3], Kakinoki, Ryosuke[4], Kasai, Yasunari[5], Maekawa, Taira[5], Tada, Harue[6], Teramukai, Satoshi[6], Nakamura, Takashi[7], Matsuda, Shuichi[7]

[1]Department of Tissue Regeneration, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan, [2]Department of Physical Therapy, Human Health Sciences, Kyoto University, Kyoto City, Japan, [3]Department Orthopaedic Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [4]Department of Rehabilitation Medicine, Kyoto University Hospital, Kyoto University, Kyoto, Japan, [5]Center for Cell and Molecular Therapy, Kyoto University Hospital, Kyoto University, Kyoto, Japan, [6]Department of Clinical Trial Design and Management, Translational Research Center, Kyoto University, Kyoto, Japan, [7]Department of Orthopaedic Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Idiopathic osteonecrosis of femoral head (ION) is a painful disorder that progresses to collapse of the femoral head and destruction of the hip joint. Its precise pathology remains unknown, however the loss of blood supply causing the loss of living bone-forming cells is a hallmark of the pathophysiology of osteonecrosis. Transplantation of multipotent mesenchymal stromal cells (MSCs) is a promising tool for the regenerating the musculoskeletal system. The aim of the present study was to assess the safety and efficacy of transplantation of cultured autologous bone marrow-derived MSCs mixed with β-tricalcium phosphate (β-TCP) in combination with vascularized bone grafts for the treatment of advanced stage ION in a clinical trial. This was the first clinical study approved by the Ministry of Health, Labor and Welfare of Japanese goverment under the guidance of clinical study using human stem cells. Ten patients with stage 3 ION were enrolled in this study. Autologous bone marrow-derived MSCs were cultured with autologous serum, and cells were transplanted after mixing with β-TCP granules in combination with vascularized iliac bone grafts. Patients were assessed 24 months after treatment. The primary and secondary endpoints were progression of the radiological stage and changes in bone volume at the femoral head, and clinical score, respectively. Nine of ten patients completed the protocol, seven of which remained at the same radiological stage. The remaining two cases progressed to stage 4. Average bone volume increased from 56.5±8.5cm3to 57.7±10.6cm3. The average clinical score according to the Japan Orthopaedic Association improved from 65.6±25.5 points to 87.9±19.0 points. Sixty-seven adverse events in ten patients were observed, all of which were correlated with surgical procedures, not with cell transplantation. All procedures were successfully performed and some young patients with extensive necrotic lesions with pain demonstrated good bone regeneration with amelioration of symptoms. Further improvements in our method using MSC and the proper selection of patients will open a new approach for the treatment of this refractory disease.

JUNE 20, 2014

FRIDAY
POSTER PRESENTATIONS

6:00 - 7:00 ODD numbered posters presented
7:00 - 8:00 EVEN numbered posters presented

PANCREATIC CELLS

F-1007
RE-ENGINEERING THE 3D STEM-CELL NICHE: CO-CULTURING DIFFERENTIATING HUMAN EMBRYONIC STEM CELLS WITH ENDOTHELIAL CELLS IN DECELLULARIZED PANCREATIC SCAFFOLD

Goh, Saik Kia[1], Bertera, Suzanne[2], Banerjee, Ipsita[3]
[1]Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA, [2]Children's Hospital of Pittsburgh, Pittsburgh, PA, USA, [3]University of Pittsburgh, Pittsburgh, PA, USA

Introduction: The shortage of donor islets for treating diabetes highlights the urgent need for generating insulin-producing β-cell from human embryonic stem cells (hESCs). Despite the improvement in β-cell differentiation cultures, a major gap remains in the lack of cellular maturity. This maturation defect may be partly due to the lack of 3D structure and interactions with stromal cells, both of which influence embryonic pancreatic development in vivo. Whole organ decellularization preserves the native 3D structural and biochemical information of an organ and provides an attractive 3D template for stem cell differentiation. Hence, we hypothesize that 3D ultrastructure and co-culture of relevant cell types are critical for β-cell phenotype and functionality. Toward this end, we propose using the decellularized pancreas as a favorable 3D ultrastructure and combining with endothelial cells to provide the optimal microstructure and microenvironment niche to promote β-cell functionality. Materials and Methods: Cadaveric pancreata were isolated from adult mice (n=8) and decellularized via detergent (2% Triton-X, 1% DOC) perfusion. Resulting decellularized matrices were characterized for acellularity, preservation of ECM and microstructures. Pancreatic progenitor (PP) cells were generated from step-wise differentiation of hESC into definitive endoderm (Activin and Wnt for 4 days) followed by sonic hedgehog inhibition (cyclopamine) and retinoic acid induction for another 4 days. For recellularization, hESC-derived PP cells ($3x10^6$ cells) were combined with HUVEC ($3x10^6$ cells) and introduced into the acellular 3D pancreatic scaffold via portal vein in 3 steps, with 30 min interval between each step. The recell 3D construct were then mounted on a perfusion bioreactor to allow dynamic culture for 7 days. The recellularized constructs were evaluated via immunostaining, TUNEL, and qRT-PCR. Results and Discussion: Perfusion-decellularization of pancreas with detergent resulted in complete removal of cells. Characterization of the decellularized pancreas revealed preservation of ECM and 3D architecture as previously described [1]. qRT-PCR analysis revealed that cells in decellularized pancreas (n=3) had significantly increased expression of early pancreatic marker genes (PDX1, NKX6.1, NGN3, ISL1) and maturation markers (MAFA, INSULIN) compared to cells in matrigel (n=6) and control (n=6). Consistent with this, immunostaining result showed significantly higher number of PDX1+ cells ($P<0.023$) were found in recellularized pancreas construct compared to matrigel. In addition, immunostaining result also showed that majority of PDX1+ cells (n=10, 82±11%) were detected in close proximity with endothelial cells (CD31+ cells), suggesting the involvement of stromal-cell-dependent factors in pancreatic maturation. Conclusion: Perfusion-decellularization of pancreas efficiently removes cells while retaining ECM protein and microstructure. This perfusable 3D whole organ scaffold yields complex 3D tissue construct that favored pancreatic differentiation when seeded with hESC-PP and HUVEC. This is a first step proof of concept to show that the 3D pancreatic maturation can be recapitulated in vitro by culturing pancreatic progenitors with endothelial cells in decellularized pancreas.

F-1008
NUTRIENT REGULATION BY CONTINUOUS FEEDING REMOVES LIMITATIONS ON CELL YIELD IN THE LARGE-SCALE EXPANSION OF MAMMALIAN BETA CELL AND IPS CELL SPHEROIDS

Firpo, Meri T., Weegman, Brad, Nash, Peter, Carlson, Alexandra, Voltzke, Kristin, Geng, Zhaohui, Janhani Kondori, Marjan, Papas, Klearchos
University of Minnesota, Minneapolis, MN, USA

Cellular therapies are emerging as a standard approach for the treatment of several diseases. However, realizing the promise of cellular therapies across the full range of treatable disorders will require large-scale, controlled, reproducible culture methods. Bioreactor systems offer the scale-up and monitoring needed, but standard stirred bioreactor cultures do not allow for the real-time regulation of key nutrients in the medium. In this study, β-TC6 insulinoma cells and human iPS cells were aggregated and cultured for 3 weeks as a model of manufacturing a mammalian cell product, as these cells are particularly sensitive to nutrient levels in the environment. Cells were compared in static, stirred suspension bioreactors, and continuously fed stirred suspension bioreactors for cell expansion rates and medium nutrient levels. While stirred bioreactors cultures facilitated increased culture volumes, no increase in cell yields were observed, partly due to limitations in key nutrients, such as glucose, which were consumed by the cultures between feedings. Glucose levels are particularly important for the differentiation, function and survival of beta cells, since they are sensitive to both high and low concentrations of this key nutrient. For these cultures, even when glucose levels were increased to prevent depletion between feedings, dramatic fluctuations in glucose levels were observed. Continuous feeding eliminated fluctuations and improved cell expansion when compared with both static and stirred bioreactor culture methods. Further improvements in growth rates and differentiation profiles were observed after adjusting the feed rate based on calculated nutrient depletion, which maintained physiological glucose levels for the duration of the expansion. Adjusting the feed rate in a continuous medium replacement system can maintain the consistent nutrient levels required for the large-scale application of many cell products. Thus, a perfusion feeding system of continuously fed bioreactor systems combined with feed rate regulation can be used to improve the yield and reproducibility of mammalian cell cultures for biological products and cellular therapies, and will facilitate the translation of cell culture from the research lab to clinical applications.

F-1009
ARTIFICIAL EXTRACELLULAR MATRIX CONTAINING LAMININ-IKVAV SEQUENCE PROMOTES DIFFERENTIATION OF INSULIN-EXPRESSING COLONIES

Ghazalli, Nadiah[1], Mahdavi, Alborz[2], Jin, Liang[1], Feng, Tao[1], Jasper, Hsu[1], David, Tirrell[2], Riggs, Arthur[1], Ku, H. Teresa[1]
[1]Department of Diabetes and Metabolic Diseases Research, City of Hope, Duarte, CA, USA, [2]Department of Bioengineering, California Institute of Technology, Pasadena, CA, USA

Pancreatic stem and progenitor cells are potential sources to produce insulin-expressing beta cells to treat Type 1 Diabetes. We recently established a Matrigel-based culture system that allows the formation of multi-lineage colonies from single cells isolated from the adult pancreas. These colonies differentiate towards endocrine lineage when cultured in an artificial extracellular matrix protein (aECM) that contains the functional sequence of laminin called IKVAV. Whether or not IKVAV is necessary for the colony-forming activity and endocrine differentiation is unknown. We used postnatal pancreas, a rich source for progenitor cells, to test the ability of aECM (one containing IKVAV sequence, aECM-lam; one containing scrambled sequence, aECM-scr) and Matrigel to induce endocrine differentiation in vitro. To determine colony-forming ability, single cells from postnatal pancreas were cultured for 7 days in cultures containing either Matrigel, aECM-scr, or aECM-lam. We found that colonies were able to form in all materials, but efficiency was the highest in aECM-lam. Colonies grown in aECM-lam expressed high levels of the endocrine markers Insulin1, Insulin2 and Glucagon compared to aECM-scr and Matrigel. This result indicates that although IKVAV sequence is not required for colony formation, it does enhance endocrine gene expression. In contrast, Matrigel was found to be inhibitory towards formation of endocrine colonies. Further analysis of the colonies in aECM-lam showed that they display the hallmarks of functional beta cells: mature beta cell granules and secretion of C-peptide in response to high glucose. We conclude that postnatal pancreas contains colony-forming activity and aECM-lam promotes differentiation of functional insulin-expressing colonies.

F-1010
THE ARX/PAX4 REGULATORY LOOP IS ESSENTIAL FOR THE EX VIVO GENERATION OF FUNCTIONAL BETA CELLS TRANSDIFFERENTIATED FROM HUMAN EXOCRINE TISSUE

Lima, Maria Joao, Muir, Kenneth R., Docherty, Hilary M., Docherty, Kevin
University of Aberdeen, Aberdeen, United Kingdom

The shortage of donor material has driven research towards finding a replenishable supply of islets for transplantation. We have previously shown that the exocrine material that results as a by-product of the islet isolation procedure can be reprogrammed towards functional islet-like structures by overexpression of the pancreatic transcription factors Pdx1, MafA, Ngn3 and Pax4 (4TFs), followed by culture in the presence of betacellulin, nicotinamide and exendin-4. The transdifferentiated cells secreted insulin in a glucose responsive regulated manner and rescued diabetes in a streptozotocin-induced diabetic mouse model, but expressed only 1% of the insulin levels found in a mature adult islet. The difference in insulin expression observed between mature islets and transdifferentiated cells suggested that the latter had not reached the same maturation status as adult islets. Thus, in order to improve the transdifferentiation outcome, thirty different combinations of pancreatic TFs were tested. Interestingly, similar levels of insulin expression were obtained when replacing Pax4 by either Nkx6.1 or NeuroD, however, only in the presence of Pax4 were the transdifferentiated cells able to secrete insulin in a glucose dependent manner, indicating that Pax4 plays a crucial role in establishing the functionality of mature beta cells in humans. RT-QPCR of late beta cell markers further demonstrated that MafA expression was only present in cells transdifferentiated with the 4TF combination, indicating that its expression is a key factor for beta cell functionality. These results are in accordance with earlier studies in the developing mouse, where it has been shown that the transcription factors Pax4 and Arx play a pivotal role in the final maturation of the beta and alpha cell lineages, where the beta cell lineage is established in part through inhibition of Arx by Pax4. RT-QPCR has shown that Arx is expressed during the differentiation protocol and may favour the development of alpha versus beta cells during reprogramming of the exocrine derived material. To investigate this possibility Arx expression was inhibited by siRNA at the late stages of the transdifferentiation protocol, resulting in a ~60 fold increase in insulin expression levels compared to cells treated with control siRNA, bringing insulin expression levels much closer to those of mature beta cells. Additionally, glucagon expression was significantly down-regulated after inhibition of ARX expression. Specific ELISAs for human insulin and C-Peptide demonstrated that the transdifferentiated cells were able to efficiently store and process insulin, secreting C-peptide in a regulated glucose-responsive manner, with levels comparable to those found in human islets. To further support its role in the functionality and maturation of the reprogrammed beta cells, removal of Pax4 from the transdifferentiation protocol resulted in the abolishment of C-peptide release in response to an increased glucose concentration. These studies indicate that the regulatory loop between Arx and Pax4 during the final stages of pancreatic development is essential for the late maturation stages of human beta cells generated *in vitro*. We have shown for the first time that inhibition of Arx, along with Pax4 overexpression, is crucial for the transdifferentiation of human exocrine cells towards mature, glucose responsive beta-like cells that have the potential to be used in future cell therapy for type 1 diabetes.

F-1011
EPIGENETIC COMPARISON OF DIFFERENTIATING HESCS AND THE DEVELOPING HUMAN PANCREAS TO IDENTIFY CRITICAL REGULATORS OF PANCREATIC DIFFERENTIATION

Mora-Castilla, Sergio[1], Liao, Xiaoyan[2], Laurent, Louise[2]
[1]Reproductive Medicine, University San Diego California, San Diego, CA, USA, [2]University of California, San Diego, La Jolla, CA, USA

In order to understand the epigenetic specification of pancreatic tissue identity, we performed genome-wide transcriptome and methylome analysis of stepwise in vitro differentiation of human embryonic stem cells (WA09), human fetal pancreas tissue and adult islets, in the context of a variety of other fetal tissues. Heirarchical clustering, group-wise analysis, and transcriptome/methylome anticorrelation analyses were combined with functional enrichment analysis DAVID and G.R.E.A.T. to identify candidate regulators of pancreatic differentiation. GO analysis revealed that the set of genes that was both hypomethylated in adult islets and became hypomethylated during pancreatic differentiation of hESCs was enriched for genes associated with HNF1a-HNF4a signaling pathway, suggesting an important role for these transcription factors in pancreatic differentiation. Consistent with previous studies, we confirmed that the cluster of genes that became hypermethylated during hESC differentiated was enriched for pluripotency-associated genes (including OCT4 and NANOG). In addition, there we two small clusters displaying endoderm-specific hypo- and hyper-methylation; neither of these genes showed significant enrichments. To validate the de novo hypomethylation of the HNF1a/

HNF4a signaling pathway and hypermethylation of pluripotency genes during pancreatic differentiation of hESCs, we performed bisulfite sequencing of the promoter regions of OCT4, NANOG, NROB2 and HNF4A. Results from these experiments showed a progressive increase in DNA methylation at the OCT4 and NANOG promoters and loss of NROB2 and HNF4A methylation over the course of hESC differentiation, as well as hypomethylation in adult islets. Our results demonstrate that comparison of transcriptome and methylome data from differentiating hESCs and corresponding human tissues can be used discover of candidate factors acting as hub-regulators of important signalling pathways critical for early lineage specification and cellular differentiation.

LIVER CELLS

F-1012
A NOVEL HEPATIC CORD CELL MODEL FOR DRUG HEPATOTOXICITY ASSESSMENT

Chen, Kevin, Hsu, Lih-Tao, Huang, Hui-Ting, Chang, Shiun-Yin, Chen, Wannhsin
Industrial Technology Research Institute, Hsinchu, Taiwan

Primary human hepatocytes are utilized in drug development to evaluate human specific drug properties such as drug hepatotoxicity, metabolism and drug-drug interactions. However, the demand for primary human hepatocytes far exceeds the available supply and the quality of primary hepatocytes is highly variable. Human pluripotent stem cells could provide a renewable source of human hepatocytes. However, the derivation methods described so far are inefficient to yield polarized functional hepatocytes. In this study, we developed a novel hepatic cord cell model from pluripotent stem cells via a progenitor stage in which cells were capable of extensive proliferation. Following expansion, progenitors were induced to differentiate into hepatocyte-like cells, which were arranged in irregular columns, resembling liver cords. The hepatocyte-like cells were observed to express mature hepatocyte markers and exhibited hepatic functions including membrane polarity, CYP3A4 activity, LDL uptake, albumin secretion, glycogen and lipid storage. To evaluate the capability of this cell model for prediction of drug-induced hepatotoxicity, cells were treated with a human hepatotoxicant troglitazone at various concentrations. Following treatment, troglitazone caused an increase in cytotoxicity, demonstrating the feasibility of this model for evaluation of drug hepatotoxicity. We are currently testing other hepatotoxicants using the hepatic cord cell model.

F-1013
HEPATIC PROGENITOR CELLS CONTRIBUTE TO THE PROGRESSION OF LIVER FIBROSIS INDUCED BY 2-AAF/ CCL4 IN RATS THROUGH THE NON-CANONICAL WNT PATHWAY

Chen, Jiamei[1], Mu, Yongping[1], Duan, Yuyou[2], Liu, Ping[1]
[1]Institute of Liver Diseases, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China, [2] University of California Davis Medical Center, Sacramento, CA, USA

Hepatic progenitor cells, known as oval cells in rodents, can differentiate into hepatocytes or cholangiocytes when mature hepatocyte proliferation is limited or suppressed during liver injury. It appears that hepatic progenitor cells may be transformed into myofibroblasts and contribute a profibrotic effect in sustaining the progression towards cirrhosis. The Wnt signaling pathway plays an important role in regulating progenitor cell proliferation and cell fate decisions; however, the relationship between hepatic progenitor cell differentiation and Wnt signaling has not been established in the pathological microenvironment of progressive cirrhosis. In this study, we developed a rodent cirrhosis model induced by subcutaneous injection with 50% of carbon tetrachloride (CCl4) for 8 weeks, followed by the continuous injection with 30% of CCl4 for additional 4 weeks. In order to inhibit mature hepatocytes regeneration and increase hepatic progenitor cell expansion and differentiation, the treated rats were fed with 2-acetylaminofluorine (2-AAF) during the period of cirrhosis progression. The results showed that the expressions of hepatic oval cell markers (OV6 and CK19) were increased significantly during fibrosis progression. In the CCl4/ 2-AAF treated rats, OV6 positive cells and CK19 positive cells extended across the liver lobule, formed bridges between portal tracts and divided the parenchyma into smaller pseudo-lobules, as determined by immunohistochemical staining. Double staining showed that OV6 was largely colocated with α-SMA positive cells, and the number of cells positive for both OV6 and α-SMA was obviously increased after administration of 2-AAF. The expressions of Wnt4, Wnt5b, frizzled2, frizzled3 and frizzled6 were markedly increased after administration of 2-AAF (p<0.01). Immunohistochemistry showed that β-catenin protein, a canonical Wnt pathway marker, was mostly localized to the nucleus of cells before administration of 2-AAF; however, β-catenin was found predominantly within the cytoplasm after administration of 2-AAF. In addition, the expression level of βcatenin was not changed by the administration of 2-AAF, suggesting that the activation of Wnt pathways was not mediated through the classical β-catenin pathway. Moreover, after administration of 2-AAF, gene expression of frizzled1 and frizzled4 was markedly decreased (p<0.01); however frizzled5 expression was not significantly changed, indicating that non-canonical Wnt signaling rather than Wnt/β-catenin signaling was primarily activated. We also determined that the expression of TGF-β1 was markedly increased in vivo after administration of 2-AAF. Expression of α-SMA and F-actin, as well as collagen types I and IV were significantly increased after the WB-F344 cell line, a mouse hepatic stem/progenitor cell line, was treated with TGB-β1 for 24 hours. Additional investigation revealed that both Wnt5b and frizzled2 expression were significantly increased in WB-F344 cells after treatment with TGF-β1 (p < 0.01), and β-catenin expression was not up-regulated during the treatment. Thus, these in vitro results confirmed our finding in vivo. In conclusion, our results indicate that hepatic progenitor cells appear to transdifferentiate into myofibroblasts and exhibit a profibrotic effect in the fibrogenic process through activating the non-canonical Wnt signaling pathway.

F-1014
HUMAN AMNIOTIC EPITHELIAL CELLS AND CONDITIONED MEDIA HAVE ANTI-FIBROTIC AND PRO-REGENERATIVE EFFECTS ON LIVER FIBROSIS

Lim, Rebecca[1], Hodge, Alexander[2], Lourensz, Dinushka[1], Tchongue, Jorge[2], Yeoh, George C.[3], Sievert, William[2]
[1]The Ritchie Centre, Clayton VIC, Australia, [2]Gastroenterology, Monash Medical Centre, Clayton VIC, Australia, [3]Western Australian Institute for Medical Research, Crawley WA, Australia

Recovery from cirrhosis requires extra-cellular matrix degradation plus hepatocyte regeneration. Decreased hepatic stellate cell (HSC) activation and expression of matrix degrading enzymes by both macrophages (MP) and HSC create a fibrolytic environment. Hepatocyte regeneration by liver progenitor cells (LPC) requires interactions among LPC, HSC and MP. We have shown that cell therapy with human amniotic epithelial cells (hAEC) ameliorates hepatic fibrosis in a murine model by decreasing HSC activation and inducing MP-derived matrix metalloproteinases. This study

investigated whether factors secreted by hAEC contained in 'cell-free' conditioned media (CM) (i) reduce hepatic fibrosis in vivo, (ii) exert an anti-fibrotic effect on HSC and MP and (iii) promote LPC proliferation. Mice given carbon tetrachloride for 12 weeks (wk) were administered intravenous hAEC CM 3 times/wk for the last 4 wk of the model. To compare CM to cell therapy, hAEC were injected at 8 wk. Liver fibrosis area was determined by Sirius red staining. The effect of hAEC CM in vitro was explored using human HSC (LX2 cells) and murine bone marrow derived MP exposed to CM for 24 or 48 hour treatments, or mouse LPC (BMOL cell line) given CM given for 4 or 8 days. Differentiation media was applied to LPC as a positive control. Matrix metalloproteinase 9 (MMP9), alpha-fetoprotein (AFP), albumin and cytokeratin-19 (CK-19) gene expression was assessed by RT-PCR. Intracellular collagen was determined by 3H-proline incorporation. Ly6Chi MP were identified by FACS. Proliferation with determined by Brd-U incorporation. Hepatic fibrosis area reduced by 26% ($P<0.05$) in CM treated animals, equivalent to hAEC cell therapy. hAEC CM treatment reduced MP and HSC numbers but LPC persisted showing that the anti-inflammatory effect of hAEC CM did not impair LPC proliferation. Treatment of HSC by CM resulted in significant reduction in collagen production and increased MMP9 expression. CM treated MP showed a significant reduction in Ly6Chi expression and increase in MMP9 expression. CM treated LPC demonstrated increased proliferation and expression of albumin and AFP but not CK-19. hAEC CM therapy significantly reduced liver fibrosis in vivo and induced a fibrolytic phenotype in HSC and MP. hAEC CM treatment of LPC resulted in proliferation and differentiation down the hepatocyte lineage while markers of cholangiocyte regeneration remained unchanged. hAEC-based therapy appears promising for patients with chronic liver disease.

F-1015
HNF4A AND FOXA2 UNDERGO DIFFERENTIATION-DEPENDENT ENHANCER SWITCHING TO AFFECT GLOBAL GENE EXPRESSION DURING HEPATOCYTE MATURATION.

Alder, Olivia[1], Cullum, Rebecca[1], Lee, Sam[1], Bilenky, Misha[2], Griffith, Malachi[2], Morrissy, A. Sorrano[2], Robertson, Gordon[2], Thiessen, Nina[2], Zhao, Yongjun[2], Chen, Qian[3], Pan, Duojia[3], Jones, Steven J.M.[4], Marra, Marco A.[4], Hoodless, Pamela[4]

[1]Terry Fox Laboratory, Vancouver, BC, Canada, [2]BC Cancer Agency, Michael Smith Genome Sciences Centre, Vancouver, BC, Canada, [3]Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD, USA, [4]Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada

Cell fate acquisition is heavily influenced by direct interactions between master regulators and tissue-specific enhancers. However, it remains unclear how lineage-specifying transcription factors, often expressed in both progenitor and mature cell populations, influence cell differentiation. Using in vivo mouse liver development as a model, we identified thousands of enhancers that are bound by the master regulators, HNF4A and FOXA2, in a differentiation-dependent manner, subject to chromatin remodeling and associated with differentially expressed target genes. Enhancers exclusively occupied in the embryo were found to be responsive to developmentally regulated TEAD2 and co-activator YAP1. Our data suggests Hippo signaling may affect hepatocyte differentiation by influencing HNF4A and FOXA2 interactions with temporal enhancers. In summary, transcription factor-enhancer interactions are not only tissue-specific but differentiation-dependent, an important consideration for those studying cancer biology, mammalian development and/or using transformed cell lines.

F-1016
ALGINATE ENCAPSULATION OF STEM CELL DERIVED HEPATOCYTES FOR LIVER REPAIR

Cameron, Kate[1], Lucendo Villarin, Baltasar[1], szkolnicka, Dagmara[1], Dhawan, Anil[2], Wilmut, Ian[3], Forbes, Stuart[4], Hay, David[1]

[1]MRC Centre for Regenerative Medicine, Edinburgh, United Kingdom, [2]Paediatric Liver Centre, King's College Hospital, London, United Kingdom, [3]MRC Centre for Regenerative Medicine, Edinburgh, United Kingdom, [4]University of Edinburgh, Edinburgh, United Kingdom

Introduction: The increase in human liver disease worldwide is of major concern. At present, the only successful mode of treatment for failing livers is organ transplantation. While highly successful, donor organs are a limited resource that cannot meet demand. In addition, the immunosuppression associated, with organ transplantation, can lead to an increase in developing cancer and cardiovascular and renal disease, amongst others. A cell based approach to supporting human liver function that does not require lifelong immunosuppression is therefore of great interest and would overcome the current limitations in hepatocyte supply. While human hepatocytes can be readily isolated from cadavers, treatment strategies are limited due to availability of donor organs and the viability of purified hepatocytes. As such, an alternative source of hepatocytes is required to meet demand. Pluripotent stem cells represent a promising alternative due to their unlimited proliferative and differentiation capacity in culture. Currently PSCs can be readily differentiated towards hepatocytes with approximately 80-90% efficiency. However, these cells are limited by their instability and immature phenotype when cultured on biological cell culture matrices. We believe this must be overcome if stem cell derived hepatocytes are to be scaled and used for therapy. Aim: In this study we aim to deliver mature hepatocyte populations in three dimensional niches employing encapsulation. Method: Human PSCs were differentiated to the hepatoblast stage, as described in (Szknolnicka et al 2014), expanded in Williams essential medium containing wnt inhibitory factor (WIF) on laminin coated culture plates. PSC derived hepatoblasts were encapsulated in 1.5% (w/v) clinical grade sodium alginate at a density of $2.5x10^6$ cells/ml, resulting in microbeads 400-500μm in diameter (Sarkis et al 2001). The cells were matured within the beads in hepatoZYME serum free medium containing HGF and OSM for up to 20 days. Hepatic progenitors were re-plated onto matrigel as a control. Results: Hepatoblasts were successfully generated from H9 hESCs and a GMP grade cell line, Man11. PSC derived hepatoblasts were readily expandable, produced high levels of AFP and retained the ability to differentiate into both hepatocyte-like cells and cholangiocyte-like cells over five passages. Clinical grade alginate-encapsulated hepatoblasts were matured and remained viable up to 10 days post encapsulation as shown via FDA/EtBr staining. Post-encapsulation, cells in alginate demonstrated decreased levels of early hepatoblast genes, produced significantly lower AFP and greater albumin protein production when compared to control cells replated on matrigel. Conclusion: These data demonstrate an expandable and scalable population of bipotent hepatoblasts can be established from PSCs and in a GMP grade ES cell line. Furthermore, encapsulation of hepatoblasts in alginate promotes their maturation towards functional hepatocytes, but as yet does not support viability over longer term culture. We hypothesise that this may be overcome through co-encapsulation of supporting non-parenchymal cell types.

F-1017

REPROGRAMMING OF ADULT HEPATOCYTES TO BIPOTENTIAL PROGENITORS IN SPHEROID CULTURES UTILIZING POLYVINYL ALCOHOL SUBSTRATES

Chien, Chiao-Yun[1], Lee, I-Chi[2], Chang, Fang-Pei[1], Tsai, Hsuan–Ang[1], Chang, Ying-Chih[1], Lee, Hsuan-Shu[3], Shen, Chia-Ning[1]

[1]Genomics Research Center, Academic Sinica, Taipei, Taiwan, [2]Biochemical and Biomedical Engineering, Chang-Gung University, Taoyuan, Taiwan, [3]Institute of Biotechnology, National Taiwan University, Taipei, Taiwan

The liver is an organ with an enormous capability of regeneration upon injury. Recent progress has demonstrated mature hepatocytes can contribute to regeneration process via dedifferentiation to progenitor cell status. However, whether the dedifferentiation of mature hepatocytes to progenitor's fate can be triggered in vitro in a controllable manner still needed be determined. Initially, hepatocytes were seeded on polyvinyl alcohol (PVA) substrates in either serum-containing hepatocyte medium or serum-free medium supplementary with B27 and growth factors. We demonstrated that, when adult hepatocytes were grown on PVA-coated glassware with serum-free medium supplementary with B27 and growth factors, adult hepatocytes formed spheres accompanied by downregulation of hepatic specific features such as expression of asialoglycoprotein receptors and cytochrome P450 enzymes. These sphere cells expressed a panel of stem-cell markers including CD133, EpCAM, CD49f, AFP, and SOX9 and had the potency to differentiate into hepatocytes and cholangiocytes in vitro. Moreover, transplantation of GFP-labeled sphere cells into to the mice treated with carbon tetrachloride (CCL4) could replenish damaged hepatocytes. Lineage-tracing experiments utilizing CK19-CreERT2;UBC-floxedDsRedT3-emGFP mice and Alb-Cre;R26R-CAG-Brainbow2.1 further identified that only fluorescent-labeled (RFP-labeled) hepatocytes isolated from two-stage liver perfusion could directly give rise to sphere cells on PVA substrates which share phenotypic similarities with liver stem/progenitor cells, but not GFP-labeled cholangiocytes. Indeed, PVA is commonly used in medical devices due to its low protein adsorption characteristics. It has also been shown that hydrophilic nature of PVA prevents liver cells adhering to the substrate which therefore forced cells to grow as spheres. It, in turn, triggers mature hepatocytes to reprogram in a serum-free three-dimensional culture system. In conclusion, the findings the capability of reprogramming adult hepatocytes to bipotential progenitors and insulin-producing clusters may potentially have an implication on developing translation therapies for patients with liver diseases and diabetes.

F-1018

ENHANCING MOBILIZATION OF MENOPAUSAL ENDOMETRIUM-DERIVED IPSC DIFFERENTIATED HEPATOCYTES AND LIVER REGENERATION BY INJECTABLE HGF RELEASING HYDROGEL IN FULMINANT HEPATIC FAILURE

Chou, Shih-Jie[1], Chiou, Shih-Hwa[2]

[1]Pharmacology NYMU, Taipei, Taiwan, [2] Department of Medical Research and Education, Taipei Veterans General Hospital, Taipei, Taiwan

Acute hepatic failure (AHF) is a severe liver injury with sustained damage and complications. Whether induced pluripotent stem cells (iPSCs) are an alternative option for the treatment of AHF remains uncertain. In this study, we reprogrammed menopausal women-derived endometrial fibroblasts into iPSCs (MP-iPSCs), which exhibited pluripotency and ability for differentiation into tridermal lineages, including hepatocyte-like cells (iPSC-Heps). These iPSC-Heps resembled human ESC-derived hepatocyte-like cells in gene signature and hepatic markers/functions. To improve iPSC-Heps engraftment, we next developed an injectable carboxymethyl-hexanoyl chitosan hydrogel (CHC) with sustained HGF release (HGF-CHC) and investigated the hepatoprotective activity of HGF-CHC-delivered iPSC-Heps in vitro and in an immunocompromised AHF mouse model induced by thioacetamide (TAA). Intrasplenic delivery of HGF-CHC-iPSC-Heps reduced TAA-induced hepatic necrotic area, and rescued liver function and recipient viability. Comparing to PBS-delivered iPSC-Heps, using HGF-CHC as delivery vehicle enhanced mobilization, matrix metalloproteinases (MMPs) secretion, and antioxidant and anti-apoptotic ability of iPSC-Heps. Importantly, these HGF-CHC-mediated responses could be abolished by administrating HGF neutralizing antibodies. In conclusion, our findings demonstrated that HGF predominantly mediated the effect that enhanced iPSC-Hep migration and hepatoprotection, and HGF-CHC may serve as an excellent vehicle for iPSC-Hep engraftment in iPSC-based therapy against AHF.

F-1019

POTENTIAL OF UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELL CO-CULTURE WITH PRIMARY HUMAN HEPATOCYTES FOR ALGINATE MICROBEAD ENCAPSULATION

Filippi, Celine[1], Barbero-Klem, Fabiane[2], Jitraruch, Suttiruk[2], Lehec, Sharon[2], Fitzpatrick, Emer[2], Mitry, Ragai R.[2], Hughes, Robin D.[2], Dhawan, Anil[2]

[1]Institute of Liver Studies, Biomedical Research Centre at Guy's and St Thomas' and King's College London, London, United Kingdom, [2]Institute of Liver Studies, King's College London, London, United Kingdom

Background: Our group has developed a GMP technique of primary hepatocyte alginate bead transplantation for the treatment of patients with acute liver failure. However, there are concerns about the maintenance of viability and metabolic functions of primary hepatocytes over time. Previous studies have shown the positive effect of mesenchymal stem cells (MSCs) on various primary cell types, improving their viability and function ex vivo. We hypothesized that MSCs would improve the viability and function of cryopreserved primary human hepatocytes isolated from unused donor livers and could be beneficial for alginate-encapsulated hepatocyte transplantation. Aim: To study the effects of MSCs co-culture on primary human hepatocyte function (i) in 2D culture and (ii) in 3D-alginate microbeads. Materials and methods: Human or rat hepatocytes (n=3 each) were plated onto collagen-coated culture plates with or without umbilical-cord derived human MSCs, at ratios of 10:1, 10:2 or 10:3. Supernatants were collected every second day for 23 days for measurement of cell function (albumin synthesis), cell attachment and overall survival (SRB test). Immunofluorescence and albumin ELISA were used to detect potential MSC differentiation into hepatocytes. For microbead tests, hepatocytes and MSC (10:1, n=3) were encapsulated at 3.5.10e6 cell/ml in ultrapure GMP alginate, before measurements of cell numbers and metabolism over 11 days. Results: 1 - In 2D culture, MSC addition dramatically improved hepatocyte attachment, increasing SRB values 7 folds from day 1 ($p<0.05$). MSC proliferated throughout the experiment either in mono or co-cultures. Whilst MSC co-culture did not initially have any effect on hepatocyte function, by day 5 the production of albumin was increased 3 folds and progressing to 40 folds by day 23 ($p<0.002$ vs hepatocyte monoculture). This was not due to MSC differentiation into hepatocytes, as proven by both immuno-fluorescence and further experiments using rat hepatocytes and human MSCs, where no human albumin was detected. 2 - Co-

encapsulation of MSCs and hepatocytes in alginate beads resulted in a total loss of the benefit of MSCs first observed in 2D cultures, with no improvement in albumin synthesis or urea synthesis. MSC stopped proliferating in beads, which could contribute to this loss of function. Conclusion: MSCs have a strong effect on primary human hepatocytes in conventional 2D cultures, which is lost when the cells are encapsulated in pure alginate beads. This dramatic loss of function should warrant further study into the effects of alginate gel formula modifications and cellular interactions.

F-1020
GENERATION OF A TALEN-MEDIATED IPSC MODEL TO STUDY NON-ALCOHOLIC FATTY LIVER DISEASE ASSOCIATED WITH A PNPLA3 POLYMORPHISM

Goyal, Nidhi[1], Ordonez, Maria Paulina[2], Goldstein, Lawrence S.B.[3]
[1]Pediatrics, University of California, San Diego, La Jolla, CA, USA, [2]Pediatrics, University of California, San Diego, San Diego, CA, USA, [3]Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA, USA

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease in the adult and pediatric population and is associated with co-morbidities including obesity, hypertension, type 2 diabetes, and cardiovascular disease, thereby increasing the risk of premature death. NAFLD denotes a histological spectrum of disease ranging from fat accumulation in the liver (steatosis) to fibrosis. NAFLD is a complex disease that has both environmental and genetic components and therapeutic options are limited. Genome-wide association studies (GWAS) have identified a polymorphism in the gene PNPLA3 that has a strong association with risk and severity of NAFLD, with the variant allele of PNPLA3 being associated with more severe biochemical and histological abnormalities. The protein product of PNPLA3, or adiponutrin, is involved in lipid metabolism, but its exact function in humans remains unclear. The pattern of expression of adiponutrin is different in mice and humans, making it difficult to extrapolate findings from animal models. Using TAL effector nuclease (TALEN) technology, we designed TALENs specific to the PNPLA3 SNP. Subsequently, we have now generated isogenic lines of human induced pluripotent cells (hIPSCs) from a known genetic background with the variant and wild-type homozygous alleles of PNPLA3 using these site specific TALENs. We are able to induce differentiation of hIPSC to hepatocyte like cells (HLC) that have typical morphology and lineage specific markers. We will use IPSC derived HLCs with the wild type and risk alleles of PNPLA3 to test the hypothesis that polymorphisms of PNPLA3 induce abnormal lipid processing as a potential early pathogenic event in NAFLD. To our knowledge, this is the first set of isogenic lines of hiPSCs designed specifically with the PNPLA3 wild type and variant alleles. Our approach translates a population-based GWAS into an in vitro human model to study the pathophysiology of NAFLD at a fundamental level.

INTESTINAL / GUT CELLS

F-1022
TRANSCRIPTIONAL AND FUNCTIONAL DECLINE OF THE INTESTINAL STEM CELL NICHE DURING AGEING.

Nefzger, Christian M.[1], Horvay, Katja[2], Jarde, Thierry[2], Knaupp, Anja Sylvia[1], Abud, Helen E.[2], Polo, Jose Maria[1]
[1]Anatomy and Developmental Biology/Australian Regenerative Medicine Institute, Monash University, Clayton, Australia, [2]Anatomy and Developmental Biology, Monash University, Clayton, Australia

Organ homeostasis and regeneration are facilitated by multipotent tissue stem cells that give rise to all the mature cell types of a specific organ. During an organism's life, adult stem cell pools are maintained by cell division requiring both the genome and the epigenome to be faithfully copied. However, according to the relatively few studies conducted to date, changes in the epigenome and in consequence gene expression, can be detected in a large number of aged organs/tissues. The intestinal epithelium is an ideal model to study ageing as it constitutes a high-turn-over tissue that is renewed every 4-5 days. Epigenetic changes are likely to accumulate in the intestinal stem cell (ISC) pool due to its high rate of cell division, and indeed age-related changes in DNA methylation status have been shown for whole organ preparations. Coincidentally, incidence of many types of cancer (e.g. colorectal cancer) also increase with age, moreover alterations of the epigenome have been suggested to be the underlying causes. Age related changes also impact on functional properties of the intestinal epithelium as evidenced by a reduced capacity to take up essential nutrients like calcium and phosphorus. Herein, we show that in vitro age related decline is reflected by a reduced capacity of intestinal crypts isolated from aged mice to give rise to organoids, intestinal structures with crypt and villus-like epithelial domains that contain ISCs, transit amplifying cells and all differentiated cell types. Furthermore, expression array analysis of the aged crypt compartment shows profound transcriptional changes. To further investigate the underlying causes of functional and transcriptional changes within different cell types we utilise a set of cell surface markers (EPHB2, Epcam, CD44, GRP78, CD24, CD44, CD31) to isolate ISCs, transient amplifying cells and Paneth cells from young and aged animals. Using the Lgr5-GFP reporter mouse and qRT-PCR analysis we demonstrated that our FACS strategy efficiently enriches for Lgr5-GFPhi cells with a stem cell signature while excluding differentiated cell types. ISCs isolated with our sorting strategy are also able to give rise to organoids when embedded in matrigel and exposed to growth factors (Wnt3a, CHIR, Noggin, R-Spondin, Rock inhibitor).

F-1023
NICHE APPROPRIATION BY DROSOPHILA INTESTINAL STEM CELL TUMORS

Patel, Parthive H., Dutto, Devanjali, Edgar, Bruce A.
German Cancer Research Center (DKFZ) and Center for Molecular Biology Heidelberg, Heidelberg, Germany

The importance of immune cells, fibroblasts, and vasculature recruited to the tumor microenvironment is widely appreciated, but how stem cell-derived tumor initiating cells interact with the stem cell niche prior to this, during tumor initiation, is poorly understood. Here we investigate intestinal stem cell (ISC) tumors generated in Drosophila by blocking Notch signaling. These differentiation-defective cells produce an autocrine, progenitor cell-specific EGFR ligand (Spitz), which supports early tumor growth. Neighboring enterocytes initially restrict tumor growth but after achieving a critical mass, tumors induce

JNK signaling, apoptosis, and cytokine (Upd2,3) expression in these enterocytes, and another EGFR ligand (Vein) in visceral muscle. These paracrine signals, which are normally used within the niche to support regenerative growth, propel tumor growth. We propose that niche appropriation by differentiation-defective stem cells may be a common mechanism of tumor initiation.

F-1024

STRUCTURAL BASIS OF WNT SIGNALING IN ADULT STEM CELLS MODULATED BY LGR5, R-SPONDIN 1 AND E3 LIGASE ZNRF3

Peng, Weng Chuan
Utrecht University, Utrecht, Netherlands

The Wnt target gene leucine-rich repeat G-protein coupled receptor 5 (Lgr5) encodes a receptor that is exquisitely specific to adult stem cells in small intestine and colon, stomach, hair follicle, liver, kidney and mammary gland. R-spondin, a potent Wnt agonist, triggers adult stem cell proliferation in vivo and in intestinal organoid culture. Recently, transmembrane E3 ligases ZNRF3 and its homolog RNF43 were found to negatively regulate Wnt signaling by ubiquitinating Wnt/LRP6/Frizzled complexes. RSPO1 was proposed to target ZNRF3 for removal from cell surface in an LGR5-dependent manner, thus increasing the availability of Wnt complexes for signaling. We present here crystal structures of RSPO1 bounds to the receptor LGR5 ectodomain and co-receptor ZNRF3 ectodomain. Two furin-like domains (Fu1 and Fu2) of RSPO1 mediate binding and signaling. RSPO1 binds LGR5 on its concave surface through Fu2 domain and engages ZNRF3 through Fu1 domain. Surface plasmon resonance binding studies shows that RSPO1 binds LGR5 strongly but interact weakly with ZNRF3. LGR5 ectodomain serves as high affinity receptors to recruit RSPO1 on cell surface while the transmembrane domain mediates membrane clearance of ZNRF3. Structural analysis indicates that RSPO1 would compete with Frizzled for binding to ZNRF3. We further performed mutagenesis studies to understand how disease mutations in anonychia patients affect signaling. In addition, crystal structures revealed unexpected quaternary arrangement and the physiological relevance of this would be discussed. Thus, based on structural and functional studies, we elucidate how LGR5, RSPO1 and E3 ligase ZNRF3 regulate Wnt signaling in adult stem cells.

F-1025

INVESTIGATING NICHE DEPENDANCY OF EARLY AND LATE STEM CELL-DERIVED TUMORS OF THE ADULT FLY INTESTINE

Petersson, Monika, Patel, Parthive H., Edgar, Bruce A.
German Cancer Research Center (DKFZ) and Center for Molecular Biology Heidelberg, Heidelberg, Germany

The complex issue of how the microenvironment interacts with tumor cells to propel tumor progression is still not well understood. Here, we utilize a Drosophila intestinal stem cell (ISC)-derived tumor system as a tool to decipher signalling cues governing tumor and niche interactions during tumor progression. Previously, it has been shown that deficient Notch signalling in ISC lineages results in tumorous growth. Recent data from our lab strongly suggest that during early steps of tumor initiation, ISC-derived tumor cells secrete EGF ligands thereby activating EGF/MAPK signalling autonomously to promote their own growth. However, after achieving a critical mass, tumor cells stimulate neighboring cells (dying absorptive enterocytes) to secrete cytokines to additionally promote tumor outgrowth suggesting the requirement of a niche for tumor progression. Additionally, we found that Notch-deficient tumors fragments fail to progress to large tumor masses following transplantation. In contrast, we discovered that transplanted Notch deficient ISC-derived tumor cells expressing oncogenic RasV12 can grow outside their original stem cell niche. Manipulating distinct signalling cues known to be required for tumor initiation in situ (e.g. cytokines, EGF) in the host and subsequent transplantation of ISC-derived tumor cells into the manipulated host will uncover niche-ISC-derived interactions to further delineate the role of the tumor microenvironment during tumor progression. Interestingly, transplanted Notch deficient RasV12 tumor cells seem to recruit a new microenvironment after progressing further. This involves the recruitment of trachea, which supply oxygen, indicating that ISC-derived tumor cells allocate factors to promote their growth. Intriguingly, we also observed a colonization of distal tissues. Future studies could reveal how late progressed and colonized ISC-derived tumors recruit factors or cell types to set up a novel niche.

F-1026

ENTEROENDOCRINE CELLS CONTROL THE PLASTICITY OF THE STEM CELL NICHE VIA BURSICON/DLGR2 PARACRINE SIGNALING IN THE DROSOPHILA MIDGUT

Scopelliti, Alessandro[1], Cordero, Julia[1], Fengqiu, Diao[2], Strathdee, Karen[1], White, Benjamin[2], Samson, Owen[1], Vidal, Marcos[1]
[1]Cancer Research UK Beatson Institute, Glasgow, United Kingdom, [2]NIMH, Bethesda, MD, USA

Stem cells maintain tissue homeostasis by adapting their activity to microenvironmental or "niche" signals. The molecular and cellular mediators of niche signaling are, in general, poorly understood. Mammalian Leucine-rich repeat-containing G protein-coupled Receptors (LGRs) have been recently characterized as somatic stem cell markers in multiple epithelial tissues. However, their biological role in adult tissues remains largely unexplored. The epithelium of the Drosophila adult midgut is replenished by dedicated intestinal stem cells (ISCs). Drosophila LGR2 (DLGR2), encoded by the rickets (rk) gene, is orthologue to mammalian LGR4, 5 and 6. Described functions of DLGR2 and its ligand, the neuroendocrine hormone Bursicon, are restricted to the regulation of post-molting cuticle hardening and wing maturation. Using the adult Drosophila midgut, here we demonstrate a novel paracrine signaling system, in which the secretory linage, the enteroendocrine (ee) cells, and the visceral muscle (VM) cooperate to constrain proliferation of ISCs in homeostatic conditions. Midgut ee cells secrete Bursicon, which acts as a paracrine factor on the VM through DLGR2. Bursicon/DLGR2 regulate ISC proliferation through the expression of the VM-derived EGF-like growth factor Vein. Impaired Bursicon or DLGR2 results in ISC hyperproliferation and cell accumulation. Reciprocally, overexpression of Bursicon suppresses ISC proliferation in young growing, regenerating and ageing midguts. Our results identify a novel cellular and molecular network involved in the regulation of ISC quiescence through modifications of the stem cell niche, which uncovers a novel role of ee cells and the conserved ligand/receptor Bursicon/DLGR2.

F-1027

REGULATION OF STRING DURING DROSOPHILA INTESTINAL STEM CELL PROLIFERATION

Xiang, Jinyi[1], Edgar, Bruce A.[2]
[1]Cell Proliferation and Growth, German Cancer Research Center and Heidelberg University, Heidelberg, Germany, [2]German Cancer Research Center (DKFZ) and Center for Molecular Biology Heidelberg, Heidelberg, Germany

The adult Drosophila midgut is a highly regenerative organ that is maintained by intestinal stem cells (ISC). ISC division produces new

ISCs and enteroblast (EB) daughters, which differentiate into the major midgut cell types, enterocytes (EC) and enteroendocrine (EE) cells. The mono-layered epithelium of the midgut is regularly replenished by stem cells once every two weeks under normal physiological circumstances. However in response to epithelial damage or stress conditions, such as bacterial infection, DNA damaging agents, oxidative stress, induced apoptosis, or JNK stress signaling activation, damaged ECs produce ligands of EGFR and JAK/STAT signaling to activate these pathways in ISCs and EBs, thus promoting their division and differentiation to compensate for epithelial cell loss. During this feedback, we find that the string (stg) gene, a Drosophila homologue of Cdc25 phosphatase that is the ultimate regulator of mitosis in most eukaryotic cells, is strongly increased in expression. Gain- and loss- of function studies show that stg is necessary for midgut stem cell proliferation to response to the extrinsic growth signals from the midgut epithelium. Moreover, we find that one small DNA fragment (4.3kb) of the very large stg control region (>30kb) is sufficient to drive stg transcription in midgut stem cells. To elucidate the mechanism of how extrinsic signals regulate this key cell cycle gene, we will use the yeast 1 hybrid (Y1H) technique to screen for candidate transcription factors that can directly bind this specific enhancer and regulate stg expression in intestinal stem cells. With this work, we expect to find the essential link between signal transduction factors and cell cycle control in stem cells.

LUNG CELLS

F-1029
DIRECT DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO MATURE AIRWAY EPITHELIUM CELLS FOR DISEASE MODELING, IN VITRO DRUG TESTING AND LUNG ENGINEERING.

Ghaedi, Mahboobe, Laura E. Niklason
Department of Anesthesia and Biomedical Engineering, Yale University, New Haven CT, USA

Background and Aims: It is postulated that the use of induced pluripotent stem (iPS) cells may be the most effective strategy to develop patient-specific respiratory epithelial cells that may be valuable in lung-related cell therapy and lung tissue engineering. Efforts to differentiate human iPS cells into lung epithelia focused on the generation of the distal airway epithelial phenotypes Fewer studies have targeted the differentiation of airway epithelial cells despite the fact that airway diseases such as asthnma, cystic fibrosis are more prevalent than the disease of alveoli such as emphysema Methods: In this study we successfully differentiated human iPS cells toward epithelial airway cells using our efficient and consistent, step-wise differentiation method. The dynamics of differentiation were examined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), flow cytometry and immunocytochemistry. Results: Differentiated cells had phenotypic properties similar to mature human airway cells. As determined by flow cytometry up to 97.5% of cells were positive for P63, and 100% of cells were positive for CCSP, (Clara cells), SOX2 and FOXJ1(ciliated cells) 92.2 % and 62% of cells were positive for CK5 and CFTR respectively. Close to 24% of cells were positive for goblet cell marker, Mucin 5AC and 56.9% of cells were positive for basal cell marker C151. Quantitative RT-PCR also revealed that CK5, CCSP, CFTR, P63, FOXJ1 and Mucin-5AC were highly expressed in iPS derived airway epithelial cells, with the relative levels compared to freshly isolated human airway cells. Conclusion: Our study provides a method for generating patient-specific airway epithelial cells and develops new strategies to use autologous cells (iPSC derived alveolar epithelium) for disease modeling, drug discovery and human lung regeneration *in vitro*

F-1030
GENERATION OF ALVEOLAR EPITHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Gotoh, Shimpei[1], Ito, Isao[1], Nagasaki, Tadao[1], Yamamoto, Yuki[1], Konishi, Satoshi[1], Korogi, Yohei[1], Matsumoto, Hisako[1], Muro, Shigero[1], Hirai, Toyohiro[1], Mae, Shin-Ichi[2], Funato, Michinori[2], Toyoda, Taro[2], Sato-Otsubo, Aiko[3], Ogawa, Seishi[3], Osafune, Kenji[2], Mishima, Michiaki[1]
[1]Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan, [2]Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan, [3]Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Type II alveolar epithelial cells (AECs) are a major epithelial component of the peripheral lung, where they secrete pulmonary surfactant and are differentiated into type I AECs covering the majority of the surface area of human alveoli. The methods for differentiating and isolating type II AECs from human pluripotent stem cells (hPSCs) are in demand in order to understand the complicated differentiation of human alveolar cells and future application. In this study, we stepwisely induced $CXCR4^+SOX17^+$ definitive endoderm cells at an efficiency of >=80%, $SOX2^+FOXA2^+$ anterior foregut cells at an efficiency of >=88%, and $NKX2\text{-}1^+FOXA2^+$ ventral anterior foregut cells at an efficiency of 57.0~77.5% for several hPSC lines, including H9 human embryoinic stem cells, and 201B7 and 585A1 human induced pluripotent stem cells (hiPSCs). Then $SFTPB^+$ and $SFTPC^+$ cells were induced through further differentiation. Next, we generated SFTPC-GFP knock-in reporter hPSCs to detect and isolate $SFTPC^+$ cells, using bacterial artificial chromosome (BAC)-based recombination technique. By differentiating the reporter cell-line, we could detect $SFTPC^+$ cells in two- and three-dimensional differentiation. Furthermore, we found that alveolar differentiation was more specific in three-dimensional differentiation than two-dimentional differentiation, in terms of suppressing the expression of club cell marker protein, SCGB1A1 and SCGB3A2. These findings would accelerate understanding of the complicated differentiation of human alveolar cells and open the door to the development of new strategies for in vitro toxicology and, cell replacement therapy as well as screening of therapeutic drug compounds in the future.

F-1031
SEVERAL COMPONENTS AFFECT THE IN VITRO SPHERE FORMATION OF MOUSE AIRWAY EPITHELIAL STEM CELLS

Hegab, Ahmed E.M., Arai, Daisuke, Kuroda, Aoi, Soejima, Kenzo, Betsuyaku, Tomoko
Keio University School of Medicine, Tokyo, Japan

The niche interacts with stem cells to regulate their homeostasis. Sphere formation assay has been widely used to study stem cell behavior in vitro based on its capacity to evaluate self-renewal, differentiation and effect of various medium supplements, drug treatments and co-culture. Objectives:To explore the effect of endothelial cells, fibroblasts, fibroblasts growth factor (FGF)-2, 9 and 10, FGF-receptors (FGFRs) and leukemia inhibitory factor (LIF) on both proximal (basal cells) and distal (Clara/alveolar cells) lung stem cells in vitro growth and differentiation, as a possible contributors to their niche.Methods:Freshly collected airway basal cells and distal lung epithelium were mixed with Matrigel and seeded in transwells with or without mouse fibroblasts or endothelial cells, and various treatments

were supplied to medium in lower chamber. Sphere numbers and diameters were assessed from images observed at 2-5 weeks. Sphere wells paraffin sections were stained with H and E and various cellular markers to study proliferation and differentiation.Results: Distal lung epithelium requires co-culture with fibroblasts, but not with endothelial cells, to undergo clonal proliferation and form spheres in vitro. Histological analysis of spheres showed that fibroblasts tightly wrapped them. Treating co-culture wells with FGF2, FGF9, FGF10 and LIF increased the sphere formation efficiency. FGF treatments resulted in loss of the tight wrapping of fibroblasts around the spheres. However, treating distal lung epithelium (without fibroblast co-culture) with FGF2, FGF9, FGF10, fibroblasts-conditioned medium or culturing fibroblasts in lower chamber; all failed to support sphere formation. Treating distal lung epithelial cells co-cultured with fibroblasts with the FGFR blockers PD173074 or AZD4547 partially inhibited sphere formation and abolished the FGFs-induced increase in sphere formation indicating that one or more of FGFs/FGFRs is/are involved in the fibroblasts' support for epithelial progenitors. Our distal lung epithelial spheres showed the previously described three distinct types of epithelial colonies, the round luminal spheres arising from proximal conducting airway, and the large saccular and small dense spheres arising from terminal bronchioles and alveoli (Chen et al Stem cells 2012). Interestingly, co-culture with fibroblasts isolated from the mouse trachea favored the growth of the round luminal spheres while using fibroblasts isolated from the mouse lung favored the growth of the large saccular and small dense spheres. Tracheal basal cell sphere formation did not require fibroblast or endothelial cells co-culture, but still fibroblasts co-culture could enhance sphere formation efficiency. Treating basal cells with FGFs increased sphere formation while FGFR blockers reduced number and size of spheres and abolished the FGFs-induced effect on sphere numbers. RT-PCR analysis revealed the mRNA expression of FGF2, FGFR1 and FGFR2, but not FGF9, FGF10 or FGFR3 in fibroblasts. Both tracheal and distal airway epithelium mainly expressed FGFR3. Conclusion: Taken together, these data confirm the requirement of physical proximity of fibroblasts for distal lung epithelial sphere formation.The fibroblasts' ability to support distal lung epithelial sphere is influenced by the tissue of origin of fibroblasts. FGFs/FGFRs signaling are involved in regulating sphere formation of both basal cells and distal lung epithelial cells.

EPITHELIAL CELLS (NOT SKIN)

F-1032
DUAL ROLES FOR ID4 IN THE REGULATION OF ESTROGEN SIGNALING IN THE MAMMARY GLAND AND OVARY

Best, Sarah Ann[1], Hutt, Karla J.[2], Liew, Seng H.[2], Lindeman, Geoffrey J.[1], Visvader, Jane E.[1]
[1]ACRF Stem Cells and Cancer Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, [2]Ovarian Biology, Prince Henry's Institute of Medical Research, Melbourne, Australia

The HLH transcriptional regulator Id4 exerts important roles in different organs including the neural compartment, where Id4 loss usually results in early lethality. To circumvent such effects and explore the role of this basally-restricted transcription factor in the mammary gland, we generated a cre-inducible mouse model. MMTV-cre mediated deletion led to a delay in ductal morphogenesis, consistent with previous findings using a germ-line knock-out model. A striking increase in the expression of ER, PR and FoxA1 was observed in both the basal and luminal cellular subsets of Id4-deficient mammary glands, implicating Id4 as a negative regulator of the ERα axis. Ovaries of targeted mice were nodular and contained aberrant mature follicles. Moreover, expression of the cascade of enzymes critical for estrogen biosynthesis in the ovary was decreased, suggesting that the mammary ductal defects in Id4-deficient mice are secondary to compromised ovarian function and decreased circulating estrogen. Together, these data identify Id4 as a novel regulator of estrogen signaling, in which Id4 restrains ERα expression in the basal and luminal cellular compartments of the mammary gland, and regulates estrogen biosynthesis in the ovary.

F-1033
THE CROSS-TALK BETWEEN NOTCH AND WNT SIGNALING REGULATE LUMINAL CELL FATE IN THE MAMMARY GLAND

Bhat, Vasudeva[1], Sun, YuJia[1], Weger, Steven[1], Raouf, Afshin[2]
[1]Department of Immunology, University of Manitoba, Winnipeg, MB, Canada, [2]University of Manitoba, Winnipeg, MB, Canada

Human mammary gland is composed of networks of ducts and alveolar structures that are formed during puberty. Luminal and the myoepithelial cells make up the functional cells of the mammary gland. At the time of lactation, the mammary epithelial cell numbers increase rapidly and subsequently regress to their non-pregnant state. This dynamic process of expansion and regression shows that the mammary gland has a unique regenerative capability that allows multiple cycles of pregnancies. Recent evidence suggests that the regenerative potential of the mammary gland is due to a small self-renewing population of stem cells. Stem cells differentiate into bipotential progenitors that give rise to lineage-restricted luminal or myoepithelial progenitors. These restricted progenitors in turn produce the mature luminal and the myoepithelial cells. Evolutionary conserved signaling pathways control the special functions of these stem and progenitor cells. Current research suggests the Wnt signaling pathway plays essential roles in regulating the self-renewal capacity of the breast stem cells. We previously demonstrated that signaling through NOTCH3 receptor (NR3) specifically commits the bipotent progenitors to the luminal cell fate. In contrast to the Notch signaling paradigm, our observation suggested that NR3 acts through non-redundant target genes. In this study then, we set out to identify the unique targets of NR3. For this purpose, we used short hairpin RNA to knockdown the transcript levels of each of the 4 Notch Receptors (NRs) in human mammary epithelial cells (HMECs) as well as 184-hTERT cells (a non-malignant human breast epithelial cell line). We examined the successful and specific decrease in the expression of each receptor at the RNA and the protein levels. Next, changes to the transcript levels of known Notch target genes due to the loss of each Notch receptor were examined using the Notch pathway-specific pPCR Array. The qPCR array data identified a set of genes whose transcript expression was specifically and exclusively regulated by NR3 signaling; namely, DLL1, JAG2, MMP7, Keratin1 and FZD7. We had previously found that FZD7, a Wnt signaling receptor, and NR3 were co-expressed in the luminal progenitors and therefore we studied the role of NR3 in regulating FZD7 expression further. Utilizing gain of function studies we demonstrated that only a constitutively activated form of NR3 and not any other NRs could up regulate FZD7 gene expression at RNA and the protein levels. Also, we show that while only 1-1.5% of the normal human breast cells express FZD7, the luminal progenitors show a higher expression of this Wnt receptor (2 fold) compared to the undifferentiated bipotent progenitors. Interestingly, when we examined all other 9 Wnt receptor family members, none were regulated by the Notch signaling. The role of Wnt signaling in luminal cell fate determination had not been investigated before. Our findings suggest that a potential cross-

talk between the Notch and Wnt signaling pathway might be involved the luminal cell fate determination. We are at the moment trying to ascertain if the canonical or non-canonical activation of FZD7 is involved in regulating the commitment of the undifferentiated bipotent progenitors to the luminal cell fate. These finding are likely to be important in understanding how alterations to the Notch-Wnt signaling network may lead to the development and/or progression of breast cancer tumors.

F-1034
INTEGRATION OF SIGNALING CUES DURING RESPIRATORY LINEAGE SPECIFICATION

Budjan, Christoph[1], Martinez Arias, Alfonso[2], Rawlins, Emma[1]
[1]Gurdon Institute, University of Cambridge, Cambridge, United Kingdom, [2]Department of Genetics, University of Cambridge, Cambridge, United Kingdom

How do cells integrate and compute various extrinsic signals to make a decision about their fate? I am using the specification of the respiratory lineage in the developing endoderm as a general paradigm to understand how cells integrate signal inputs in a spatio-temporal manner to make a fate decision. Specifically, do individual signaling pathways make quantitative or functional contributions to specifying progenitor cells? Additionally, how does the tem-poral dynamics of signaling activity influence cell fate decision making? Answering these questions will be important and highly relevant to developing directed differentiation approaches using iPS cells. I am using a combination of ex vivo chemical genetics and in vivo genetics to investigate functional and quantitative contributions of Fgf, Wnt and Bmp signaling pathways in respiratory lineage specification. Modulation of signaling strength, duration and timing allows me to dissect individual quantitative, as well as functional contributions, of signaling pathways in this process. I have shown that specification of respiratory progenitors is characterized by highly dynamic signaling signatures of Fgf/Erk, Bmp and Wnt signaling pathway activities in vivo. Strikingly, onset of respiratory marker gene expres-sion in the developing embryo coincides with a transient pulse of canonical Wnt signaling activity. Manipulation of FGFR and Wnt signaling leads to mis-patterning and switches in cell fates in a time and dose-dependent manner. Moreover, I have shown that competence to differentiate towards the respiratory lineage in response to cues is restricted to a subset of anterior endoderm cells. Taken together, my in vivo and ex vivo analysis reveals a critical time window during which anterior endoderm cells can respond to the key signaling inputs and commit to respiratory progenitor cell fate. How integration of multiple signals and signaling dynamics is computed on a cellular and tissue level will not only be critical for understanding how cell diversity is achieved during the development of an organism but will also be important for developing rational differentiation and reprogramming protocols for regenerative medicine.

F-1035
LOSS OF IGFBP7 LEADS TO THE EXPANSION OF LUMINAL PROGENITOR POOL THROUGH DIMINISHED DIFFERENTIATION POTENTIAL OF THE STROMAL FIBROBLASTS

Chatterjee, Sumanta[1], Yang, W.Y.[2], Spyropoulus, D.[3], Bacopulos, Stephanie[2], Seth, Arun[2], Raouf, Afshin[1]
[1]Immunology, Regenerative Medicine Program and Cancer Care Manitoba, University of Manitoba, Winnipeg, MB, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]University of South Carolina, South Carolina, SC, USA

Insulin like growth factor binding protein 7, Igfbp7, is a secreted peptide that in addition to modulating insulin and insulin-like growth factor signaling, acts as a tumor suppressor gene in breast and other cancers. To elucidate the role of Igfbp7 in regulating the proliferation and differentiation of mammary epithelial cells, we recently examined the development and maturation of mammary gland in Igfbp7-null mice. We found that these mice showed significant overall retardation in mammary gland development, in particular during the alveolar differentiation stage. This phenotype suggested to us that the luminal progenitor cell functions may be effected due to the lack of Igfbp7 expression. In this study we characterized the proliferation and differentiation potential of the Igfbp7-null luminal progenitors. For this purpose, we used the robust colony forming cell assay to quantify the total number of luminal progenitors in the Igfbp7-null and the Wild-Type (WT) mammary glands in 11-week old virgin mice. Surprisingly, observed a 3±0.25 fold expansion of the luminal progenitors in the Igfbp7-null glands which is not in keeping with the growth retardation of that is observed in these glands. To further characterize the expansion potential of the Igfbp7-null luminal progenitors, we placed the WT or Igfbp7-null luminal progenitors in matrigel assays. Interestingly, after 7 days, the Igfbp7-null luminal progenitors showed on average a 3.5-4 fold expansion potential compared to only 2-fold expansion in the WT luminal progenitors. We then hypothesized that the Igfbp7-null mammary glands might provide an environment that is not supportive of the luminal progenitor cell differentiation. To test this hypothesis, we examined the ability of WT or Igfbp7-null stromal fibroblasts to support luminal progenitor differentiation potential using the CFC assays. When WT luminal progenitors were placed in CFC assays in co-cultures with Igfbp7-null fibroblasts we found a significant decrease in the number of colonies formed compared to the WT fibroblasts co-cultures. The Igbp7-null luminal progenitors generated fully developed luminal colonies in co-cultures with the WT fibroblasts but failed to develop substantial colonies in co-cultures with Igfbp7-null fibroblasts. It is interesting to note that the Igfb7-null luminal progenitors formed larger colonies compared to the WT luminal progenitors and that each colony contained significantly more cells per colony. To understand the molecular mechanisms that may be involved in the enhanced proliferation potential of the Igfbp7-null luminal progenitors, we examined the expression of known breast oncogenes in these progenitors. Interestingly, we found that compared to the WT progenitors the Ighfbp7-null luminal progenitors showed significantly increased transcript expression of Lin28 and Her2/Neu while they showed significantly decreased expression of Brca1. These findings suggest that loss of a potential tumor suppressor gene (i.e. Igfbp7) may provide an efficient mechanism to produce a pool of highly proliferative breast cells through cell intrinsic effects on the progenitors cells but also by attenuating the stromal fibroblasts ability to support their differentiation.

F-1036

MULTI-LINEAGE POTENTIAL AND SELF-RENEWAL DEFINE AN EPITHELIAL PROGENITOR CELL POPULATION IN THE ADULT THYMUS

Chidgey, Ann[1], Wong, Kahlia[1], Seach, Natalie[1], Barsanti, Marco[1], Lim, Joanna Mei Ch'wan[1], Hammett, Maree[1], Khong, Danika[1], Gray, Daniel H.[2], Boyd, Richard[1]

[1]Anatomy and Developmental Biology, Monash University, Melbourne, Australia, [2]Walter and Eliza Hall Institute, Melbourne VIC, Australia

Thymic epithelial cells are critical for T cell development and self-tolerance. However, the thymus gradually involutes with age, which paradoxically begins early in life, leading to >75% loss of function by mid-life and hence profound decrease in naïve T cell production. A central feature to thymic involution is the loss in epithelial cells, the mechanisms for which remain unresolved. While the fetal thymus has been widely investigated, progress in the adult has been hindered due to difficulties in isolation techniques of thymic epithelial cells, which make up less than 1% of the total adult thymus cellularity, and lack of adult TEC functional validation assays. The existence of thymic epithelial progenitor or stem cells in the adult has been inferred, however, they have not been phenotypically identified or characterised, nor has the sequence of postnatal cortical and medullary epithelial cell generation been determined. Through a combination of comprehensive cellular and molecular characterisation, *in vivo* turnover and functional analysis by 3D *in vivo* reaggregate organ culture and our newly described *in vitro* culture system, we have for the first time identified an adult thymic epithelial progenitor population contained within a subset of phenotypically immature cortical epithelium. We have demonstrated their self-renewal, colony forming potential and importantly, the generation of mature cortical and medullary cell lineages, including the autoimmune regulator (Aire)+ medullary subset. We have also demonstrated for the first time that generation of adult thymic epithelial cell lineages shows a similar hierarchy to fetal thymus, with medullary lineages differentiating from cortical precursors. The identification of adult thymic epithelial progenitor cells will provide an important catalyst for the field to progress investigations into the molecular regulation of these critical cells during homeostasis, differentiation and regeneration following damage, and degeneration with age. In the longer term, this will enable more strategically targeted approaches for therapeutic thymic regeneration.

F-1038

TELOMERASE REVERSE TRANSCRIPTASE EXPRESSION IDENTIFIES ENDOMETRIAL PROGENITORS WITH EPITHELIAL DIFFERENTIATION POTENTIAL

Gargett, Caroline E.[1], Breault, David T.[2], Deane, James[1]

[1]The Ritchie Centre, MIMR-PHI Institute of Medical Research, Clayton VIC, Australia, [2]Boston Children's Hospital, Boston, MA, USA

Rare epithelial and stromal stem/progenitor cells (SPC) have recently been identified in the endometrial lining of the uterus, which undergoes cyclical regeneration in the adult female mouse each estrus cycle. However the role of these SPC in endometrial regeneration is not clear due to the lack of a traceable marker. The objective of this study was to use telomerase reverse transcriptase (mTert) reporter mice to identify mouse endometrial SPC and examine their epithelial differentiation potential. mTert-GFP mice were examined for GFP expression using endogenous GFP fluorescence or GFP antibody, and confocal microscopy. Cell lineage tracing used mTert-CreER::R26R mice, injected with tamoxifen on postnatal day 3 to permanently mark the mTert lineage with LacZ expression, and harvested 10 (prepubertal, n=4) and >40 days (cycling, n=12) later . Mouse uteri were also dissociated and examined by flow cytometry to identify intrinsic non-leukocyte (CD45 -ve) mTert+ stromal cells. Dual colour fluorescence microscopy was used to co-localise mTert+ cells with proliferating (Ki-67), BrdU+ label retaining, or estrogen receptor-α (ERα) expressing cells. A rare population of intrinsic (CD45 -ve) endometrial stromal cells expressed mTert reporter (GFP) during development and in adult mTert reporter mice. Rare focal regions of epithelial GFP expression were observed in both the glandular and luminal epithelium in adult mice. These mTert+ cells were distinct from stromal and epithelial label-retaining cells previously identified in mouse endometrium. Stromal mTert+ cells did not express ERα but epithelial mTert+ cells did. Epithelial mTert+ cells were not slow cycling and displayed a level of proliferation comparable to mTert -ve epithelium. Lineage tracing during development showed that mTert lineage cells were confined to the stroma of prepubertal endometrium, but contributed to glandular and luminal epithelium in the endometrium of adult cycling mice. In adult mice, removal of endogenous ovarian hormones by ovariectomy resulted in endometrial atrophy and blocked expansion of the epithelial mTert lineage. Our data suggest that epithelial structures of the adult cycling endometrium originate from an mTert+ stromal stem/progenitor cells that undergoes mesenchymal-to-epithelial transition during cyclic endometrial regeneration. This is the first report of a traceable marker for endometrial progenitors with epithelial differentiation potential.

F-1039

MICROARRAY ANALYSES COCHLEA-DERIVED OTOSPHERES REVEAL PUTATIVE TRANSCRIPTION FACTORS WHICH REGULATE CHARACTERS OF THE OTOSPHERES

Iki, Takehiro, Tanaka, Michihiro, Saito, Megumu, Fujibuchi, Wataru, Nakahata, Tatsutoshi

Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

About 360 million people are suffered from hearing loss in the world. There are two types of hearing loss. One of them, sensorineural hearing loss is permanent because auditory hair cells in the inner ear cannot regenerate in mammals generally. It is difficult to obtain the auditory tissues from a living human because the inner ear is encapsulated with hard bone, and the puncture to that capsule for biopsy results in the serious deterioration of the inner ear function. Even if biopsy could be done, the amount of the inner ear tissue would be limited. These problems prevent the progress of human hearing loss research. Therefore, it is useful to generate hair cells in vitro for analysis of hearing loss. Recently several reports demonstrated successful differentiation into hair cells from pluripotent stem cells. However, there is no protocol with reliable results using human iPS cells. Instead of hair cells, we obtained otospheres included with otic stem/progenitor cells from neonatal mice cochlea sensory epithelia (CSE). Otospheres have some advantages that they can be cultured for long term and in defined medium, and can be differentiated into cochlea cells including hair cells. It is necessary to understand the gene expression of otospheres for producing them from human cells, although little is known. To reveal the gene expression profile of otospheres, we performed transcriptome analyses of neonatal mice derived-otospheres with cDNA microarrays. We found that gene expression analysis showed each otosphere samples were relatively homogeneous in principal component analysis. Expression profiles of otosphere, CSE and mouse ES cell samples were not overlapped in hierarchical clustering analysis. There were 8810 probes corresponding to varying gene expression between otospheres and CSE. Gene ontology analysis demonstrated the difference of properties between CSE and otospheres. We also confirmed the

difference of transcriptional factors between two groups. Some transcription factors including in those genes would be key factors to characterize properties of otospheres.

EPIDERMAL CELLS

F-1040
UNDERSTANDING THE MECHANISMS OF TISSUE REPAIR BY LIVE IMAGING

Park, Sangbum[1], Greco, Valentina[2]
[1]Genetics, Yale Medical School, New Haven, CT, USA, [2]Yale Medical School, New Haven, CT, USA

The most important role of skin is to protect our body against the outer environment. Maintaining skin homeostasis is directly connected to both disease and survival. Skin and its appendages, including hair follicles, display a high turnover in homeostasis and can readily regenerate after injury. As in other adult tissues, regeneration of adult skin is dependent on the regulation of stem cells by a diversity of molecular signals. While we are beginning to shed light on how signaling pathways regulate skin regeneration, the dynamic behaviors of specific stem cell populations controlled by these signals during injury repair remain unclear. The challenge in addressing these questions has been the inability to follow the same cells in vivo to understand how their interactions with neighboring cells contribute to tissue regeneration. In a major advancement to the field, our lab was the first to establish the ability to visualize and manipulate stem cells and their environment in an intact animal using two-photon microscopy and laser ablation (Rompolas et al. 2012; Rompolas et al. 2013). This unique approach enables us to study hair follicle regeneration in live mice and to elucidate the cellular mechanisms driving these complex processes. In this study, we identify the roles of skin stem cells by combining live imaging and wound repair. This study will provide a novel set of approaches for understanding fundamental mechanisms of tissue regeneration.

F-1041
MECHANISMS OF STEM CELL FATE IN THE HAIR FOLLICLE NICHE

Rompolas, Panteleimon
Genetics, Yale School of Medicine, New Haven, CT, USA

In adult tissues, stem cell niches constitute a spatially distinct microenvironment, including neighboring cells, signals and extracellular material, which is crucial for regulating stem cell behavior and function. Anatomical and molecular heterogeneity appears to be a common feature between mammalian stem cell niches across different tissues; however, it is not clear whether the specific location that a stem cell occupies within the niche also determines its function. To address this question we utilize the highly compartmentalized hair follicle stem cell niche because of its stereotypic regeneration and accessibility for direct microscopic observation in vivo. Thus, we devised a novel approach by combining intravital microscopy with genetic lineage tracing tools, which enables us to first, mark single stem cells in different positions within the niche and second, re-visit the same cells and monitor their lineages throughout regeneration over the period of several weeks to months. Using this method we show directly that the location of a stem cell within the hair follicle niche determines its fate. The hair follicle niche is spatially organized so that we can identify three functionally distinct compartments; the upper and lower bulge and the hair germ. Depending where a stem cell resided at the onset of regeneration reproducibly determined its fate during hair growth. Furthermore, we uses laser-induced cell ablation to test whether hair follicle stem cells are required for hair regeneration and to address how injury-induced cell mobility between different niches affects their fate. We found that when a hair stem cell compartment is ablated, the niche is able to recover the lost population and proceed with hair regeneration. Surprisingly, epithelial populations that do not normally participate in hair growth are mobilized to enter the hair follicle niche. These new cells contribute to both re-establish the lost stem cell pool and sustain hair regeneration for several months. Furthermore, we provide evidence towards a) the origin of the hair germ, b) the cellular mechanisms utilized for stem cell clone expansion during hair growth and c) functional independence of the stem cell niche compartments during hair growth. This study provides a general paradigm for niche-induced fate determination in adult tissues.

F-1042
MIR184 REPRESSES K15 AND INDUCES EPITHELIAL STEM CELL DIFFERENTIATION

Putin, Daria[1], Nagosa, Sara[1], Serror, Laura[1], Aberdam, Daniel[2], **Shalom-Feuerstein, Ruby**[1]
[1]Technion - Israel Institute of Technology, Haifa, Israel, [2]INSERM U633, Evry, France

The skin and the cornea are situated at the surface of our body and eye, providing us protection against external insults such as microorganisms, toxicants and physical injury. These tissues share many features including structure, function and common molecular pathways in health and disease. We have recently developed novel culture systems for differentiating induced pluripotent stem cells (iPSCs) into corneal and epidermal keratinocytes. These models recapitulate major steps of skin/corneal embryogenesis and have been efficiently used for revealing new pathways of microRNAs and p63 in normal development, stem cell homeostasis and pathology. Here we studied miR-184, a highly evolutionary conserved microRNA which was induced at early iPSC differentiation in vitro and during skin and eye morphogenesis in vivo. The knockdown of miR-184 resulted in a decrease in corneal embryonic commitment of iPSCs. In post natal mice, miR-184 was predominantly expressed in the corneal epithelium, epidermis and hair follicles at growth phase. The expression of miR-184 was restricted to progenitors or early differentiated cells in vivo and in vitro but was absent from the stem or terminally differentiated cell compartment. We further demonstrated that miR-184 is repressing the stem cell marker cytokeratin 15 (K15), promoted Notch activation and keratinocyte differentiation. Interestingly, in corneal pannus collected from patients that suffer from severe limbal stem cell deficiency, we observed an uncontrolled increase in miR-184 expression that was coupled with a decrease in K15. Indeed, ectopic expression of miR-184 in limbal stem cell-enriched culture resulted in a dramatic decrease in clonogenic potential. Altogether, these data indicate that miR-184 induces an escape from 'stemness' state while abnormal expression of miR-184 may lead to a decline in epithelial stem cell reservoir.

F-1043
HOW DO EPIDERMAL STEM CELLS AGE? AN APPROACH FROM THE CIRCADIAN CLOCK DESYNCHRONIZATION

Solanas, Guiomar[1], Janich, Peggy[2], Toufighi, Kiana[3], Aznar-Benitah, Salvador[1]
[1]Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain, [2]Center for Integrative Genomics, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland, [3]Center for Genomic Regulation, Barcelona, Spain

Adult stem cells (SCs) localize to specific niches where they remain unspecified. Upon requirement for tissue

replenishment, they egress the niche and proliferate to contribute to the differentiated compartment. Tissue homeostasis is, thus, crucially dependent on correct adult SC function. The behaviour of SCs is dictated by their genetic program and environment. Several morphogens play essential roles in SC function; nevertheless, little is understood about their hierarchy, their spatiotemporal coordination within the system, and how they read and translate whole organismal cues into a specific stem cell function. Little is known about how and why adult SCs become affected upon aging and neoplastic transformation. Aging has been related to a combination of telomere shortening, high levels of reactive oxygen species and accumulation of DNA damage. However, recent studies show that SCs present mechanisms to counteract those aggressions. Hence, the causal role for SC malfunctioning in aging remains a largely speculative subject. We plan to tackle the question whether misregulation of SCs is indeed causative of aging, while deciphering the molecular mechanisms that govern progressive adult SC deterioration. Using skin as a model to study the function and behaviour of adult SC, our lab has recently identified that the molecular clock machinery orchestrates epidermal SC (epSC) function. We have shown that different populations of Bulge SCs coexist at different phases of their molecular clock, which predispose their response to activating and inactivating cues dictated by their microenvironment. Moreover, we have combined timed high-throughput transcriptome analysis and functional data to determine that the clock machinery modulates the behaviour of human epidermal stem cells and their differentiated counterparts. Core clock genes peaking in a concatenated manner along the 24 hours of the day establish 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 epSCs to respond to specific cues, such as those that trigger their proliferation or differentiation. This complex mechanism ensures epidermal homeostasis by providing epSCs with temporal functional cues along the day. However, the clock does not only coordinate the spatiotemporal function of epSCs during homeostasis but we have also shown that desynchronization of the stem cell clock in vivo disrupts tissue homeostasis, accelerates tissue aging, and predisposes the tissue to tumorigenesis. Interestingly, the epSC clock loses oscillating amplitude and becomes phase-shifted upon aging. Our aim is to understand how and why the epidermal stem cell clock is shifted and dampened with age. We want to understand if such dampening/shifting of the clock is due to intrinsic causes (i.e. due to epigenetic modifications in the epSCs themselves) or extrinsic changes. If the reasons were extrinsic to the SCs, either the niche cells or the whole organism could be responsible for sending the wrong signals to the epidermal stem cells. For this reason we are studying the role of epSC niche cells and metabolism in the entrainment/dampening of the epidermal stem cell clock.

F-1044
BNIP3 PLAYS CRUCIAL ROLES IN THE DIFFERENTIATION AND MAINTENANCE OF EPIDERMAL KERATINOCYTES

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

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

F-1045
GSDMA3, A MURINE GENE ASSOCIATED WITH SKIN INFLAMMATION AND ALOPECIA, ENCODES AN N-TERMINAL CELL DEATH-INDUCING DOMAIN AND A C-TERMINAL REGULATORY DOMAIN

Yang, Liang-Tung, Lin, Pei-Hsuan, Lin, Hsien-Yi, Wu, Shu-Hui
Institute of Cellular and System Medicine, National Health Research Institutes, Miaoli County, Taiwan

Mutations in *Gasdermin A3* (*Gsdma3*) have been reported to be responsible for mouse skin disease and alopecia. *Gsdma3* belongs to a structurally related *Gsdma*/*GSDMA* family, which is expressed mainly in differentiated cells of the skin and upper gastrointestinal tract. The physiological function of Gsdma3 remains unclear, although phenotypes of *Gsdma3* mutants deduce that *Gsdma3* functions in regulating epithelial homeostasis. Nine dominant mutations of *Gsdma3*, including three spontaneous and six ENU mutations, have been reported and these mutants displayed epidermal hyperkeratosis,

sebaceous gland abnormality, and hair cycle disorders. In many cases, the mutations were located in the C-terminal part, indicating that this segment is important for executing the biological function of *Gsdma3*. The autosomal dominant phenotype of *Gsdma3* mutants could be resulted from haploinsufficiency or gain-of-function mutation. Since these dominant mutations are nonsense- or missense-mutations, the haploinsufficiency theory proposed that the mutant protein may not be expressed due to nonsense-mediated decay of the mRNA or was rapidly degraded caused by missense-mediated instability. In contrast, gene knockout of *Gsdma1* and *Gsdmd* displayed neither epithelial hyperplasia nor carcinoma, suggesting that a new function is generated by the dominant mutation, representing a gain-of-function mutation. Still, the mechanism underlying how Gsdma3 exerts its dominant effect and what is the cellular target of Gsdma3 needs to be clarified. Here, we study protein domain interactions between Gsdma3 and Gsdma3 mutants using biochemistry pull down assay. Moreover, subcellular localization of the protein domains was examined by confocal microscopy and the cellular function of Gsdma3 and its mutants was characterized by flow cytometry in combination with pharmacological inhibitors. Our data demonstrate that Gsdsma3 is kept in a relative dormant state by the interactions of N-terminal and C-terminal parts. The mutation in the C-terminal part disables its association with N-terminal part, and the unmasked N-terminal part can self-associate and elicit the dominant-active on wild type Gsdma3. The N-terminal part of Gsdma3 partially co-localized with mitochondria and induced mitochondrial oxidative stress, which leads to mitochondrial membrane potential dissipation and cell death. Blockade of ROS generation by NAC alleviates the cytotoxicity of Gsdma3 mutants. In addition, overexpression of C-terminal part of Gsdma3 can rescue the cell death and the expression level of Gsdma3 mutants. In conclusion, we revealed that the N-terminal domain of Gsdma3 exerts its pro-apoptotic activity through affecting mitochondrial function and the C-terminal domain of Gsdma3 can mask the function of N-terminal domain through an intra-molecular or inter-molecular association.

F-1046
SPONTANEOUS TUMOR REGRESSION IS DRIVEN BY WNT /RETINOIC ACID SIGNALING CROSS-TALK

Zito, Giovanni, Saotome, Ichiko, Liu, Zongzhi, Ferro, Enrico G., Sun, Thomas Y., Nguyen, Don X., Bilguvar, Kaya, Ko, Christine J., Greco, Valentina
Yale University, New Haven, CT, USA

A fundamental goal in cancer biology is to identify the cells and signaling pathways that are key to induce tumor regression. Here, we use a spontaneously self-regressing tumor, cutaneous keratoacanthoma, to identify physiological mechanisms that drive tumor regression. By using a mouse model system that recapitulates the behavior of human keratoacanthomas, we show that self-regressing tumors shift their balance to a differentiation program during regression. Furthermore, we demonstrate that developmental programs utilized for skin hair follicle regeneration, such as Wnt, are hijacked to sustain tumor growth and that the Retinoic Acid signaling pathway promotes tumor regression by inhibiting Wnt signaling. Finally, we found that Retinoic Acid signaling can induce regression of malignant tumors that do not normally spontaneously regress, such as Squamous Cell Carcinomas. These findings provide new insights into the physiological mechanisms of tumor regression and suggest therapeutic strategies to induce tumor regression.

ENDOTHELIAL CELLS / HEMAGIOBLASTS

F-1049
SOX17 IS TRANSIENTLY EXPRESSED DURING HUMAN HEMANGIOBLAST COLONY FORMATION

Bruveris, Freya Faith[1], Ng, Elizabeth S.[1], Elefanty, Andrew George[2], Stanley, Ed[3], Azzola, Lisa[1]
[1]Murdoch Children's Research Institute, Melbourne, Australia, [2]Murdoch Childrens Research Institute, Parkville VIC, Australia, [3]Murdoch Childrens Research Institute The Royal Children's Hospital, Parkville VIC, Australia

The earliest hematopoietic progenitor, termed the blast-colony forming cell [BL-CFC], has been isolated from differentiating human pluripotent stem cells [hPSCs] under the influence of specific growth factors. This tripotential precursor generates blood, endothelium and smooth muscle through a transient hemogenic endothelial intermediate. Previous studies have identified a crucial role for Sox17 in the specification and regulation of blood cell formation from hemogenic endothelium at later developmental time points in both mouse and human, but this has not been studied in developing blast colonies. To facilitate the examination of the role of SOX17 in blood cell development, we generated a SOX17 reporter line [SOX17mCherry/w] in which the gene encoding mCherry was inserted via homologous recombination into the SOX17 locus in H9 hESCs. Although SOX17 was expressed in a subset of cells after three days of differentiation, BL-CFCs were predominantly generated from cells expressing the mesodermal marker PDGFRa but not SOX17 [mCherry-PDGFRa+]. When the expression of SOX17 was examined during blast colony growth over the ensuing days, a subset of cells in most colonies transiently expressed the mCherry SOX17 reporter. In most cases, this occurred during the formation of the mesodermal 'core' that characterizes blast colony development. As the colonies matured, the proportion of SOX17 cells decreased, with residual SOX17+ cells retaining an adherent, endothelial morphology. These data are consistent with the expression of SOX17 in a transient intermediate endothelial stage of differentiation during blast colony formation and argue for the necessary reduction in SOX17 expression during the transition to blood cell formation from endothelium. We are currently planning studies to investigate the requirement for SOX17 during this presumptive endothelial precursor stage.

F-1050
MODELING HYPER IGE SYNDROME (HIES) BY USING INDUCED PLURIPOTENT STEM CELL (IPSCS) REVEALS AN IMPORTANT ROLE OF STAT3 IN VASCULAR REMODELING AND ANGIOGENESIS

Chen, Guibin, Walts, Avraw, Jin, Hui, Freeman, Alexandra, Holland, Steven, Boehm, Manfred
National Institutes of Health, Bethesda, MD, USA

Stat3 plays an important role in early development by regulating cell proliferation and differentiation. Autosomal dominant Hyper IgE Syndrome (HIES) is a rare, primary immunodeficiency that arises from mutations in Stat3 and is characterized by elevated IgE, dermatitis, and recurrent lung and skin infections. HIES patients have skeletal and connective tissue abnormalities, and generalized wound healing defects. Inadequate wound healing and aneurysm formation in HIES patients suggest a role of Stat3 in the vascular remodeling and angiogenesis. We generated human induced pluripotent stem cell (iPSCs) from cells isolated from HIES patients to study these findings in more complex multi cell type disease models. By using viral-mediated

infection of transcription factors Oct4, Sox2, Klf4, and c-Myc, multiple HIES patient-specific iPSC lines (HIES-iPSC) were created from both HUVEC cells and fibroblasts. We found that the reprogramming efficiency of both HUVECs and fibroblasts is significantly reduce in HIES comparison with that of health donor, indicating that human Stat3 is indispensable in reprogramming. However once generated, these iPSCs can be further expanded, with growth rates comparable to that of iPSCs derived from health donors (WT-iPSCs). The HIES-iPSCs maintained normal karyotype and exhibit expression of pluripotency markers as measured by immunocytochemistry, flow cytometry and RT-PCR. HIES-iPSCs are able to successfully differentiate into all three germ layers in vitro. However, the proportion of endothelial and hematopoietic cells induction was significantly decreased compare to WT-iPS cells. Over-expression Stat3 in HIES-iPSCs by using ZFN or TALEN technology did improve the efficiency of endothelial and hematopoietic differentiation in HIES-iPSCs. Furthermore, the HIES-iPSCs are defective in their ability to develop teratomas in vivo. The numbers of endothelia cells in HIES-iPSCs derived teratomas, marked as CD31 and CD144, shown significantly lower than that of WT-iPSCs. The results imply that Stat3 signaling may play an important role in endothelia cells development and vascular angiogenesis. Future investigations will address the molecular mechanisms of Stat3 in differentiating cells derived from HIES-iPS cells in vivo and angiogenesis in vitro to improve our understanding of biofunction of Stat3 and pathophoysiology of HIES.

F-1051
STEM CELLS, DEVELOPMENT, IMMUNOTOXICITY, AND THE TERATOGENIC HYPOTHESIS

Harbi, Shaghayegh
New York University, New York, NY, USA

Infantile hemangioma (IH) is the most common tumor of infancy. The progenitor hemangioma-derived stem cell (HemSC) is a vascular stem/progenitor cell whose proliferation is dysregulated, but not a fully transformed cell, which orchestrates hemangioma pathophysiology via a sophisticated activation of multiple signaling and regulatory networks. Hemangioma-genesis follows a model where growth and progression of the tumor is driven by a small subpopulation of cells of progenitor cells, but what is the origin of the stem cell (e.g. germ cell, embryonic, placental, angioblast, mesangioblast, hemangioblast)? What regulatory process is involved or altered: epigenetic regulation (for example, zinc finger domain as target for toxic metal ions), genetic aberrations, or both? What is the interaction between environmental cues (such as hypoxia, hormones, immunity, stress, toxicity), progenitor cells, and associated signaling pathways (such as the involvement of endocrine disruptors, xenoestrogens, or metalloestrogens)? Virchow (1860) proposed an angioblastic origin while Pack and Miller (1950) described the origin as sequestered embryonic tissue. Current theories on the origin and pathogenesis of IH include: 1) placenta theory, 2) metastatic theory, 3) progenitor cell theory (a hemangioma-derived multipotential stem cell), 4) extrinsic factor theory (based on a hypoxic environment), 5) neural crest/pericyte stem cell theory, and 6) metastatic niche theory. The goal of this investigation is to understand the process of hemangioma-genesis by exploring the teratogenic hypothesis on the origin and pathogenesis of IH. The teratogenic hypothesis: immature, incompletely differentiated, dysregulated progenitor cells (*HemSCs*) are arrested in development (with vasculogenic, angiogenic, tumorigenic, and teratogenic potential) due to exposure to teratogenic agents. For this purpose, a toxicogenomics screen was performed to explore gene expression variations using a validated human immunotoxicity/cytotoxicity classifier genes assay. The assay employs an in vitro, high throughput approach for mRNA expression profiling based off of a set of classifier genes. The results described show that relative to normal human controls, HemSCs are characterized by higher expression of genes involved in toxicity (such as cytochrome P-450 and methallothionein genes). Exposure to a teratogenic insult results with an adverse in utero environment. For example, exposure to *extrinsic factors* (endocrine disruptors, xenoestrogens, or metalloestrogens) disrupt *intrinsic factors* (transcriptional and epigenetic regulators) via molecular mimicry. Specifically, targeted disruption of stem cells by extrinsic factors alter the genetic program with mutagenic, cytotoxic, and embryotoxic effects. The onset of pathogenesis at birth, due to a teratogenic insult in utero, may be triggered by the onset of immunocompetence at birth - as an immunogenic reactive, response. Prenatal and neonatal periods of development are characterized by well-orchestrated cues, gracefully transitioning the steps of immunogenesis - a stepwise maturation process from immunodeficiency to immunotolerance to immunocompetence. With the transition from immunotolerance to immunocompetence, immune cells transition from *tolerogenic* properties to a mechanism of *attack*. Thus immunotherapy may serve as a therapeutic option to challenge IH tumorigenesis and proliferation at the initial onset of growth.

F-1052
SCALABLE AND HIGHLY EFFICIENT ENDOTHELIAL CELL DIFFERENTIATION METHODS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS BASED ON MONOLAYER AND SERUM-FREE CULTURE FOR REALIZATION OF REGENERATIVE MEDICINE

Ikuno, Takeshi[1], Masumoto, Hidetoshi[1], Marui, Akira[2], MInakata, Kenji[2], Ikeda, Tadashi[2], Sakata, Ryuzo[2], Yamashita, Jun[1]
[1]Center for iPS Cell Research and Application (CIRA), Kyoto University, Kyoto, Japan, [2]Department of Cardiovascular Surgery, Kyoto University Graduate School of Medicine, Kyoto, Japan

Background: Efficient induction methods of endothelial cells (ECs) from human induced pluripotent stem cell (hiPSC) are essential for vascular regenerative medicine and disease modeling. Recently, ECs is also required to hiPSC-based tissue engineering for organgeneration such as the heart and liver to generate larger sized grafts and complicated structures. Previously, with a mouse ES cell system, we reported that simultaneous stimulation of vascular endothelial growth factor (VEGF) and cyclic adenosine monophosphate (cAMP) signaling in mesoderm cells drastically enhanced EC differentiation. We also established an efficient and scalable monolayer high density culture-based cardiomyocyte differentiation protocol from hiPSCs (with modifications on a differentiation method for human ESCs. Combining these methods, here we tried to control hiPSC differentiation to ECs with stage-specific supplementation of VEGF and cAMP. Methods and Results: Various concentrations of VEGF and 8-bromo-cAMP were supplemented at time points around the possible mesoderm emergence, then efficiency of EC induction was evaluated with flow cytometry on differentiation day 9. Vascular endothelial (VE)-cadherin-positive EC population was significantly increased with addition of VEGF (100ng/ml, from differentiation differentiation day 4-9) and transient stimulation with 8bromo-cAMP (1mM, from differentiation day 4-6) compared to those with VEGF alone, or those with no VEGF and cAMP (56.2±12.5% vs 11.8±7.2% vs 2.3±2.4% of total cells, P=0.000017, n=4). Calculated EC count was also notably increased. On differentiation differentiation day 9, 0.8 ECs were collected through cell sorting from 1 hiPSC. We further modulated the method to once purify vascular endothelial growth factor receptor-2 (VEGFR2)-positive cells at differentiation day 6, and then reculture them with VEGF and cAMP. Purified VEGFR2-positive cells gave

rise to ECs with more than 99% efficiency at differentiation day 9. Moreover, yield of ECs at differentiation day 9 was increased in 4 times than the former method. Purified ECs were able to be recultured and expanded 2.5 times during additional 5 days. Finally, we could obtain 8.5 ECs from 1 iPSCs at differentiation day 14. This method increased the yield of ECs to 20-folds than previous reports. We confirmed the similar efficiencies of EC differentiation in multiple hiPSC lines with this method. Induced ECs were able to be cryopreserved. Conclusions: We established an efficient and scalable EC differentiation method from hiPSCs based on monolayer and serum-free culture. This method would be applicable to regenerative strategies and modeling for vascular diseases.

F-1053
GENETIC AND EPIGENETIC LANDSCAPE OF ENDOTHELIAL CELLS DIFFERENTITATION REVEAL THE TRANSCRIPTIONAL FACTORS NETWORK

Kanki, Yasuharu[1], Matsunaga, Taichi[2], Nakaki, Ryo[1], Yamamizu, Kohei[3], Shimamura, Teppei[1], Wada, Youichiro[1], Yamashita, Jun[4], Minami, Takashi[1]

[1]The University of Tokyo, Tokyo, Japan, [2]Kyoto University, Kyoto, Japan, [3]NIA / NIH, Baltimore, MD, USA, [4]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Until now, many studies of vascular development have consisted of gene knockout and knockdown studies using mice and zebrafish. Recently, direct conversion from amniotic cells to vascular endothelial cells has been reported. In this article, cascade of ETS transcription factors and inhibition of TGFb signaling are important for long-lasting functional vascular vessels. Although these works led to the new discoveries of vascular development in mammals, they could not sufficiently identify the precise molecular cascade on the vascular endothelial cell (ECs) differentiation and one big problem is that getting the amniotic cells is difficult for adults. Therefore, our aim is to establish the more efficient and practical ECs differentiation system from embryonic stem (ES) or induced pluripotent stem (iPS) cells. On the above aim, we used ES cell differentiation system. Using this system, we can systematically induce ECs in vitro and dissect their differentiating processes in detail. 96 hours after induction of differentiation from mouse ES cells, Flk (VEGF, vascular endothelial cell growth factors, receptor 2) -positive mesoderm cells are sorted by MACS using anti-Flk antibody. If these cells are stimulated by VEGF (50 ng/ml), cells have commitment to ECs. On the other hand, cells differentiate to smooth muscle cells (SMCs) without VEGF stimuli. To elucidate comprehensive gene expression profiles during ECs or SMCs differentiation, we performed sequential mRNA-seq experiments 6,12,24,48 hours after with or without VEGF stimulation. As a result, transcription factors (Gata2, Etv2, Sox7, Sox18, Fli1) were induced at early time points, cell-specific markers (Icam2, VE-Cadherin, Endomucin) were induced at late time points only under VEGF stimuli. Next, in order to depict epigenetic landscape about vascular development, we conducted ChIP-seq (Chromatin immunoprecipitation with next generation sequencing) using H3K4me3 and H3K27me3 specific antibodies and FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements) -seq for open chromatin regions. From ChIP-seq and FAIRE-seq, we found out some bivalent genes (double positive, H3K4me3 and H3K27me3) such as Etv2, Gata2, and Sox18 which are upregulated at early time points and GATA2 motif (T/A GATA A/G) is most enriched from the analysis of RNA-seq and FAIRE-seq. Next, to test whether above bivalent genes have important roles on vascular ECs differentiation, we transfected si-RNA and investigated the effect. Then we demonstrated that 4 transcription factors including Gata2 has essential roles for ECs development. Especially, we have reported GATA2 is indispensable for the maintenance of matured ECs specificity (Kanki Y et al 2011 EMBO J), so, taken together we can think that Gata2 is important transcription factor for both differentiation and maintenance of vascular ECs. These findings have suggested that determination of cell fate is based on not only master transcription factors but also epigenetic histone modifications. Overall, we have demonstrated ECs specific epigenetic switch on the differentiation from mouse embryonic stem cells.

F-1054
MATURATION OF HUMAN IPS CELL-DERIVED ENDOTHELIAL CELLS INTO BRAIN ENDOTHELIAL CELLS FOR ESTABLISHMENT OF IN VITRO BLOOD BRAIN BARRIER MODEL

Yamaguchi, Tomoko[1], Tashiro, Katsuhisa[1], Minami, Haruka[1], Mizuguchi, Hiroyuki[2], **Kawabata, Kenji**[1]

[1]Laboratory of Stem Cell Regulation, National Institute of Biomedical Innovation, Osaka, Japan, [2]Laboratory of Biochemistry and Molecular Biology, Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan

Blood brain barrier (BBB) is formed by brain capillary endothelial cells working together with astrocytes and pericytes. Brain endothelial cells (BECs) regulate strictly the penetration of many kinds of molecules into the brain by forming tight junctions and expressing various transporters. Although in vitro BBB models have widely been reported with primary BECs isolated from animals, the results obtained from animal models do not always reflect the pharmacokinetics in human, because the expression patterns and levels of transporters in BECs are different between human and animals. To properly predict the disposition characteristics of drugs, human BBB models have also been developed using primary BECs isolated from freshly resected brain specimens. However, it is difficult to obtain a large amount of BECs for the construction of in vitro BBB models. To overcome this problem, we generated the BECs from human induced pluripotent stem cells (iPSCs). CD34-positive cells were induced from human iPSCs by embryoid body formation method, and they were subsequently isolated and cultured on fibronectin-coated plates. These cells showed the tube formation and uptake of acetylated LDL, indicating that endothelial cells (ECs) could be successfully generated from human iPSCs. Previous studies demonstrated that ECs acquire the tissue-specific properties in response to stimulation from surrounding cells. Therefore, to generate BECs, iPS cell derived-ECs (iPS-ECs) were co-cultured with C6 glioma cells, which are known to have a property of astrocytes. As a control, iPS-ECs were cultured without C6 cells. After 5 days culture, the level of transendothelial electric resistance (TEER) in iPS-ECs co-cultured with C6 cells (iPS-ECs-C6) was significantly increased as compared with that in control cells. Quantitative PCR analysis also revealed the elevated expression of genes associated with tight junction complexes, such as claudin-5, in iPS-ECs-C6. It is of note that permeability to dextran was obviously decreased in iPS-ECs-C6. These data indicate that human iPS-ECs could robustly form tight junction by co-culturing with C6 cells. Furthermore, iPS-ECs-C6 expressed various transporters, such as MDR-1, BCRP, MRP-4 and Glut1, and displayed the functional expression of MDR-1. Thus, our data showed that maturation of iPS-ECs into BECs could be promoted by co-culturing with C6 cells. We also found that iPS-ECs were also maturated into BECs by using C6 cell-conditioned medium, indicating that C6 cell-derived soluble factor would be responsible for induction of BECs. Importantly, BECs could not be generated from human umbilical vein endothelial cell (HUVEC) even co-cultured with C6 cells, suggesting the possibility that iPS-ECs but not other normal ECs would probably be suitable for generating BECs. In summary, we

successfully developed the two-step protocols for generating the BECs form human iPSCs. Human iPSC-derived BECs would be a cell source for generation of in vitro BBB models, and also expected to application to drug-screening.

HEMATOPOIETIC CELLS

F-1055
PROTEOMICS ANALYSIS OF HEMATOPOIETIC STEM CELLS FOR IDENTIFICATION OF TREATMENT PREDICTIVE BIOMARKERS IN CHRONIC MYELOID LEUKEMIA (CML) PATIENTS

Alaiya, Ayodele[1], Chaudhri, Naeem[1], Owaidah, Tarek[1], Fox, Jonathan[2], Shinwari, Zakia[1], Barhoush, Eman[1], Alsharif, Fahad[1], Mohamed, Said[1], Rasheed, Walid[1], Osman, Ahmed Syed[1], Alfraih, Feras[1], Al-Jurf, Mahmoud[1]

[1]King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia, [2]Waters U.K. Limited, Atlas Park, Manchester, United Kingdom

Purpose: Presently, there is lack of less invasive measurable biomarkers for management of most hematological malignancies. This study aim to analyze global protein expression profiles in hematopoietic stem cells derived from bone marrow and peripheral blood samples. The goal was to identify potential protein biomarkers for choice of therapy, accurate prediction of treatment response and better prediction of disease prognosis in patients diagnosed with Chronic Myeloid Leukemia. Experimental Design: Proteomics mining of plasma or serum provide unique ability to identify biomarkers requiring less invasive procedures, with ease for monitoring disease progression and effective prediction of response to therapy in relatively early stages. We have analyzed global protein expression profiles of bone marrow and serum, samples from 28 patients with newly diagnosed chronic-phase CML using the classical expression proteomics platforms (2-DE and label free quantitative liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The goal was to evaluate for early treatment response and discovery disease-specific /disease-associated proteins for prognostic monitoring and patient's response to therapy at protein level. Results: Expression proteomics data from 2-DE analysis predicts accurately patients with major molecular, cytogenetic and hematological responses from patients with either partial or no response to treatment using unsupervised principal component analysis. In addition, differentially expression protein profiles discriminates patients response at 6 months of Imatinib (Gleevec) treatment from patients that ultimately required 2nd generation tyrosine kinase inhibitor therapy. Some of the results were independently validated using label free quantitative liquid chromatography tandem mass spectrometry. We have so far identified a panel of 15 differentially expressed proteins (> 2- ∞- fold change, $p < 0.001$) from 28 CML patients that achieved Major Molecular Response (MMR) vs. No-Major Molecular Response (NoMMR) at 6 months treatment with imatinib. Some of the identified proteins were implicated in hematological diseases and includes Group-specific component (vitamin D binding protein), haptoglobin and vitronectin. Others were inter-alpha-trypsin inhibitor heavy chain 1, leucine-rich alpha-2-glycoprotein 1 and metallophosphoesterase 1. Conclusion: Our results highlight the power of proteome analysis of cell fractions derived from fresh clinical specimens towards discovery of surrogate biomarkers for more objective monitoring treatment response in CML patients. This data indicates that multivariate analysis of quantitative proteome data can potentially be useful as a means of unsupervised artificial intelligence algorithm for objective classification and stratification of clinical CML patients. These proteins might be valuable, once validated, to complement the currently existing parameters for reliable and objective prediction of disease progression, monitoring treatment response and clinical outcome of CML patients.

F-1056
DISTINCT METABOLIC GENE EXPRESSION PROFILE MAY BE KEY IN DISTINGUISHING LEUKEMIC STEM CELLS FROM NORMAL HEMATOPOIETIC STEM AND PROGENITOR CELLS

Ghaffari, Saghi, Rimmele, Pauline, Liang, Raymond, Papatsenko, Dmitri, Bigarella, Carolina

Icahn School of Medicine at Mount Sinai, New York, NY, USA

Leukemic stem cells (LSCs) share many of the same properties of normal hematopoietic stem cells (HSCs) including their highly quiescent state, capacity to self-renew, low levels of reactive oxygen species (ROS) and enhanced DNA repair program. These properties make the efficient and specific eradication of these cells challenging. P53 and Foxo3 are two transcription factors essential for the modulation of HSC quiescence and self-renewal. While Foxo3 is inhibited by several oncoproteins and crucial for the maintenance of the LSCs in both chronic and acute myeloid leukemia (CML and AML respectively), mutations of p53 although rare, are associated with poor prognosis in advanced stages of these diseases. In vivo ROS-mediated activation of p53 is known to lead to loss of quiescence, alterations of cell cycle and exhaustion of the Foxo3-/- HSC pool. Seeking to understand the contribution of p53 to Foxo3-/- HSC cycling defects, we analyzed p53+/-/ Foxo3-/- LSK (Lin-Sca1+cKit+) cells. To our surprise we found the bone marrow (BM) frequency of both p53+/-Foxo3-/- and p53-/-Foxo3-/- LSK and long-term-HSC (LT-HSC, LSK Flk2-CD34-) populations were greatly increased as compared to their Foxo3-/- counterparts (n=5 mice per genotype; $p<0.05$). Using Ki67 and DAPI staining we found that loss of only one allele of p53 rescued the cell cycle defects of Foxo3-/- HSC and increased the frequency of LSK cells in Go by 2-fold. Loss of p53 also rescued the impaired capacity of Foxo3-/- HSC to competitively repopulate multilineage blood over 16 weeks, as shown by the higher frequency of p53+/-Foxo3-/- and p53-/-Foxo3-/- donor-derived cells in the peripheral blood of recipient animals. Furthermore, loss of p53 significantly improved the compromised self-renewal of Foxo3 mutant HSC in serial BM transplantations. In our quest to identify mechanisms whereby p53 depletion improves Foxo3-/- HSC function, we noticed that the DNA damage accumulated in Foxo3-/- HSC at the steady-state was remarkably ameliorated by removal of one or both alleles of p53 from Foxo3-/- HSCs, as measured by flow cytometry levels of phospho-H2AX (gamma-H2AX) and DNA breaks by comet assay (n=3, $p<0.05$). Unexpectedly, ROS levels were also significantly reduced in p53+/-Foxo3-/- in comparison to Foxo3-/- LSK cells, while ROS levels in p53+/- LSK cells were similar to that in WT cells. These "quasi normal HSC" exhibit a striking aberrant gene expression profile including abnormal expression of oncogenes and tumor suppressors, metabolic genes and anti-oxidant genes. Specifically these cells were enriched in a fatty acid metabolism-related gene cluster as analyzed by Gene Set Enrichment Analysis (GSEA) of the microarray (ES=0.746; $p<0.01$). Their gene expression profile exhibited a remarkable overlap with genes exclusively upregulated in a LSC-gene signature and was highly reminiscent of malignant transformation recapitulating many features of pre-leukemic stem cells. In agreement with this, their susceptibility to develop malignancy was increased as we found faster onset of BCR-ABL-transformed p53+/-Foxo3-/- HSC in establishing CML in mice, more rapid progression to blast crisis and decreased survival of p53+/-Foxo3-/- recipient mice as compared to controls. Finally we identified upregulation of Bmi1 in p53+/-Foxo3-/- HSC as a potential mediator of their LSC function. Our results suggest that gene expression profile specifically of metabolic genes may be key in

distinguishing normal HSC from leukemic stem cells.

F-1057

BONE CELLS GOVERN T LYMPHOPOIESIS BY REGULATING THYMIC EMIGRANTS FROM BONE MARROW

Yu, Vionnie[1], Saez, Borja[1], Lotinun, Sutada[2], Cook, Colleen[1], Yusuf, Rushdia[1], Ferraro, Francesca[1], Stefania, Lymperi[1], Wang, Ying-Hua[1], Pardo-Saganta, Ana[1], Raaijmakers, Marc[3], Zhou, Lan[4], Rajagopal, Jayaraj[1], Kronenberg, Henry[5], Baron, Roland[2], Scadden, David T.[1]
[1]Massachusetts General Hospital/Harvard Stem Cell Institute, Boston, MA, USA, [2]Harvard School of Dental Medicine, Boston, MA, USA, [3]Erasmus Stem Cell Institute, Rotterdam, Netherlands, [4]Case Western Reserve University, Cleveland, OH, USA, [5]Endocrine Unit, Massachusetts General Hospital, Boston, MA, USA

Mesenchymal cell direction of parenchymal cell activity is hypothesized to be critical for adult tissue function, but remains poorly defined. We examined how specific subsets of osteolineage mesenchymal cells control parenchymal hematopoietic cells in the bone marrow using selective cell depletion genetic murine models. Unexpectedly, mature bone cells expressing osteocalcin (Ocn+) were found to be critical for T lymphopoiesis through the modulation of cells destined for thymic emigration. Specific depletion of Ocn+ cells reduced the number of adult bone marrow T-lymphoid biased progenitors by reduced endosteal DLL4 production and corresponding hematopoietic progenitor Notch activation. Thymic emigrants were compromised in association with reduced chemotactic molecules CCR7 and PSGL1, yet were capable of normal T lineage differentiation upon adoptive transfer to the thymus. Other hematopoietic lineages and hematopoietic stem cell numbers were unperturbed. Therefore, mature osteolineage cells have a highly constrained effect on T lymphoid cells, altering T cell production by regulation of thymic emigrants and their ability to translocate to the site of maturation. These data reveal a new role for bone in the homeostasis of the immune system and demonstrate how perturbation of bone cells can critically impact adult immunity.

F-1058

THE FABULOUS FATE OF CD34: DEFINING A NOVEL ROLE FOR CD34 IN DIRECTING THE MIGRATION OF HEMATOPOIETIC STEM CELLS.

AbuSamra, Dina Bashir, Chin, Chee Jia Julia, Merzaban, Jasmeen
Bioscience, King Abdullah University for Science and Technology, Thuwal, Saudi Arabia

E-selectin is constitutively expressed on human bone marrow microvasculature and is implicated in mediating the homing of hematopoietic stem/progenitor cells (HSPCs) to bone via interactions with glycoprotein ligands. Given that the identity of these glycoprotein E-selectin ligands (E-selL) on human HSPCs is limited, we used mass spectrometry (MS)-based proteomics to fully characterize all potential ligands expressed on 1) primary human CD34+ HSPCs isolated from umbilical cord blood (CB-HSPCs) and 2) CD34+ Kg1a cell line (human acute myelogenous leukemia that serves as a model for human HSPCs). We obtained a very rich resource for further investigation and chose to highlight CD34 as a candidate E-selL. The cell surface glycoprotein CD34 is widely recognized as a marker of hematopoietic stem/progenitor cells (HSPCs), but its physiological role on HSPCs remains elusive. Although CD34 is well recognized for mediating homing of L-selectin+ naïve T cells to high endothelial venules of secondary lymphoid organs, the natural ligand for HSPC specific-CD34 is unknown. We developed a novel-binding assay to capture endogenous candidate glycoprotein ligands from whole cell lysates prepared from human HSPCs (CB-HSPC and Kg1a) onto a biacore chip and characterized their interaction with recombinant E-selectin. Using this assay we demonstrate that CD34 binds to recombinant E-selectin with affinity comparable to the well-described E-selectin ligand, CD44/HCELL. Further biochemical analysis demonstrated that E-selectin binding was restricted to a specific glycoform of full-length CD34 that expresses sialyl Lewis X (sLex). This interaction was dependent on calcium, sialylation and O-glycans as removal of these abrogated binding to E-selectin. CD34 also binds vascular P-selectin, in a sulfation and O-glycan dependent manner and hence defining CD34 as the first selectin ligand since PSGL-1 (P-selectin glycoprotein ligand 1) to bind all three selectin family members. Among the stem cell specific processes such as self-renewal and differentiation that CD34 has been implicated to have a role in, these data are the first to define a role for human CD34 in mediating the migration of HSPCs to selectins expressed on vascular beds such as the bone marrow and suggests that a natural ligand for CD34 on human HSPCs are the vascular selectins.

F-1059

NOVEL INTEGRATED CLONE TRACKING IN NONHUMAN PRIMATES SUGGESTS A MINIMAL POPULATION OF MULTIPOTENTIAL HEMATOPOITEIC STEM CELLS WITH LONG-TERM REPOPULATING POTENTIAL IN CD34-ENRICHED CELL POOLS

Adair, Jennifer Eileen[1], Porter, Shaina N.[2], Porteus, Matthew H.[3], Kiem, Hans-Peter[1]
[1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA, [2]Washington University, St. Louis, MO, USA, [3]Stanford University, Stanford, CA, USA

While the total number of allogeneic cells required to reliably reconstitute hematopoiesis following hematopoietic stem cell transplantation (HSCT) has been established, we know very little about the actual number or characteristics of true long-term, multipotential autologous HSCs, critical for any gene therapy or cord blood transplantation approach. Integration site (IS) analysis in gene therapy trials using integrating retroviral vectors (RV) and lentiviral vectors (LV), suggest very few transplanted clones contribute, but underestimates clonal diversity due to procedural bias. However, IS analysis can identify insertional events which may influence hematopoietic reconstitution kinetics. DNA barcoding via RV tagging has successfully quantitated hematopoietic reconstitution in mice, but relevance to human hematopoiesis is limited and DNA barcoded vector libraries reported to date lack complexity to recapitulate quantitative tracking in clinically-relevant large animal models. Additionally, DNA barcode analysis alone cannot identify insertional events influencing hematopoiesis. We hypothesized that IS analysis and DNA barcoding together would provide more robust HSCT reconstitution data, and that application of this approach in the nonhuman primate model would be most informative for developing strategies to improve HSCT in humans. We developed a high complexity (1.2 million), DNA barcoded LV library encoding the chemotherapy resistance gene MGMT(P140K) and enhanced green fluorescent protein (GFP) to measure hematopoietic reconstitution following autologous HSCT in pigtailed macaques. A total of 3.6E+06 autologous CD34+ cells (3 x library complexity) were transduced at a MOI of 20 (10 x 2) and infused into two animals following myeloablative total body irradiation. We observed ~2 and 12% GFP+ peripheral blood white blood cells (PB WBCs) following hematopoietic recovery in these animals, respectively. We performed barcode and IS analysis from PB WBCs collected early (1 month) after HSCT and observed <0.001% of infused clones contributing to hematopoiesis by DNA barcoding, and 42% fewer clones via IS analysis of the same sample. The most dominant clones identified by each individual method contributed to

5-6% of resulting clonal sequences, suggesting both methods efficiently captured more abundant clones. Over 1 year following HSCT, a total of 5,089 (0.4%) and 20,799 (1.7%) infused clones contributed to hematopoiesis in each animal, respectively, by DNA barcode analysis, with polyclonal contribution patterns by both methods. At 1 year post-transplant, we analyzed PB WBC subsets and identified a total of 116 and 2,246 clones respectively contributing to each of three lineages (T, B, and granulocyte), suggesting <0.2% of infused CD34+ cells displayed multi-lineage potential in either animal. Adjusting for marking levels we estimate between 5000-20,000 clones gave rise to trilineage hematopoiesis at 1 year post-HSCT. These findings underscore the need to quantitatively assess repopulation kinetics of HSCs under various conditions aimed at increasing gene modified cell engraftment. Here we provide a tool by which to comprehensively analyze efficacy and safety of approaches such as HSC expansion or MGMT(P140K)-mediated in vivo chemoselection, and a baseline for comparison and evaluation in the clinically-relevant nonhuman primate model.

F-1061
A METABOLIC MAP OF HEMATOPOIETIC STEM CELLS

Agathocleous, Michalis, Hu, Zeping, Morrison, Sean J.
Children's Research Institute at UT Southwestern, Dallas, TX, USA

A general problem in biology is whether different types of cells in the same tissue are metabolically different to each other, and whether such differences are important for cellular function. Experiments that can comprehensively measure the cellular metabolome currently require millions of cultured cells and cannot be used with small numbers of cells freshly isolated from heterogeneous populations in vivo. In particular, hematopoietic stem cells (HSCs) have been intensively studied for decades but their metabolic composition is largely unknown. We have developed a method to measure metabolites in small numbers of HSCs. The HSC isolation method and mass spectrometry method have been optimized for maintenance of the metabolome during purification, sensitivity and robustness. About 50 metabolites can be quantified from 10,000 HSCs, covering a wide spectrum of the cellular metabolome. Several metabolic differences exist between HSCs and other bone marrow cells, including restricted progenitors. I am investigating the role of these metabolic differences in HSC function. The ability to profile the metabolome of rare cells isolated directly from an organism opens the possibility to metabolically compare stem cells to other purified populations of cells at different stages of differentiation, to test the metabolic consequences of physiological challenges on specific populations of cells, and to test whether other rare cells, like cancer stem cells, are metabolically distinctive.

F-1062
ANDROGEN/ANDROGEN RECEPTOR INDUCE CD4+CD25+FOXP3+ REGULATORY T CELLS VIA MESENCHYMAL STEM CELLS (MSC)TGF-BETA PATHWAYS

Al Harbi, Ayman Saeed[1], Al Awad, Abdullah[2], Al Jarbou, Ahmad[3], Chang, Chawnshang[4], Zou, Wiping[5], Al Masri, Nidal[6], Ala, Hejazi[6], Rudat, Volker[7], Muhammad Ali, Maria[7], Al Sobayil, Fahd[8], Altuwaijri, Saleh[7]
[1]Taif University, Taif, Saudi Arabia, [2]King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia, [3]Al Ghad International Medical Sciences Colleges, Qassim, Saudi Arabia, [4]Rochester University, Rochester, NY, USA, [5]University of Michigan Health Systems, Ann Arbor, MI, USA, [6]Pathology and Laboratory Medicine, Saad Specialist Hospital, Al Khobar, Saudi Arabia, [7]Saad Research and Development Center, Saad Specialist Hospital, Al Khobar, Saudi Arabia, [8]Qassim University, Qassim, Saudi Arabia

Females have a higher incidence of autoimmune diseases than males for reasons that are unknown. Naturally occurring $CD4^+CD25^+FOXP3^+$ regulatory T cells (Treg cells) play a pivotal role in immune tolerance. We hypothesize that androgen/androgen receptor (AR) may play a role in Treg cell differentiaton and function. Eight to 12-week-old wild ($AR^{+/+}$) and androgen receptor knock out ($AR^{-/-}$) mice were used. Immune cells including Treg cells in different organ compartments were isolated with easySep selection kit and sorted with FACSAria. Mesenchymal Stem Cells (MSC) were obtained from bone marrow and cultured in vitro. The phenotype and function of Treg cells were determined by flow cytometry analysis (FACS) and in vitro immune suppressive assay. We observed similar levels of thymic Treg cells in $AR^{+/+}$ and $AR^{-/-}$ male mice. However, the levels of peripheral Treg cells were lower in $AR^{-/-}$ mice than $AR^{+/+}$ mice. Sorted $AR^{-/-}$ Treg cells were functionally less suppressive than their counterparts in wild type mice. Further, $AR^{-/-}$ MSC were less efficient to induce Treg cell expansion than $AR^{+/+}$ MSC. Additional studies demonstrated that $AR^{+/+}$ MSC-induced Treg cell expansion was partially impaired by blocking androgen receptor signal. Furthermore, the levels of TGF-β were lower in the T cell coculture with $AR^{-/-}$ MSC compared to $AR^{+/+}$ MSC. Our data suggest that androgen/AR and TGF-β signal pathway may be implicated in Treg cell expansion and function.

F-1063
LINEAGE NEGATIVE BMCS INDUCE REGENERATION PROMOTING EFFECTS IN BOTH PTERYGOPALANTINE ARTERY (PPA) LIGATION AND NMDA INDUCED RGC DEPLETION MOUSE MODELS

Anand, Akshay[1], Minhas, Gillipsie[1], Prabhakar, Sudesh[1], Mukhopadhyay, Asok[2]
[1]Neuroscience Research Lab, Post Graduate Institute of Medical Education and Research, Chandigarh, India, [2]National Institute of Immunology, New Delhi, India

Background: Current treatments available for RGC damage, caused either due to retinal ischemia or Glaucoma, are not sufficient to restore the visual functions. Different animal models have been generated to study in retinal repair and validate therapies but the efficacy of stem cell in multiple animal models is rarely tested simultaneously. Purpose: We improvised pterygopalatine artery (PPA) ligation model to study transient retinal ischemia concurrently with established NMDA induced RGC depletion mouse model in order to test the effect of non VSEL stem cells derived from mouse bone marrow. Methodology: Age and sex-matched C57BL/6J mice were subjected to PPA ligation. The external carotid artery and the PPA were ligated for 3.5 hours reducing the ocular blood flow analysed by Laser Doppler, fluorescein

angiography and ERG. Similarly, 2 ul of 50nm and 100nm doses of NMDA were intravitreally injected to induce dose dependent RGC depletion. The retinal damage was assessed using morphometry and histological analysis besides real time PCR, immunohistochemical and electrophysiological techniques, with fellow eye serving as control. The Sca 1+, lineage negative CD45+ stem cells, herein designated as non VSEL stem cells, were purified using magnetic associated cell sorter (MACS) and FACS. About 100,000 CFDA stained donor cells were transplanted intravenously or intravitreously, after characterising Sca-1, CD34, CD 45 and CD117 expression by flow-cytometry. Results: Results were obtained at 7, 14, 21 and 28 days for NMDA model and 5 and 10 days for PPA model. Thinning of retinal layers was observed consequent to retinal ischemia as well as NMDA damage. Immunohistochemistry revealed decrease in Brn3b and increase in doublecortin, Nestin, GFAP and CNTF in the NMDA model. After transplantation, BDNF expression was found to be decreased while CNTF was elevated. In case of PPA model BDNF, Nestin FGF2, GFAP increased post injury when analysed by real-time PCR and IHC at 5 days. A significant decrease in GFAP and HIF expression expression was observed after 10 days of transplantation when analysed by PCR and IHC, while BDNF expression was found to be increased when analysed by PCR alongwith FGF2 and Nestin. The transplantation of lineage-negative BMCs were found to survive up to 21 days of transplantation. Conclusions**:** The lineage negative BMCs derived from mouse bone-marrow exert regeneration promoting effects on damaged rodent retina.

F-1064
RECOVERY OF FUNCTIONAL DISABILITIES IN MULTIPLE SCLEROSIS PATIENTS FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANT SUGGESTS REGIONAL DIFFERENCE IN REPAIR CAPACITY OF THE CENTRAL NERVOUS SYSTEM.

Atkins, Harold[1], Bowman, Marjorie[1], Allan, David[2], Arnold, Douglas[3], Bar-Or, Amit[3], Bence-Bruckler, Isabelle[4], Bredeson, Christopher[4], Hamelin, Linda[4], Huebsch, Lothar[4], McDiarmid, Sheryl[4], Sabloff, Mitchell[4], Sheppard, Dawn[4], Tay, Jason[4], Walker, Lisa[5], Freedman, Mark[6]

[1]Ottawa Hospital Research Institute, Ottawa, ON, Canada, [2]Blood and Marrow Transplant Programme, Ottawa Hospital Research Institute, Ottawa, ON, Canada, [3]Montreal Neurological Institute, Montreal, QC, Canada, [4]Blood and Marrow Transplant Programme, Ottawa Hospital, Ottawa, ON, Canada, [5]Ottawa Hospital, Ottawa, ON, Canada, [6]Multiple Sclerosis Clinic, Ottawa Hospital, Ottawa, ON, Canada

Multiple Sclerosis (MS) is an immune mediated disease characterized by episodic inflammatory lesions in the central nervous system (CNS) that ultimately result in permanent damage to CNS tissues. The relapses and remissions of early MS are replaced by sustained accumulation of disabilities as time passes. MS disability is scored during a neurological examination by a trained evaluator in specific domains resulting in 7 functional system scores (FSS). These scores and mobility data are combined to give an overall ordinal score, the Expanded Disability Status Scale (EDSS). Increasing disability results in higher scores. Chemotherapy and anti-thymocyte globulin have been used to destroy the autoreactive immune system. Reconstitution of a naïve immune system follows autologous Hematopoietic Stem Cell Transplantation (HSCT). Twenty four patients with aggressive MS were treated with Busulphan, Cyclophosphamide, Thymoglobulin and a CD34 selected autologous apheresis hematopoietic stem cell graft. The EDSS and FSS measured prior to treatment and 3 years after HSCT were available for 21 patients. While MS is often marked by sustained accumulation of disability with a rising EDSS over time, the EDSS was improved in 9 patients (42%), stable in 6 patients (29%) and worsened in 6 patients (29%). Overall 38% of the FSS improved over the 3 year follow-up period. Even patients with higher EDSS scores had improvement in 24% of the FSS scores. Improvement was seen in 28% of patients in the Visual FSS and Brainstem FSS, 42% of patients in the Sensory FSS and Mental FSS, in 47% of patients in the Pyramidal FSS and in 58% of patients in the Cerebellar and Bowel/Bladder FSS. Taken together, these results suggest that patients with MS can recover from longstanding disabilities after Hematopoietic Stem Cell Transplantation - a rare outcome following most standard drug treatments for MS. Furthermore, this unique clinical situation - recovery of disabilities caused by disseminated lesions throughout the CNS - highlights differences in the frequency of improvement between the FSS and provides clinical evidence that there is regional variation in the reparative capacity of the CNS.

F-1065
DEVELOPMENTAL DOWNREGULATION OF LIN28 AND HMGA2 EXPRESSION IN PRIMITIVE NORMAL HUMAN HEMATOPOIETIC CELLS

Babovic, Sonja, Knapp, David J.H.F., Beer, Philip Anthony, Copley, Michael, Eaves, Connie

Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Hematopoietic stem cells (HSCs) are functionally identified by their ability to regenerate and sustain the production of mature blood cells for long periods of time, usually assessed in myelosuppressed transplanted recipients. Previous studies have shown that in the mouse such cells can have intrinsically determined differences in their properties, some of which are developmentally regulated. These include a heightened ability of fetal HSCs to expand their numbers in irradiated adult recipient mice, and a greater sensitivity of fetal HSCs to Steel factor (SF), which is known to promote HSC self-renewal. We have previously demonstrated that increased expression of *Lin28b* (an inhibitor of let-7 miRNA biogenesis), and *Hmga2* (a transcriptional regulator and let-7 target) underpins the heightened *in vivo* expansion of fetal HSC, and forced overexpression of *Lin28* or *Hmga2* in adult HSCs is sufficient to give them a "fetal-like" HSC expansion phenotype when they are then transplanted into irradiated hosts. Abnormally increased expression of *LIN28* has been documented in the cells responsible for the blast crisis of chronic myeloid leukemia, and increased expression of *HMGA2* has been observed in the dominant clonal hematopoietic cells responsible for paroxysmal nocturnal hemoglobinuria. Insertional activation of *HMGA2* expression has also been associated with amplified clonal hematopoiesis in human and monkeys transplanted with transduced cells. Together, these findings suggest that this axis may also regulate human HSC self-renewal. However, whether and how the elements of this axis may change their expression in primitive normal human hematopoietic cells present at different stages of development has not been investigated. To address this question, we used qRT-PCR to measure and compare the levels of transcripts for *LIN28A, LIN28B, HMGA2*, as well as *IGF2BP1*, *IGF2BP2*, and *IGF2BP3*, several known molecular targets of HMGA2, in purified $CD34^+CD38^-$ hematopoietic cells isolated from human fetal liver (FL), cord blood (CB), and adult bone marrow (ABM). *LIN28B, HMGA2, IGF2BP1*, and *IGF2BP3* transcript levels were significantly higher in FL compared to CB and/or ABM ($p<0.05$). Experiments to determine whether the forced overexpression of *HMGA2* and *LIN28B* in human CB and ABM HSCs will alter their *in vivo* self-renewal in transplanted irradiated immunodeficient recipient mice are ongoing. These studies will provide new insights into the developmental control of HSC properties, potential contributors to disease progression in human leukemia, and new strategies for expanding human HSCs *ex*

vivo.

F-1066
ENDOGLIN REGULATES MESODERM SPECIFICATION BY MODULATING WNT AND BMP SIGNALING

Baik, June, Perlingeiro, Rita C.R.
University of Minnesota, Minneapolis, MN, USA

Signaling by the transforming growth factor-beta (TGF-β) superfamily regulates embryogenesis, adult homeostasis, and disease. In mice, the absence of the TGF-β type lll receptor endoglin (Eng) results in embryonic lethality due to impaired primitive hematopoiesis and cardiovascular defects. This indicates that Eng plays an essential role in embryogenesis but the mechanism underlining these early functions remains elusive. Using a doxycycline-inducible Eng ES cell line, we have observed that Eng up-regulation results in increased hematopoiesis, which happens at the expense of the cardiac lineage. Interestingly the effect of Eng overexpression in cardiac differentiation is stage specific, suggesting that Eng may control lineage specification from uncommitted mesodermal progenitors. To understand the molecular mechanism behind Eng gain-of-function at this stage, we have performed transcriptional profiling analyses of non-induced vs. Eng-induced embryoid bodies (EBs). Among the up-regulated genes, we focused our attention on Dishevelled 1 (Dvl1), a cytoplasmic effector of Wnt signaling. Considering that Wnt signaling is critical during heart development, we hypothesized that Wnt activation may be involved in Eng-mediated cardiac repression. To investigate this idea, we tested the effect of the canonical Wnt signaling inhibitor IWR-1, in Eng-induced EB cultures. In agreement with our hypothesis, the cardiac repression observed following Eng overexpression is abrogated by inhibition of the canonical Wnt signaling pathway. Surprisingly we also found that Wnt inhibition negatively affects the ability of Eng to induce hematopoiesis, indicating that the function of Eng at this early stage of development may require active Wnt signaling. To determine whether the activation of Wnt signaling is controlled by Eng, we have introduced a β-catenin-dependent reporter in the Eng-inducible ES cell line. We observed distinct up-regulation of reporter activity as well as the amount of active β-catenin in Eng-induced EBs compared with non-induced EBs, showing Wnt activation is directly regulated by Eng. Importantly, by using the BMP signaling inhibitor Dorsomorphin, we have also demonstrated that the positive effect of Eng in hematopoiesis occurs through BMP signaling. Considering that the modulation of Wnt activity at this stage establishes competence to form either heart or blood in response to the BMP signal, our findings suggest that Eng may promote the commitment of early mesodermal progenitors to the hematopoietic lineage at the expense of the cardiac lineage via modulation of Wnt and BMP signaling. This is corroborated by our recent studies, using the mesodermal reporter *brachyury*-GFP ES cell line, which revealed that GFP^+Eng^+ cells are endowed with both hematopoietic and cardiac potential. Our study uncovers a novel role of endoglin as a potential mediator between BMP and Wnt signaling during mesoderm specification, and contributes to broaden our understanding of TGF-β signaling in cell fate decision.

F-1067
GENETIC DISRUPTION OF IKAROS ENHANCES THE IN VIVO REGENERATION OF HUMAN HEMATOPOIETIC STEM CELLS FROM CD34+ CORD BLOOD CELLS WITHOUT ALTERING THEIR DIFFERENTIATION

Beer, Philip Anthony, Knapp, David JHF, Kannan, Nagarajan, Miller, Paul Harry, Babovic, Sonja, Aghaeepour, Nima, Gabrielle, Rabu, Rostamirad, Shabnam, Kingsley, Shih, Wei, Lisa, Eaves, Connie
Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

IKAROS (encoded by *Ikzf1*) is a transcription factor essential for lymphopoiesis in mice, with expression of dominant negative variants producing T-cell tumors. In humans, however, inactivating mutations of *IKZF1* are recurrent events in a subset of myeloid as well as lymphoid leukemias, suggesting that IKAROS may have as yet uncovered roles in regulating normal human hematopoiesis. To address this question, we first analyzed IKAROS expression in multiple subsets of human cord blood (CB) cells. High levels of IKAROS protein were found in the hematopoietic stem cell (HSC)-enriched $CD49f^+$ subset of $CD34^+$ cells. These levels remained unchanged in later progenitor or differentiating cells until a marked decrease was seen in maturing neutrophils and erythroid cells. Expression of a lentivirally introduced dominant-negative isoform of IKAROS (IK6) in $CD34^+$ CB cells blocked the normal nuclear localization of IKAROS. This validated the use of this construct to examine the effects of IKAROS disruption on the *in vivo* regenerative activity of CB cells transplanted into NOD/SCID-IL-2Rγc-null (NSG) mice or NSG mice constitutively producing human IL-3, GM-CSF and Steel factor (NSG-3GS mice). Analysis of 24 mice co-transplanted with IK6- and control-transduced cells showed the $IK6^+$ cells generated on average 13-fold more B-lineage ($CD19^+$) cells in the bone marrow and spleen after 10 weeks, without effects on B-cell differentiation (similarly increased ratios of surface IgM^+ B-cells). Human NK-cells ($CD56^+$) and T-cells ($CD3^+$), including naïve ($CD45RA^+$) and memory ($CD45RO^+$) subsets, were also readily detectable, but their numbers were not altered by IK6 expression. However, IK6 also consistently enhanced human granulopoiesis in these mice (3-fold increase in $CD33^+$ cells). Continued monitoring of 14 NSG recipients showed that all of these cell-autonomous effects of IK6 persisted for 6 months, along with a sustained increase in the production of primitive $IK6^+$ $CD34^+CD38^-$ cells. Limiting dilution transplants in secondary mice revealed a 10-fold greater output in the primary mice of HSCs with multi-lineage differentiation activity. IK6 specifically enhanced the IL-3 and GM-CSF-dependent production of granulocytes *in vitro* by increasing the sensitivity of their progenitors to these cytokines. Phosphoflow analysis showed basal and cytokine-stimulated activation of several signaling intermediates was also enhanced in $IK6^+$ progenitors. Most notable of these was cAMP Response Element-Binding Protein 1 (CREB) which was accompanied by increased transcription of known CREB targets in $IK6^+$ $CD34^+$ cells, and included higher levels of c-FOS protein and an increased frequency of Cyclin B1+ cells. Transcriptome analysis of primitive ($CD34^+CD38^-$) $IK6^+$ and control cells confirmed the differential expression of CREB target genes in the $IK6^+$ cells and revealed their premature initiation of a B-lineage transcriptional program without alteration of an HSC gene signature. These results provide multiple lines of evidence of a previously unknown inhibitory role of IKAROS in the homeostasis of primitive human hematopoietic cells, mediated in part by a negative regulation of intracellular signaling. Together, these findings raise the interesting possibility that mutations targeting *IKZF1* may contribute to the leukemic transformation of primitive human hematopoietic cells with myeloid and/or lymphoid potential.

F-1068

FORCED EXPRESSION OF C-MYC ENHANCES THE GROWTH OF PRIMITIVE HUMAN NORMAL AND CML HEMATOPOIETIC CELLS IN SINGLE-CELL AND BULK CULTURES

Bulaeva, Elizabeth, Beer, Philip A., Eaves, Connie
Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

MYC is a well-studied oncogenic transcription factor implicated in the control of self-renewal in studies of mouse hematopoietic stem cells (HSCs) and human cell line models. MYC expression is frequently deregulated in human leukemias, and increased MYC activity has been found to inhibit differentiation in human leukemia-derived cell lines. In particular, increased expression of MYC has been reported in chronic myeloid leukemia (CML) patients and may be associated with the progression of this malignancy. However, the role of MYC in primitive normal human hematopoietic cells or in the pathogenesis of CML has not been previously investigated. The present study was designed to address these questions. As a first step we compared the effects of lentivirally-mediated overexpression of MYC on the extent and duration of cell production from control (YFP+) and test (GFP+) transduced CD34+ cells. These cells were isolated from 2 separate pooled samples of normal human cord blood (CB) and from a chronic phase CML patient sample in which all primitive cells harbored a BCR-ABL1 fusion. The transduced cells were co-cultured for up to 16 weeks on mouse stromal feeders engineered to express human FLT3L, Steel factor (SF), IL-3 and G-CSF, and assessed periodically for the number of mature (nonadherent) cells present. All endpoints measured showed that MYC-transduction of both cell sources caused a marked and sustained (at least 12 weeks) competitive enhancement of their growth under these conditions. Previous studies suggesting that high intracellular levels of MYC may promote cell death prompted us to next examine whether such a phenomenon might be affecting the overall outcomes measured in bulk cultures of transduced human hematopoietic cells. Accordingly, we isolated MYC-high and MYC-low transduced (as inferred from their GFP fluorescence) CD34+ CB cells by fluorescence-activated cell sorting, and incubated them in single-cell cultures containing 50 ng/ml SF, 20 ng/ml GM-CSF, IL-3, IL-6 and G-CSF, and 3 units/ml EPO for 12 days. The efficiency of clone formation was similar in all groups in both experiments performed, but the modal size of the colonies produced by cells with the highest MYC levels was on average ~10-fold larger than that of the controls. The size of the colonies produced by the MYC-low cells appeared intermediate. These findings highlight a marked enhancing effect of MYC expression on the clonal outputs of single progenitors, but speak against a pro-apoptotic effect of MYC in this system. These findings suggest that the presence of supra-normal levels of MYC in human CML and normal CB CD34+ cells activates pathways that significantly, rapidly and sustainably increase cell outputs from both these sources. Moreover, the levels of MYC achieved when it is expressed under the control of the MND promoter (from our lentiviral vector) do not appear to compromise cell survival.

F-1069

ASYMMETRY IN SKELETAL DISTRIBUTION OF MURINE HEMATOPOIETIC STEM CELL CLONES AND THEIR EQUILIBRATION BY MOBILIZING CYTOKINES

Bystrykh, Leonid, Verovskaya, Evgenia, Broekhuis, Mathilde J.C., Zwart, Erik, Weersing, Ellen, Ritsema, Martha, van Poele, Theo, Bosman, Lisette J., De Haan, Gerald
European Research Institute for the Biology of Ageing, Laboratory of Ageing Biology and Stem Cells, University Medical Centre Groningen, Groningen, Netherlands

Hematopoietic stem cells (HSCs) are able to migrate through the blood stream and engraft bone marrow niches. These features are key factors for successful stem cell transplantations that are used in cancer patients and in gene therapy protocols. It is unknown to what extent transplanted HSCs distribute throughout different anatomical niches in the bone marrow, and whether this changes with age. Here we determine the degree of hematopoietic migration at a clonal level by transplanting individual young and aged murine HSCs labeled with barcoded viral vector, followed by assessing the skeletal distribution of hundreds of HSC clones. We detected highly skewed representation of individual clones in different bones at least 11 months post-transplantation. Importantly, a single challenge with the clinically relevant mobilizing agent granulocyte-colony stimulating factor caused rapid redistribution of HSCs across the skeletal compartments. Old and young HSC clones showed a similar level of migratory behavior. Clonal make-up of blood of secondary recipients recapitulates the barcode composition of HSCs in the bone of origin. These data demonstrate a previously unanticipated high skeletal disequilibrium of the clonal composition of HSC pool long-term after transplantation. Our findings have important implications for experimental and clinical and stem cell transplantation protocols.

F-1070

AGING IMPAIRS HEMATOPOIETIC STEM CELLS FUNCTION IN MICE

Campagnaro, Bianca Prandi[1], Ceschim, Sara Lopes[1], Porto, Marcella Leite[2], Rodrigues, Bianca Paula[2], Vasquez, Elisardo Corral[1], Meyrelles, Silvana Santos[2]
[1]University of Vila Velha, Vila Velha - ES, Brazil, [2]Federal University of Espírito Santo, Vitória, Brazil

Background: Stem cells are a unique cell population characterized by self-renewal and cellular differentiation capabilities. These characteristics make them play an important role in regenerative treatments applications. Adult stem cells such as hematopoietic stem cells can be easily isolate from bone marrow, shows multilineage differentiation capability and are largely applied in autologous transplantation which makes them a favorable candidate for clinical translation. However, very few reports evaluated stem cells from aging subjects. Therefore, the aim of this study was to evaluate the impact of aging on hematopoietic stem cells functionality. Methods: Male C57Bl/6 mice were divided into young (2-month-old, n=6) and aged (24-month-old, n=6) groups. Hematopoietic stem cells were isolated from femurs and tibias bone marrow. Differentiated hematopoietic cells were depleted using a commercial kit (BD). After, hematopoietic stem cells were stained with antibodies against Sca-1, CD117 and CD133, or matched isotype control. Oxidative stress was assessed by dihydroethidium (DHE) staining using a FACSCanto II cytometer and data were analyzed using BD FACSDiva and FCS Express 4 softwares. DNA damage was analysed by alkaline comet assay. Briefly, cell sample were mixed with low melting point agarose, spread on slides and submerged in lysis solution. Then, comet slides were placed on an

electrophoresis chamber filled with unwinding alkali buffer and later electrophoresed in the same solution. After electrophoresis, the comet slides were neutralized, visualized in a fluorescence microscope after ethidium bromide staining and analyzed by the program CASP. Data are mean±SEM. Statistical analysis was performed using Student´s t test. Results: Aging augmented cytoplasmic superoxide anion production and, consequently, DNA damage. Hematopoietic stem cells superoxide anions levels, determined by DHE staining, showed significant increase in Aged mice compared with Young (4654 ± 562 vs 2303 ± 312 MFI, p <0.05). Then, using the alkaline comet assay, we evaluated if augmented superoxide anions were able to damage DNA of hematopoietic stem cells. Aged mice showed higher DNA fragmentation compared to Young mice, which could be seen by tail moment (Aged: 62 ± 5 vs.Young: 26 ± 9 a.u., p<0.05) and % tail DNA (Aged: 43 ± 3 vs. Young: 20 ± 5 %, p<0.05). These results suggest that aging increased superoxide anions levels, leading to DNA damage in hematopoietic stem cells. Conclusion: The present study demonstrated that aging affect hematopoietic stem cells functionality. Taking into account that hematopoietic stem cells are responsible of maintaining, generating and replacing differentiated cells as a consequence of physiological cell turnover or tissue damage due to injury, the data obtained by this study suggested that anging Hve to be particularly considered if autologous transplantation is intended, since the donor cells might be altered in its functionality. Financial Support: CNPq, CAPES, FACITEC, FAPES-PRONEX.

F-1071
A MICROCHANNEL ELECTROPORATION (MEP) ARRAY FOR HETEROGENEITY STUDIES AND DRUG RESPONSE OF CHRONIC LYMPHOCYTIC LEUKEMIA STEM CELLS

Chiang, Chi-Ling, Chang, Lingqian, Lee, L. James, Muthusamy, Natarajan
Ohio State University, Columbus, OH, USA

Chronic lymphocytic leukemia (CLL), the most common leukemia in western adults is characterized by gradual accumulation of malignant CD5+CD19+sIgM+ B lymphocytes in the bone marrow, spleen, peripheral blood and lymph nodes. While target therapies using monoclonal antibody against CD20 and CD52 molecules and chemotherapies including fludarabine and chloramabucil are clinically available, none of these agents are curative. Patients exhibit transient response but ultimately relapses and thus posing a challenge. The major challenges in studying CLL relapse could be attributed to among other mechanisms or ineffective targeting of the recently described CLL hematopoietic stem cells (HSCs). However, no specific surface biomarks can identify CLL-HSC, but higher GATA2 mRNA level was recently reported in CLL-HSC than in normal HSC. The detection of GATA2 mRNA in living cells as an intracellular biomarker is difficult. To analysis cellular heterogeneity of CLL-HSCs, we developed an array-based microchannel electroporation (MEP) chip allowing injection of GATA2 mRNA molecular beacons (MB) into 1,000 living cells individually without severe cell damage. MBs with single stranded hairpin shaped oligonucleotide probe could hybridize to the target mRNAs and express blight fluorescence as the conformational change. With properly designing the hybridization site, MBs would not affect cellular function after the short detection. We fabricated 10x100 microchannel array and each pore has a pyramid-pits shape with 2 μm circular opening. Cells were trapped and confined on the microchannel array where the condensed but low electrical field (2 V/mm) applied locally for electroporation and biomolecule delivery. First, we proved intracellular identification of two cells lines, K562 with high GATA2 expression and Jurkat with low GATA2 expression by using the MEP with GATA2 MB. We then collected CD34+CD38-Lin- HSCs from three CLL patients and analyzed cellular heterogeneity based on the GATA2 expression level by the same MEP-MB approach. In three CLL patients studied, rare GATA2high HSCs (<10%) were identified from CLL HSC. We next exposed trapped cells to fludarabine, a widely used clinical chemotherapeutical drug, at different dosages of 0, 1, 10, and50 μM for 24 hours and determined the cellular viability by Calcein AM staining. Within two of three patients, GATA2high HSCs had higher resistance over the fludarabine than GATA2low cells. In conclusion, we demonstrated an on-chip platform integrated single-cell MEP and applied chemotherapeutical drug test for heterogeneous CLL HSC. This MEP platform is the next generation of intracellular detection for any other potential mRNA and microRNA for heterogeneity studies, and the on-chip platform will benefit the drug development.

F-1072
HUMAN PODOPLANIN-POSITIVE MONOCYTES AND PLATELETS ENHANCE LYMPHANGIOGENESIS THROUGH THE ACTIVATION OF THE PODOPLANIN/CLEC-2 AXIS

Choi, Jae-Il[1], Hur, Jin[2], Jang, Jae Hee[3], Oh, Il-Young[4], Yun, Ji-Yeon[1], Lee, Hwan[1], Yunjung, Jin[1], Kang, Jin-A[1], Lee, Hyun-Chae[1], Choi, Young-Eun[1], Lee, Sang Eun[1], Park, Young-Bae[3], Kim, Hyo-Soo[5]
[1]Seoul National University Hospital, Seoul, Republic of Korea, [2]Seoul National University Hospital Division of Cardiology Department of Internal Medicine, Seoul, Republic of Korea, [3]Seoul National University Hospital, Seoul, Republic of Korea, [4]Seoul National University Bundang Hospital, Bundang, Republic of Korea, [5]Seoul National University Hospital Department of Internal Medicine, Seoul, Republic of Korea

Emerging studies suggested that murine podoplanin-positive monocytes are involved in lymphangiogenesis. The goal of this study was to demonstrate the therapeutic lymphangiogenesis of human podoplanin-positive monocytes by the interaction with platelets. Aggregation culture of human peripheral blood mononuclear cells (PBMCs) resulted in cellular aggregates termed hematospheres. During 5-day culture, podoplanin-positive myeloid cells expanded exponentially and expressed several lymphatic endothelial cell-specific markers including VEGF receptor-3 and lymphangiogenic transcription factors. Next, we investigated the potential interaction of podoplanin-positive cells with platelets that had C-type lectin-like receptor-2 (CLEC-2), a receptor of podoplanin. In vitro co-culture of podoplanin-positive cells and platelets stimulated monocytes to strongly express lymphatic endothelial markers and up-regulated lymphangiogenic cytokines. Likewise, platelets stimulated by co-cultured monocytes up-regulated lymphangiogenic cytokine IL-1beta via podoplanin/CLEC-2 axis. The supernatant of co-culture was able to enhance the migration, viability and proliferation of LEC. Local injection of hematospheres with platelets significantly increased lymphatic neovascularization and facilitated wound healing in the full-thickness skin wounds of nude mice. Co-treatment with podoplanin-positive monocytes and platelets augments lymphangiogenesis through podoplanin/CLEC-2 axis, which thus would be a promising novel strategy of cell therapy to treat human lymphatic vessel disease.

F-1073

ENABLING SUCCESSFUL REPROGRAMMING OF PERIPHERAL BLOOD MONONUCLEAR CELLS TO INDUCED PLURIPOTENT STEM CELLS WITH THE CYTOTUNE™ -IPS 2.0 SENDAI REPROGRAMMING KIT

Lebakken, Connie S.[1], Reichling, Laurie[1], MacArthur, Chad C.[2], **Crean, Jennifer**[3], Lakshmipathy, Uma[2], Hammer, Bonnie[1]

[1]Research and Development, Thermo Fisher Scientific, Life Sciences Solutions, Madison, WI, USA, [2]Research and Development, Thermo Fisher Scientific, Life Sciences Solutions, Carlsbad, CA, USA, [3]Thermo Fisher Scientific, Life Sciences Solutions, Grand Island, NY, USA

Human induced pluripotent stem cells (iPSCs) derived from adult somatic cells hold great promise for disease modeling and may provide new cell sources for clinical therapies. Currently the majority of iPSCs are derived from donor fibroblast cells which are obtained by a skin biopsy and expanded prior to reprogramming. Utilizing a donor blood sample as a source for reprogramming is attractive as blood can be easily obtained from most patients and there are large banks of frozen peripheral blood mononuclear cell (PBMC) samples from patients available to researchers. Reprogramming from peripheral blood sources has been challenging due to cytotoxicity and low reprogramming efficiencies. Our goal was to enable the successful and consistent generation of iPSCs from frozen PBMCs utilizing our CytoTune™ iPS Sendai reprogramming tools. We optimized conditions throughout the workflow including culture conditions, timing of transduction and transduction methodology which led to increased reprogramming efficiencies from donor PBMCs. When these optimizations were used in conjunction with the CytoTune™ -iPS 2.0 Sendai Reprogramming Kit we observed reprogramming efficiencies of greater than 2% on MEF feeder layers in KSR-based iPSC medium and greater than 1% on Geltrex™ or Vitronectin substrates in Essential 8™ Medium. Together these improvements support the efficient and reproducible reprogramming of PBMCs and should provide a reliable tool to generate iPSCs from existing and future PBMC sources.

F-1074

THE CLINICAL TRANSLATION OF AN EX VIVO EXPANSION PROCESS FOR UMBILICAL CORD BLOOD DERIVED HAEMATOPOIETIC STEM CELLS

Csaszar, Elizabeth[1], **Prowse, Andrew[1],** Larochelle, Fannie[2], Fares, Iman[3], Cohen, Sandra[2], Sauvageau, Guy[3], Roy, Dennis-Claude[2], Timmins, Nicholas E.[1], Zandstra, Peter W.[1]

[1]CCRM, Toronto, ON, Canada, [2]Hôpital Maisonneuve-Rosemont, Montreal, QC, Canada, [3]IRIC, University of Montreal, Montreal, QC, Canada

Umbilical cord blood (UCB) has a number of advantages over donor bone marrow or mobilized peripheral blood as a cell source for haematopoietic stem cell (HSC) transplantation. However, cell numbers per UCB unit are limited, and there is a strong clinical correlation between primitive cell dose transplanted and successful transplant outcome. As such, the limitation in cell numbers ultimately restricts the use of most banked units to paediatric recipients. Ex vivo expansion of UCB derived HSCs could increase the cell dose available to patients. As well as enabling widespread use for adult recipients, this could also increase the availability of 6/6 HLA matched single UCB units, improving clinical outcomes. In addition to increasing the number of primitive haematopoietic cells available for transplantation, the ideal ex vivo expansion approach should also be scalable, economical, and amenable to automation. We have recently developed an ex vivo UCB expansion strategy that combines a fed-batch feeding regime with a small molecule additive (UM171) identified in a high-throughput screen at the University of Montreal. This combined approach was demonstrated to yield a >100-fold increase in CD34+ progenitor cells and significant expansion of both short- and long-term repopulating HSCs (as measured by a limiting dilution assay out to 30-weeks post-transplantation). This expansion technology is now in the final stages of preparation for a Canadian Clinical Trial Application to initiate a multi-site, 10-patient, expanded UCB Phase I trial, in which the ex vivo expanded UCB unit will be transplanted along with a second un-manipulated unit. In addition to primary safety end-points, this trial will seek to enhance time to neutrophil and platelet recovery as well as achieve long-term repopulation from the expanded unit, in order to assess the potential of expanding very small but highly matched UCB units. The development of the clinical manufacturing process has been a collaborative effort between the Centre for Commercialization of Regenerative Medicine (CCRM), the Université de Montréal, and Hôpital Maisonneuve Rosemont (HMR). The laboratory-scale (1 - 12 mL) ex vivo expansion technology was translated into a robust clinical-scale manufacturing process, which required a >100-fold scale-up in maximum operating volume. In order to accommodate the range of banked UCB units available, and volume increase due to feeding, the system must allow for a culture volume range of 4 - 1500 mL over a 12 day culture period. Process performance is assessed on the basis of numerical expansion of the cells, viability, and phenotype analysis. The clinical scale process has been demonstrated to reproducibly achieve cell expansion levels of >100-fold, and the expanded cells remain > 50% CD34+ and > 25% CD34+CD45RA-. During the technology transfer of the system to the clinical manufacturing site at HMR, the culture system is being integrated with steps that have been developed for the upstream cell preparation (thawing and CD34+ selection) and downstream processing (cell harvest, wash, and formulation). With this work, we have successfully developed a clinical-scale culture process for expansion of HSCs. This process is in final preparation for immediate use in a Phase I trial, and is readily amenable to full automation and closure for efficient routine manufacture in the future.

F-1076

EXAMINATION OF THE EFFECTS OF A NOVEL EP4 RECEPTOR AGONIST CONJUGATE ON MURINE HEMATOPOIETIC STEM CELL ACTIVITY AND NICHE CELLS

Eisner, Christine[1], Scott, Wilder[1], Underhill, Michael[1], Young, Robert[2], Rossi, Fabio M.V.[1]

[1]University of British Columbia, Vancouver, BC, Canada, [2]Simon Fraser University, Burnaby, BC, Canada

The bone marrow provides a unique microenvironment for hematopoietic stem cells (HSCs) termed the stem cell 'niche' that allows HSCs to maintain their multipotency but has also been suggested to limit HSC numbers. Prostaglandin E2 (PGE2) signalling has been implicated in HSC homing, survival and differentiation as well as anabolic effects on bone. Furthermore, PGE2 has been shown to act both directly on HSCs and indirectly on niche mesenchymal stromal cells (MSCs) by signalling through the EP4 receptor. Despite this, PGE2 has limited clinical uses due to its short half-life and widespread adverse systemic effects. To bypass the systemic effects of PGE2, our collaborators have developed a stable EP4 agonist conjugated through a novel linker to bone-targeting compounds (bisphosphonates) allowing for systemic administration and local, bone-targeted EP4a effects. Our main objectives are to characterize the effects of the novel EP4a conjugate (C1) on murine HSC activity and engraftment as well as the effects on niche MSCs. In order to assess the effects of C1 on HSC activity, C56Bl6 Ly 5.2 mice were treated with C1 weekly for up to 7 weeks and HSC enumerated using

a competitive limiting dilution assay (LDA). To determine the effects of C1 on HSC engraftment, a modified LDA was performed whereby lethally irradiated C56Bl6 Ly5.1 recipients were pretreated with either C1 or vehicle and received transplants of purified Lineage-, Sca1+, c-kit+ (LSK) cells from C56Bl6 Ly5.2 donors. Multilineage repopulation in transplanted mice was assessed every 2 weeks using flow cytometry. Effects of C1 on niche MSCs were assessed both in vitro and in vivo. For in vitro analysis, MSCs isolated from murine compact bone (Lin-, PDGFRa+, Sca1+) were maintained in culture and treated with C1 or vehicle. Untreated cells were differentiated using osteogenic media and treated with C1 or vehicle to assess the effects of differentiation on EP4-triggered responses. Cells were collected at various stages for RNAseq analysis and EdU proliferation assays. To assess the effects of C1 in vivo on niche MSCs, EMCcreERT2 transgenic mice expressing td.Tomato under the control of an inducible MSC-specific cre were treated with C1 or vehicle. Trabecular bone content was analyzed using microCT and histology of the femur. Immunoflourescence was also used to determine the number of MSC cells in C1 treated vs. untreated mice. By examining the ability of the novel EP4 agonist C1 to enhance HSC proliferation and engraftment as well as niche expansion, we may identify a new therapeutic tool that could be translated into a clinical setting to expand HSCs for transplant and help patients receiving transplants.

F-1077
MULTICOLOR STAINING OF GLOBIN SUBTYPES REVEALS IMPAIRED GLOBIN SWITCHING DURING ERYTHROPOIESIS IN HUMAN PLURIPOTENT STEM CELLS

Eto, Koji
Center for IPS Cell Research and Application (CIRA), Kyoto University, Kyoto, Japan

Adult hemoglobin composed of α- and β-globin reflects a change from expression of embryonic ε- and fetal γ-globin to adult β-globin in human erythroid cells, so-called globin switching. Human pluripotent stem cells (hPSCs) are a potential source for in vitro erythrocyte production, but they show prominent expression of γ-globin with little β-globin expression, which indicates incomplete globin switching. To examine the mechanism of this impaired globin switching, we optimized multicolor flow cytometry to simultaneously follow expression of different globin subtypes using different immunofluorescent probes. This enabled us to detect upregulation of β-globin and the corresponding silencing of γ-globin at single cell level during cord blood CD34+ cell-derived erythropoiesis, examined as an endogenous control.Using this approach, we initially characterized the heterogeneous β-globin expression in erythroblasts from several hPSC clones and confirmed the predominant expression of γ-globin. These hPSC-derived erythroid cells also displayed reduced expression of BCL11A-L. However, doxycycline-induced overexpression of BCL11A-L in selected hPSCs promoted γ-globin silencing without affecting β-globin. These results strongly suggest that impaired γ-globin silencing is associated with downregulated BCL11A-L in hPSC-derived erythroblasts, and that multicolor staining of globin subtypes is an effective approach to studying globin switching in vitro.

F-1078
WNT5A/NOTCH SIGNALING AFFECTS HEMATOPOIETIC STEM CELL AGING AND SYMMETRIC/ASYMMETRIC DIVISION

Florian, Maria Carolina[1], Nattamai, Kalpana J.[2], Soller, Karin[1], Marka, Gina[1], Überle, Bettina[1], Schiemann, Matthias[3], Eckl, Christina[3], Oostendorp, Robert A.J.[3], Scharffetter-Kochanek, Karin[4], Kestler, Hans A.[5], Zheng, Yi[2], Geiger, Hartmut[2]
[1]Institute of Molecular Medicine and Stem Cell Aging, University of Ulm, Ulm, Germany, [2]Cincinnati Children's Hospital Medical Center Experimental Hematology, Cincinnati, OH, USA, [3]Technical University Munich, Munchen, Germany, [4]Experimental Dermatology, University of Ulm, Ulm, Germany, [5]Department of Bioinformatics and Systems Biology, University of Ulm, Ulm, Germany

Many organs with a high cell turnover (e.g., skin, intestine and blood) are composed of short-lived cells that require continuous replenishment by somatic stem cells. Aging results in the inability of these tissues to maintain homeostasis and it is believed that somatic stem cell aging is one underlying cause of tissue attrition with age or age-related diseases. Hematopoietic stem cell (HSC) function declines upon aging and it is associated with impaired hematopoiesis in the elderly. Previously we showed a causative role for increased activity of the small RhoGTPase Cdc42 in aging of HSCs. Here we report an unexpected shift from canonical to non-canonical Wnt signaling due to elevated expression of Wnt5a in aged HSCs that causes stem cell aging via activating Cdc42. Wnt5a was expressed in young HSCs at very low levels and dramatically increased upon aging, as measured by both real time RT-PCR and immunofluorescence. Consistent with a canonical wnt signaling inhibitory role of non-canonical Wnt5a signaling, aged HSCs presented with a strongly reduced level and primarily cytoplasmatic localization of β-catenin in the majority of the cells. Interestingly, Wnt5a treatment of young HSCs with Wnt5a elicited a reduction in the level of β-catenin, similar to aged HSCs. Additionally, Wnt5a treatment of young HSCs induced aging associated stem cell apolarity, reduction of regenerative capacity and an aging-like myeloid-lymphoid differentiation skewing by increasing activity of Cdc42. Conversely, a stem cell intrinsic reduction of the aging associated elevated Wnt5a expression via a knock-down approach functionally rejuvenated aged HSCs, indicated by improved B-lymphopoiesis and reduction in myeloid skewing in both bone marrow and peripheral blood, restored frequency of donor-derived HSCs and reduced activity of Cdc42. In addition, the frequency of polarized HSCs with respect to distribution of both Cdc42 and tubulin was increased and donor-derived HSCs showed also a high expression level and nuclear localization of β-catenin, indicative of a reversion to active canonical Wnt signaling and an overall phenotype more similar to young HSCs. Surprisingly, aged LT-HSCs also presented with a striking difference in the expression of Notch ligands and receptors and the activation of the Notch pathway was to a great extent recapitulated in young LT-HSCs treated with Wnt5a. Preliminary data indicate that the Wnt/Notch signaling cross-talk via activation of Cdc42 might influence the frequency of asymmetric HSC divisions, so that upon aging HSCs originate more frequently daughter cells similar to each other through a symmetric mode of division. This increase in symmetric divisions is observed also in young HSCs treated with Wnt5a, while inhibition of Cdc42 activity in aged HSCs shifts the balance towards more asymmetric divisions. In summary, we present here as a novel and unexpected concept that aging of HSCs is driven by a shift from canonical Wnt signaling to non-canonical Wnt5a-Cdc42 signaling. Additionally, we show that Wnt5a cross talks with the Notch signaling pathway and affects the aged HSC phenotype possibly by altering the balance of symmetric/asymmetric stem cell divisions.

F-1079
ENFORCED EXPRESSION OF HOXB4 IN DIFFERENTIATING HUMAN EMBRYONIC STEM CELLS ENHANCES THE PRODUCTION OF MULTI-LINEAGE HAEMATOPOIETIC PROGENITORS BUT HAS NO EFFECT ON THE MATURATION OF ERYTHROCYTES

Forrester, Lesley M.[1], Jackson, Melany[1], Axton, Richard A.[1], Taylor, Helen A.[1], Ma, Rui[1], Olivier, Emmanuel[2], Turner, Marc[3], Mountford, Joanne Claire[2]

[1]Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, United Kingdom, [2]University of Glasgow, Glasgow, United Kingdom, [3]Scottish National Blood Transfusion Service, Edinburgh, United Kingdom

Cell-based therapies such as bone marrow transplantation and blood transfusion are used to treat diseases of the haematopoietic system but these procedures are completely reliant on a limited supply of donor tissue. Thus one goal has been to produce therapeutic haematopoietic cells from a bankable and limitless source of embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). There are numerous reports on the production of mature haematopoietic cells and haematopoietic progenitors cells (HPCs) from ESCs and iPSCs but significant challenges face this field: differentiation protocols that have been used to date are relatively inefficient and it has proven particularly difficult to produce the most potent HSCs capable of long term reconstitution (LTR-HSCs) and to generate fully mature, functional cells such as enucleated erythrocytes for transfusion. Enforced expression of transcription factors such as HOXB4 has been reported to have both quantitative and qualitative effects on haematopoietic differentiation of hESCs. However differences in HOXB4 expression levels, in the timing of HOXB4 expression and in the differentiation protocols used have resulted in highly variable outcomes (reviewed in Forrester and Jackson, 2012). We have developed a step-wise, suspension based differentiation protocol that is free of stromal cells and animal products with the ultimate aim of generating red blood cells for clinical transfusion. This highly defined protocol results in the efficient production (30-50%) of haematopoietic progenitor cells (HPCs)(CD34+/CD43+) after 10 days, the subsequent generation of a relatively pure population (≥95%) of erythroid progenitor cells (EPCs)(CD235+/CD71+) and a 350,000 fold expansion in cell numbers. We have tested the effects of a tamoxifen-inducible expression of HOXB4 on the production of HPCs and EPCs using this differentiation protocol. We demonstrate that enforced expression of HOXB4 results in a significant increase in the production of multi-lineage CFU-C but has no effect on subsequent erythrocyte maturation. Erythroid progenitors that were produced in the presence of enhanced HOXB4 expression displayed comparable globin profiles to control cells indicating that enforced expression of HOXB4 has no effect on the final maturation of erythrocytes. We are now exploring the effects of other haematopoietic transcription factors on erythrocyte production and maturation using comparable strategies.

F-1080
ANTI-CD44 ANTIBODIES INHIBIT BOTH MTORC1 AND MTORC2: A NEW RATIONALE SUPPORTING AML DIFFERENTIATION THERAPY INDUCED BY CD44

Gadhoum, Samah Zeineb, Abuelela, Ayman, Merzaban, Jasmeen

King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

While with minimal effect on normal hematopoiesis, anti-CD44 monoclonal antibodies (mAbs) have been shown to be able to reverse the blockage of differentiation responsible for the leukemic phenotype, inhibit proliferation of the leukemic clones, and in some types of leukemia, induce the apoptosis of leukemic blasts. However, the molecular mechanisms underlying these effects are yet to be resolved. The role of aberrant signaling pathways such as the PI3K/Akt and the mammalian target of rapamycin (mTOR) in the uncontrolled growth and survival in cancer including acute myeloid leukemia (AML) is now clearly demonstrated; and inhibitors of mTOR such as Rapamycin are currently being used in clinical trials for their potent anti-neoplastic effects. In this study, we show that anti-CD44 mAb-induced differentiation of HL60 cells as well as blasts isolated from AML patients, is accompanied by a marked decrease in the phosphorylation of mTOR with no effect on the total expression of mTOR. This decrease is correlated with a decrease in Akt phosphorylation on Thr308, strongly suggesting a role for the PI3K/Akt pathway in the inhibition of mTOR activity after CD44 triggering. Moreover, we observed that anti-CD44 mAbs induce a decrease in the phosphorylation of p70S6k (on Thr389) and in the phosphorylation of Akt (on Ser473), directly reflecting the activity of mTORC1 and mTORC2 respectively. Additionally, anti-CD44 treatment induced a decrease in the phosphorylation of the transcription factor FOXO-3a (a direct downstream target of AKT) and its subsequent translocation from the cytoplasm to the nucleus as evident through confocal microscopy. Phosphorylated FOXO-3a has been shown to be involved in the maintenance of blockage of differentiation as well as active proliferation of AML. Taken together, our results show for the first time that CD44 triggering inhibits the activity of mTORC1 and mTORC2 complexes as well as the PI3K/Akt/FOXO-3a pathway. Considering that these pathways have been shown to be constitutively active in most leukemic patients and that their inhibition would have an anti-leukemic effect, our findings unveil a new role for anti-CD44 mAbs as potent mTor inhibitors and further promoting the promise of CD44 as a strong therapeutic target in AML.

F-1081
SP1 IS ESSENTIAL AT EARLY STAGES OF DEVELOPMENT FOR NORMAL HAEMOPOIETIC DIFFERENTIATION

Gilmour, Jane[1], Assi, Salam A.[1], Kulu, Divine[2], van de Werken, Harmen[2], Westhead, David[3], Philipsen, Sjaak[2], Bonifer, Constanze[1]

[1]University of Birmingham, Birmingham, United Kingdom, [2]Erasmus University Department of Cell Biology and Genetics, Rotterdam, Netherlands, [3]Leeds University, Leeds, United Kingdom

The transcription factor Sp1 is essential for normal embryonic development and $Sp1^{-/-}$ mice die *in utero* of multiple heterogeneous developmental defects. However, $Sp1^{-/-}$ embryonic stem cells grow normally. To assess the effect of Sp1 knock-out on embryonic blood cell development, we differentiated them into haemopoietic cells using an *in vitro* differentiation system. Sp1 deficient mouse ES cells show a strongly reduced capacity for macrophage production from embryoid bodies grown in methylcellulose. In contrast, bone marrow from mice with a conditional deletion of Sp1 at the common myeloid progenitor stage of haemopoiesis still have the capacity to generate macrophages in an *in vitro* culture system, suggesting that the defect occurs early in development. A more detailed analysis of the block in differentiation using a blast culture differentiation system revealed that $Sp1^{-/-}$ cells can form haemopoietic progenitors from the haemogenic endothelium (HE), but further development of these progenitors to macrophages is impaired. To investigate the molecular basis of this failure, we performed global gene expression analysis using microarrays on RNA isolated from sorted cell populations representing the haemangioblast, early and late stage of HE as well as progenitor stages of haematopoietic development from both wild type and Sp1 deficient cells. This analysis was complemented by chromatin immunoprecipitation sequencing (ChIP-seq) studies to determine the direct targets of Sp1 binding in wild type cells at the Flk1+ve haemangioblast and progenitor stages.

The integrated analysis of gene expression and Sp1 binding allowed us to define the role of Sp1 throughout differentiation and to establish hierarchical relationships between different regulator genes. Our data show that gene expression profiles were highly similar between wild type and Sp1 deficient cells in Flk1+ve cells. As the cell populations progressed through differentiation, gene expression was progressively deregulated. However, we also found that even in the presence of thousands of deregulated genes, differentiation at the cellular level was surprisingly robust and only failed after multiple intermediate stages. Sp1 deficiency affected a variety of genes that were direct targets of Sp1 involving multiple pathways, including metabolism, focal adhesion and signalling processes. Interestingly, despite its ubiquitous expression, Sp1 bound to many genes in a cell-stage specific manner and this included all four Hox gene clusters which were targets of Sp1 in Flk1+ cells, but not in progenitor cells. Sp1 deficiency led to a down-regulation of almost all Hox genes as well as genes essential for the early stages of mesoderm specification, such as Wnt4, BMP4 and its inhibitor Noggin. This early down-regulation then affected the expression of multiple lineage specific genes in precursor cells including those relating to myeloid development. In conclusion, our analysis demonstrates that the ubiquitously expressed transcription factor Sp1 is essential for normal haemopoietic development and that in its absence the expression of both developmental regulators and lineage-specific factors is impaired.

F-1082
STROMA-DERIVED OSTEOPONTIN REGULATES HEMATOPOIETIC STEM AND PROGENITOR CELLS FUNCTIONS UPON AGING

Guidi, Novella[1], Sacma, Mehmet[1], Soller, Karin[1], Marka, Gina[1], Weiss, Johannes[2], Florian, Maria Carolina[1], Cancelas, Jose[3], Geiger, Hartmut[1]
[1]Institute for Molecular Medicine, University of Ulm, Ulm, Germany, [2]Dermatology and Allergic diseases, University of Ulm, Ulm, Germany, [3]Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Hematopoietic stem cells (HSCs) are located within the bone marrow (BM) in a specific microenvironment referred to as the stem cell niche. HSCs as well as more differentiated hematopoietic cells interact in the BM with non-hematopoietic stroma cells (CD45- cells). This interaction is critical for hematopoiesis, regulating cell proliferation, self-renewal, differentiation and location. Aging of HSCs is associated with a decreased immune response, an increase in the frequency of myeloid disease and a reduced regenerative capacity in hematopoiesis. HSC aging was thought to be a primarily stem cell intrinsic driven mechanisms. Only more recently also aging mechanisms extrinsic to HSCs are being discovered and appreciated. We thus hypothesized that HSC niches age, and that these aging-associated changes impair HSCs function. Heterochronic transplantation of young or old BM cells into young or old recipients revealed that an aged environment supports aging-associated lineage skewing of both young and aged HSCs (myeloid over lymphoid), a decrease in the overall level of engraftment and an increase in the frequency of long term HSCs (LT-HSCs). In the same way, a young environment is responsible for an attenuation of a large number of phenotypes associated with aged HSCs (e.g. decrease in frequency of common myeloid progenitors CMPs, decrease in frequency of LT-HSCs, decrease of myeloid cells and increase of T-cells). These data imply that HSC aging is, besides intrinsic effects, also determined by aging of the niche. Osteopontin (OPN) is a secreted glycoprotein expressed by osteoblasts located close to the endosteum. A role for osteopontin in regulating HSCs in young animals has already been described. OPN was decreased in aged stroma, both in terms of expression (by real time PCR) and secretion (by ELISA assay). This correlated with a significant decrease of the frequency of CD45-OPN+ stroma cells in aged mice. We then tested whether the decrease of OPN in stroma upon aging might be causatively linked to aging of HSCs. Transplantation of either young or aged BM cells into young OPN KO recipients revealed that similarly to results obtained when transplanted into aged recipients, young HSCs transplanted into OPN KO stroma resulted in a significant decrease in stem cell engraftment (20% less compare to control group) and an increase of the frequency of LT-HSCs and CMPs (20% more) implying a causal role for reduced OPN in aged stroma for regulating aging-associated phenotypes of HSCs. We recently reported that young HSCs are polar for cell polarity proteins as well as tubulin and acetylated HistoneH4 at Lysine16 (AcH4K16), while the majority of aged HSCs is apolar. Apolarity thus serves as novel aging-associated marker for HSCs. We thus determined the LT-HSCs polarity status from the heterochronic as well as the OPN KO transplant experiments by single cell immunofluorescence staining. Interestingly young and thus polar LT-HSCs, when transplanted into either aged or OPN KO mice turned apolar for tubulin and AcH4K16, while young HSCs transplanted in young mice were, as anticipated, remaining polar for these markers. These results further imply causality for loss of OPN in stroma upon aging for turning young HSCs into functionally old HSCs. In summary, our data provides new evidence that HSC aging is also regulated by extrinsic, BM niche derived mechanism.

F-1083
VASCULAR NICHE SPECIFICATION AND NOTCH-DEPENDENT EXPANSION OF AGM-DERIVED HSC POPULATIONS IN VITRO

Hadland, Brandon K.[1], Varnum-Finney, Barbara[1], Moon, Randall T.[2], Butler, Jason[3], Rafii, Shahin[4], Bernstein, Irwin D.[1]
[1]Fred Hutchinson Cancer Research Center, Seattle, WA, USA, [2] University of Washington, Seattle, WA, USA, [3]Weill Medical College of Cornell, New York, NY, USA, [4]Cornell Medical College and Angiocrine Bioscience, New York, NY, USA

Hematopoietic stem cells (HSC) emerge in arterial vessels such as the dorsal aorta of the Aorta-Gonad-Mesonephros (AGM) region, suggesting a role for an embryonic vascular niche in supporting initial HSC specification and self-renewal. Here, we establish that endothelial cells derived from the AGM region, imparted with constitutive Akt activation (AGM Akt-EC), provide an in vitro substrate for HSC development. Specifically, AGM Akt-EC provide signals necessary to induce long-term, adult-engrafting HSC from pre-engraftment stage E9-10 VE-Cadherin+ murine embryonic precursors, including phenotypic endothelial cells yet lacking hematopoietic surface markers. Furthermore, AGM Akt-EC co-culture promotes significant expansion of HSC numbers from E11 VE-Cadherin+CD45+ AGM-derived hematopoietic cells. To assess whether the endogenously expressed Notch ligands are critical niche signals, we induced Notch activation via exposure to immobilize Notch ligands in the absence of endothelial stroma. We demonstrate that in vitro Notch activation by ligand, together with cytokines and inhibition of the TGF-B pathway, is sufficient to increase the number of E11 AGM-derived HSC in vitro able to provide long-term, multilineage engraftment in vivo. Furthermore, in E9.5-10 pre-engraftment stage VE-Cadherin+c-kit+ populations, these conditions are sufficient to increase progenitors with myeloid and lymphoid potential in vitro, which can acquire in vivo lymphoid and myeloid engraftment potential following secondary culture on AGM Akt-EC. Altogether, these studies begin to deconstruct the necessary conditions to promote specification and expansion of HSC populations in vitro, which may contribute to future strategies for generating HSC from pluripotent or reprogrammed stem cells for therapeutic applications.

F-1084

HOMOZYGOUS EXPRESSION OF JAK2V617F DRIVES RAPID HEMATOPOIETIC STEM CELL PROLIFERATION AND DIFFERENTIATION AT THE EXPENSE OF SELF-RENEWAL AND IS ACCOMPANIED BY STARK CHANGES IN HOMING ABILITY

Kent, David[1], Li, Juan[1], Fink, Juergen[1], Prick, Janine CM[1], Hawkins, Edwin D.[2], Lo Celso, Cristina[2], Green, Anthony R.[3]

[1]Stem Cell Institute, University of Cambridge, Cambridge, United Kingdom, [2]Imperial College London, London, United Kingdom, [3] University of Cambridge, Cambridge, United Kingdom

Human myeloproliferative neoplasms (MPNs) are clonal disorders characterized by increases in myelo-erythroid lineages and they derive from a mutation, or series of mutations, in a single hematopoietic stem cell (HSC). The JAK2V617F mutation is present in the majority of MPN patients and many patients bear two copies of JAK2V617F, implicating gene dosage as a potential regulator of distinct disease subtypes. To investigate whether JAK2V617F dosage affected HSC proliferation, differentiation, self-renewal, and bone marrow homing we used knock-in mouse models where human JAK2V617F is expressed heterozygously ($JAK2^{+/V617F}$) or homozygously ($JAK2^{V617F/V617F}$) under the control of the endogenous *Jak2* promoter. Previously we showed that $JAK2^{+/V617F}$ mice have a stem cell defect in HSCs >6 months of age. Here we demonstrate that, despite having a more robust myeloproliferative phenotype including a marked erythocytosis, $JAK2^{V617F/V617F}$ mice have a more severe stem cell defect. At 8-12 weeks of age, there are already reduced HSC numbers in $JAK2^{V617F/V617F}$ mice and whole bone marrow transplantation experiments reveal reduced repopulating ability compared to heterozygous and wild-type littermate controls. Highly purified $JAK2^{V617F/V617F}$ E-SLAM HSCs ($CD45^{+}EPCR^{+}CD150^{+}CD48^{-}$) enter the first cell cycle more rapidly and display a pronounced proliferative advantage in short-term cultures. Moreover, clones derived from $JAK2^{V617F/V617F}$ HSCs were comprised of proportionally fewer stem and progenitor cells. Single cell cobblestone area forming cell (CAFC) assays also reveal a marked proliferation advantage with the appearance of early stage CAFCs from $JAK2^{V617F/V617F}$ HSCs, further suggestive of decreased long-term self-renewal activity. Transplantation of highly purified E-SLAM cells affirm that $JAK2^{V617F/V617F}$ HSCs have a reduced per cell repopulation capacity. To investigate whether this reduced in vivo activity was the result of $JAK2^{V617F/V617F}$ stem and progenitor cells localizing to a distinct hematopoietic stem cell niche, we performed in vivo homing assays which show that HSCs home equally well to the BM with 2 days post-transplantation. However, more advanced *in vivo* homing assays to study the HSC niche using live imaging of osteoblast-restricted collagen 1α promoter (Col2.3-GFP) reporter mice show that $JAK2^{V617F/V617F}$ $Lin^{-}CD48^{-}CD150^{+}$ are located significantly further from osteoblasts within 2 days post-transplantation, suggesting a potential mechanistic role for the niche in regulating the HSC defect. Taken together these results indicate that homozygous expression of JAK2V617F compromises HSC self-renewal by increasing proliferation and differentiation and affects niche-selection upon homing, leading to HSC exhaustion amidst expansion of terminally differentiated cells.

F-1085

THE GLOBAL RNA AND PROTEIN LANDSCAPE OF HEMATOPOIETIC STEM CELLS AND THEIR IMMEDIATE PROGENY

Klimmeck, Daniel[1], Cabezas-Wallscheid, Nina[1], Hansson, Jenny[2], Reyes, Alejandro[2], von Paleske, Lisa[1], Lipka, Daniel B.[1], Wang, Qi[1], Milsom, Michael D.[1], Plass, Christoph[1], Huber, Wolfgang[2], Krijgsveld, Jeroen[2], Trumpp, Andreas[1]

[1]Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany, [2]European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

Hematopoietic stem cells (HSC) harbor the highest self-renewal capacity and generate a series of multipotent progenitors (MPP) that differentiate into lineage-committed progenitors and subsequently mature cells. Despite intense research over the last decades the molecular basis of essential HSC features such as self-renewal and quiescence remains poorly understood. To determine the molecular programs employed by HSCs and MPPs, we performed an extensive global analysis combining quantitative proteome and transcriptome (RNA-seq) analyses on five FACS-sorted HSC and MPP populations - HSC (Linneg Sca-1+ cKit+ ,LSK, CD34- Flt3- CD150+ CD48-), MPP1 (LSK CD34+ Flt3- CD150+ CD48-), MPP2 (LSK CD34+ Flt3- CD150+ CD48+), MPP3 (LSK CD34+ Flt3- CD150- CD48+) and MPP4 (LSK CD34+ Flt3+ CD150+ CD48+) - as previously described in our laboratory (Wilson et al., Cell, 2008) and linked these to whole genome DNA methylation data. Proteomics and RNA-seq analyses identified more than 6,000 proteins and 27,000 genes demonstrating stage-specific expression clusters including Wnt and Lin28-Hmga signaling, the imprinted-gene-network, Hox genes, retinoic acid metabolism and an unexpected number of splice variants as regulatory modules installed in HSCs. Our data uncover differential expression landscapes of 493 transcription factors and 682 lncRNAs. Further, expression of Hox clusters and lncRNAs such as H19 during HSC differentiation are controlled by a progressive gain of methylation. Multipotency associated with a cell cycle/DNA repair signature identifies MPP2 as transient-amplifiers, while MPP3/4 show lineage commitment. This study provides a comprehensive genome-wide resource for functional exploration of the molecular, cellular and epigenetic processes operational at the pinnacle of the hematopoietic hierarchy.

F-1086

ASYMMETRIC DIVISION OF MOUSE FETAL AND ADULT HEMATOPOIETIC STEM CELLS

Koechlein, Claire Steeves[1], Reya, Tannishtha[2]

[1]Pharmacology, University of California, San Diego, La Jolla, CA, USA, [2]University of California San Diego School of Medicine, La Jolla, CA, USA

Hematopoietic stem cells (HSCs) are uniquely able to balance their own self-renewal with differentiation. Stem cells may be able to achieve this balance through a combination of asymmetric and symmetric division. In previous studies, we have demonstrated that HSCs have the ability to undergo both asymmetric and symmetric division. However, whether HSCs undergo asymmetric divisions in vivo, and whether this balance changes during development and adulthood is unknown. We are currently using a combination of in vitro and in vivo imaging to define the division patterns of HSCs. An improved understanding of how HSC self-renewal and differentiation is controlled may lead to the development of methods to accelerate HSC regeneration. In addition, as normal mechanisms of self-renewal become dysregulated in cancer, this work could potentially lead to new approaches to block malignant growth.

F-1087
HEPATIC LEUKEMIA FACTOR REGULATES HEMATOPOIETIC STEM CELL ACTIVITY

Komorowska, Karolina[1], Doyle, Alexander[1], Soneji, Shamit[2], Mikkola, Hanna K A[3], Larsson, Jonas[1], Magnusson, Mattias[1]
[1]Molecular Medicine and Gene Therapy, Lund Stem Cell Center, Lund, Sweden, [2]Lund Stem Cell Center, Lund, Sweden, [3]UCLA, Los Angeles, CA, USA

The transcription factor Hepatic Leukemia Factor (*HLF*) was originally identified in a chromosomal translocation with the gene *E2A*, causing a subset of childhood B-lineage acute lymphoid leukemia with poor prognosis. HLF has been described as a regulator of circadian rhythm and recent findings have implicated *HLF* as a candidate "stemness" gene in both normal and malignant stem cells. Furthermore, over-expression of HLF in human hematopoietic stem cells (HSC) results in an enhanced reconstitution capability in NOD-SCID mice. However, little is known about HLF's physiological role in hematopoiesis and HSC regulation. Using quantitative PCR, we confirmed that HLF is highly expressed in mouse (C57Bl/6) HSC and subsequently down-regulated upon differentiation. This encouraged us to further investigate HSC function in the absence of *Hlf*. The conventional *Hlf* knockout (KO) mice were viable with normal blood parameters including lineage distribution and the frequency of immunophenotypic HSCs (Lin-Sca1+ckit+CD34-Flt3-). However a significant reduction of platelet counts (WT $9.98x10^{11}$ ($\pm0.71x10^{11}$), KO $6.16x10^{11}$ ($\pm0.46x10^{11}$), $p<0.001$, n=14) was detected at steady state hematopoiesis. Upon serial competitive transplantation assay using whole bone marrow cells (200 000 cells 1:1 ratio), *Hlf* KO cells displayed a significant reduction in reconstitution capacity in primary recipients (WT 56 (±15)%, KO 40.2 (±16)%, p=0.028, n>10), which was strikingly reduced in secondary recipients (WT 87.2 (±26)%, KO 8.7 (±5.8)%, $p<0.001$, n>10) and no engraftment was detected from the *Hlf* KO cells in tertiary recipients. This was further exacerbated upon competitive transplantation assay with purified HSCs (Lin-Sca1+ckit+CD34-Flt3-), showing a 2.4 fold reduced engraftment capacity (WT 47.3 (±24)%, KO 19.4 (±25)%, p=0.16, n=9) of cells isolated from *Hlf* KO donors, in comparison to wild type (WT) control. This data indicates that HLF is an important regulator of HSCs self-renewal. Additionally, the reduced reconstitution ability was not due to reduced HSC number, as enumerated by limiting dilution transplantation assay. We then performed cell cycle analysis on *Hlf* KO mice and observed a dramatic decrease in HSC quiescence (WT: G0=73.07 (±6.54)%, G1=19.63 (±7.8)%, S/G2/M=5.99 (±1.84)%; KO: G0=54.3 (±10.88)%, G1=37.47 (±5.17)%, S/G2/M=9.1 (±0.54)%, n=3). Additionally, *Hlf* KO mice displayed a striking inability of recovery following administration of the myeloablative agent 5-Fluorouracil (5-FU). *Hlf* KO mice did not survive past day 15 post 5-FU treatment, exhibiting severely reduced HSC and progenitor levels at day 12 (LSK frequency WT $1.1x10^{-2}$ ($\pm0.3x10^{-2}$)%, KO $3.4x10^{-4}$ ($\pm0.1x10^{-4}$)%, $p<0.001$, n=5). These results strongly suggest a critical role for HLF in cell cycle regulation and HSC quiescence. Further supporting the above findings, Genome Ontology (GO) analysis of RNAseq of WT and *Hlf* KO phenotypic HSCs revealed an up-regulation in genes involved in cell cycle upon *Hlf* KO. Interestingly, down-regulated genes included many of those involved in transcriptional regulation as well as cryptochrome 2 (*Cry2*), an inhibitor of circadian clock genes. Taken together our findings show that HLF is an important regulator of HSCs activity and for maintaining HSC quiescence.

F-1088
MONOPOTENT MEGAKARYOPOIETIC PATHWAY BRIDGING HEMATOPOIETIC STEM CELLS AND MEGAKARYOCYTES

Nishikii, Hidekazu[1], Goltsev, Yury[2], Kanazawa, Yosuke[3], Umemoto, Terumasa[4], Matsuzaki, Yu[4], Matsushita, Kenji[3], Nolan, Garry[2], Negrin, Robert S.[1], Yamato, Masayuki[5], Chiba, Shigeru[3]
[1]Division of BMT, Stanford University, Stanford, CA, USA, [2]Baxer Laboratory in Stem Cell Biology, Department of Microbiology and Immunology, Stanford University, Stanford, CA, USA, [3]Department of Hematology, University of Tsukuba, Tsukuba, Ibaraki, Japan, [4]Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Shinjuku-Ku, Japan, [5]Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Tokyo, Japan

Megakaryocytes (Meg) are mapped at the downstream of bilineage progenitors for erythroid cells and megakaryocytes (BiEMP; MEP or PreMegE), whereas the bifurcation of a Meg differentiation pathway at the proximity of hematopoietic stem cells (HSC) is also postulated. Indeed, HSC and Meg share several features in common, such as surface markers, transcription factors, and cytokine signaling, particularly that through thrombopoietin (TPO)-cMpl, which is crucial for the maintenance of HSC and Meg development. Moreover, the existence of platelet-lineage biased/restricted population in HSC compartment (Pb-HSC) was discussed in the several reports. According to this background, we hypothesized that the fate decision of Meg lineage is made in the subpopulation of c-Kit+Sca1+Lin- (LSK) population and Meg could bypass BiMEP phase during their differentiation. In the single cell culture, approximately 12% of CD150+CD41-LSK (stem cell enriched population), 20% of CD150+CD41+LSK cell, and 100% of CD34+GPIbα+Lin-cells (monopotent megakaryocyte progenitor; MKP) derived from mouse bone marrow differentiated only into megakaryocytes. Single cell PCR analysis revealed that mRNA for megakaryocyte related genes (cMpl, vWF, GATA1, and GPIbα) were expressed in 20% of CD150+CD41+LSK population and all of MKP, while much less frequently in cells comprising CD150+CD41-LSK. Only very minor cells from MEP and PreMegE population expressed these genes. In situ hybridization using GPIbα mRNA probe, the signaling from GPIbα mRNA in the nucleus was detected in the subpopulation of CD150+CD41+LSK and CD150+CD41-LSK. In the hematopoietic recovery after 5-FU treatment, the GPIbα protein became detectable in the CD150+CD41+KSL population, and all most all of CD150+CD41+GPIbα+LSK cells showed monopotent megakarcytic potential. These data suggested that Meg could be differentiated directly from CD150+CD41+LSK population, bypass BiMEP phase, especially in emergency/stress condition in vivo. We next investigated the TPO dependency for MKP and MEP/PreMegE generation in the steady state and stress condition. The frequencies of CD150+CD41+LSK, MKP, and CD150+CD41+GPIbα+LSK after 5FU treatment were markedly reduced in cMpl-deficient mice. In contrast, the frequencies of both PreMegE and MEP were only mildly reduced. These findings suggest that the Meg differentiation pathway through CD150+CD41+GPIbα+LSK to MKP depends on TPO signaling more strictly than that through BiMEP pathway. As a summary, our observations imply that platelet-biased population in HSC compartment could directly differentiate into megakaryocyte lineage and bypass BiMEP stage. CD150+CD41+GPIbα+LSK, particularly recognizable during the bone marrow recovery phase, showed restricted monopotent megakaryocyte potential and might be an intermediate progenitor in this direct differentiation pathway,

strictly depending on TPO signaling.

F-1089

BALANCE OF P53 AND E2F1 MAINTAINS CHRONIC MYELOID LEUKAEMIA STEM/ PROGENITOR CELL QUIESCENCE.

Pellicano, Francesca[1], Park, Laura[1], Hopcroft, Lisa[1], Sinclair, Amy[1], Scott, Mary[1], Mark, Aspinall-O'Dea[1], Susan, Graham[1], Leone, Gustavo[2], Kranc, Kamil[3], Whetton, Anthony[4], Holyoake, Tessa[1]

[1]University of Glasgow, Glasgow, United Kingdom, [2]Ohio St University, Columbus, OH, USA, [3]University of Edinburgh, Edinburgh, United Kingdom, [4] University of Manchester, Manchester, United Kingdom

Chronic myeloid leukaemia (CML) is a myeloproliferative disease arising at the hematopoietic stem cell level. The disease results from the chromosomal translocation t(9;22)(q34;q11) that creates the BCR-ABL fusion gene, that in turn encodes for a constitutively active tyrosine kinase. CML stem/progenitor cells (SPCs, defined as CD34+38- cells) are inherently insensitive to tyrosine kinase inhibitors and their resistance is likely due to several tightly interconnected signalling pathways. Although CML SPCs express high levels of BCR-ABL, they maintain a quiescent state similar to normal SPCs. Normal and CML SPCs show a similar percentage of quiescent (G0) cells as indicated by Ki-67/7AAD staining. In agreement, mRNA levels of the cell cycle inhibitors p21, p27 and p57 were not significantly different between CML and normal SPC. By analysing the levels or the activation of the BCR-ABL downstream targets phospho-AKT, phospho-STAT5 and BCL2, we saw that BCR-ABL kinase activity, although modest, was present. To confirm the level of BCR-ABL signaling in CML and normal SPCs, phospho-STAT5 was also analysed using nano-immunoassay (NP1000), a platform that acquires detailed information relating to protein phosphorylation events using exceptionally small amounts of clinical material. We then asked the question how CML SPCs remain quiescent despite the presence of BCR-ABL activity. To identify potential regulators of this mechanism, we investigated the transcriptional differences between primitive and G0 normal and CML cells using gene expression profiling. By screening the arrays for signaling pathways having a role in cell quiescence and homeostasis in normal versus leukemic systems, the p53 and E2F1 networks were identified as significantly modulated in CML SPCs. Our investigation lead to the observation of a pivotal role for p53 and E2F1 in regulating quiescence in CML CD34+38- SPCs by maintaining a fine balance between proliferation and apoptotic signals. Knockdown of p53 in SPCs caused a marked increase in cell proliferation and in colony formation potential. Similar results were observed when p21 was knocked down, suggesting that p53 controls SPC proliferation through its canonical downstream target p21. In parallel, inhibition of E2F1 by knockdown blocked proliferation of CML SPCs and impaired their function, leading to a decrease in colony potential and increased p53-mediated cell death. When E2F1 was inhibited, p53 was phosphorylated on serine 15, leading to apoptosis. Importantly, the combination of E2F1 inhibition with p53 inhibition resulted in rescue of E2F1-mediated cell death and a decrease in colony formation, suggesting their interplay in CML SPC homeostasis. In summary, our studies suggest the importance of E2F1 and its regulated crosstalk with p53 in CML SPC survival and maintenance.

CANCER CELLS

F-1092

IMPACT OF DYSPLASTIC BONE MARROW MICROENVIRONMENT ON HUMAN OSTEOPROGENITOR CELLS

Akwaa, Frank[1], Rubinova, Rakhil[2], Frisch, Benjamin J.[3], LaMere, Mark W.[3], O'Dwyer, Kristin[4], Liesveld, Jane[4], Becker, Michael W.[5], Calvi, Laura M.[6]

[1]Division of Hematology and Oncology, Wilmot Cancer Center, University of Rochester Medical Center, Rochester, NY, USA, [2]Division of Endocrinology and Metabolism, University of Rochester Medical Center, Rochester, NY, USA, [3]Wilmot Cancer Center, University of Rochester Medical Center, Rochester, NY, USA, [4]Division of Hematology and Oncology, University of Rochester Medical Center, Rochester, NY, USA, [5]Division of Hematology and Oncology, University of Rochester School of Medicine, Rochester, NY, USA, [6]Division of Endocrine and Metabolism, University of Rochester, Rochester, NY, USA

Myelodysplastic syndromes (MDS) are clonal hematopoietic disorders with abnormal differentiation, cytopenias and risk of transformation to acute leukemia (AML). In mouse models, disruption of the osteolineage cells can contribute to initiation of ineffective hematopoiesis with phenotypic features of MDS. Osteoprogenitor cells regulate hematopoietic stem and progenitor cells (HSPCs) in response to Parathyroid hormone (PTH) treatment. However, the role of osteoprogenitor cells in human HSPC regulation is poorly understood. We hypothesized that osteoprogenitor cell number and function is impaired by dysplasia-initiated microenvironmental disruption, resulting in reduced support of HSPCs and ineffective hematopoiesis. Interventions targeting osteoprogenitor cells may improve HSPC support. The objective of our study is to characterize dysplasia-induced osteoprogenitor cell loss in human MDS and AML bone marrow and to determine the impact of PTH on HSPCs in the context of MDS/AML patient-derived osteoprogenitor cells. Human spicule associated cells (SACs) were isolated by collagenase digestion of marrow from normal subjects as well as patients with MDS or AML and cultured in mineralization media in limited dilutions. Colony forming unit osteoblasts (CFU-OBs) were scored using alkaline phosphatase and Von Kossa staining. CFU-OB frequency was calculated using L-Calc ™ (StemCell technologies). We identified non-statistically significant decrease in the frequency of CFU-OBs in bone marrow aspirates from MDS patients compared to normal donors (Mean ± SEM of 1.090e-005 ± 1.400e-006 N=2 vs. 5.024e-005 ± 1.277e-005 N=8; p= 0.179); and similar decrease in frequency of osteoprogenitor cells in the bone marrow aspirates from AML patients compared to normal donors (Mean ± SEM of 2.303e-005 ± 9.371e-006 N=3 vs. 5.024e-005 ± 1.277e-005 N=8; p= 0.251). Q-PCR analysis of osteoprogenitor markers osterix, osteocalcin, collagen1α1, in human SACs showed decrease in mRNA expression of these osteoprogenitor markers in MDS samples compared to normal and AML samples. These data support our hypothesis that osteoprogenitor cells in patients with MDS and AML are disrupted compared to normal bone marrow. To assess the potential impact of PTH, we established co-cultures of human SACs treated with vehicle or PTH, with mouse Lineage$^-$ Sca1$^+$c-Kit$^+$ (LSK) hematopoietic progenitor cells. Compared to vehicle treated SACs, we identified a non-statistically significant increase in LSK cells co-cultured with PTH treated SACs from normal donors (0.6978 ± 0.1195 vs. 0.8106 ± 0.1704, N=5, p= 0.592), and a similar trend in LSK cells co-cultured with vehicle treated SACs from AML patients compared with PTH treated SACs from the same patients (0.4482 ± 0.1591 vs. 0.6788 ± 0.2354, N=3, p= 0.429). These preliminary results suggest

that dysplastic bone marrow microenvironment results in the decline in osteoprogenitor cells, which may impact osteoprogenitor support of HPSCs. PTH treatment in our *in-vitro* model shows the potential to ameliorate this negative effect. Lack of statistical significance in our experiment is likely related to small sample size and heterogeneity in MDS/AML. Whether improved support of HSPCs with PTH treatment in these *in-vitro* experiments will translate to improvement in engraftment of these LSK hematopoietic progenitor cells is yet to be determined with transplantation experiments.

F-1093

SOX ANTAGONIZES THE HIPPO PATHWAY TO MAINTAIN CELL STEMNESS IN OSTEOSARCOMA

Basu Roy, Upal Kunal, Basilico, Claudio, Mansukhani, Alka
NYU School of Medicine, New York, NY, USA

Osteosarcomas are the most common primary, non-hematologic malignant tumors in childhood and adolescence, comprising almost 60% of the common histological subtypes of bone sarcomas. They are derived from osteoprogenitor cells and contain highly proliferative malignant cells, with a disrupted bone differentiation program. Cancer stem cells (CSCs) that have tumor-initiating properties have now been described in many solid tumors, including osteosarcomas. The growth-restraining Hippo pathway senses extracellular density cues and regulates tissue homeostasis and organ size by inhibiting the activity of its downstream effector, YAP. The differentiation- inducing signal of this pathway also restrains cancer cells where it plays a tumor suppressive role. The stem cell factor Sox2, maintains cancer stem cells and their tumorigenic properties in osteosarcomas and we have shown that Sox2 directly regulates YAP expression in mesenchymal lineage stem cells. Through a combination of approaches, including the gene expression profiles of osteosarcoma cells depleted of Sox2, we have now examined the state of the Hippo pathway in osteosarcomas. In this study, we show that the Hippo pathway is made inactive in human and mouse osteosarcomas through the upregulation of YAP and the downregulation of the Hippo pathway upstream components, Merlin (NF2) and Kibra (WWC1), that restrain the oncogenic properties of YAP. YAP depletion sharply reduces the tumorigenic properties of murine osteosarcomas. We establish that Sox2 in osteosarcoma stem cells antagonizes the tumor suppressive Hippo pathway by inducing YAP and by directly repressing expression of NF2 and WWC1, thereby increasing nuclear YAP accumulation, and inhibiting its phosphorylation. The Sox2-Hippo axis also operates in other Sox2-dependent cancers such as glioblastomas. We propose that disruption of YAP transcriptional activity reduces the cancer stem cell component and could be a potential therapeutic strategy for Sox2-dependent tumors.

F-1094

EPIGENOMIC CHARACTERIZATION OF GENE REGULATORY NETWORKS IN HUMAN OVARIAN CANCER STEM CELLS

Battle, Stephanie L.[1], Larjo, Antti[2], Lahdesmaki, Harri[2], Lieber, Andre[3], Hawkins, David[3]
[1]*Genome Sciences, University of Washington, Seattle, WA, USA,* [2]*Department of Information and Computer Science, Aalto University, Aalto, Finland,* [3]*Medical Genetics, University of Washington, Seattle, WA, USA*

Ovarian cancer is the fifth leading cause of cancer mortality among women in the United States. Despite aggressive surgery and chemotherapy, most patients relapse and develop drug-resistant tumors, leading to the <40% chance of 5yr survival in ovarian cancer patients. Evidence suggests that a small subpopulation of cells in the tumor, ovarian cancer stem cells (OvCSCs), are responsible for tumor relapse and drug resistance. OvCSCs have enhanced tumorigenicity, inherent chemoresistance, and stem-like properties such as the ability to self-renew or divide asymmetrically. These latter characteristics give CSCs an analogous role in tumors that healthy stem cells have in developing organs (adult stem cells) or entire organisms (embryonic stem cells). The goal of this study is to determine how the cancer stem cell state is regulated and by annotating the gene regulatory network of OvCSCs. In doing so, we will uncover commonalities that exist between OvCSCs and embryonic or progenitor stem cells at the molecular level. The epigenome provides the structural framework for cell-type specific gene regulation. Chromatin modifications that uniquely mark gene regulatory elements, like promoters and enhancers, are easily identified throughout the genome using ChIP-seq. ChIP-seq performed in pluripotent stem cells has revealed many epigenetic features that distinguish them from differentiated cells. In an effort to better understand CSC gene regulatory networks, we are generating comprehensive transcriptomic maps and globally identifying enhancers marked by histone 3, lysine 4 monomethylation (via RNA-seq and H3K4me1 ChIP-seq respectively) in OvCSCs and their daughter tumor cells. We are also comparing OvCSCs to previously generated data in human embryonic stem cells (hESCs) to identify shared stemness traits. OvCSCs were acquired from patient biopsies and detailed characterization identified a CD133+ CSC population within the tumor. We are using a mouse xenograft model to expand tumors in vivo. Harvested tumors are depleted of blood lineage cells and CD133+ cells (CSCs) are isolated from the remaining cells in the tumor. Our analysis of RNA-seq and enhancer maps have revealed OvCSC signatures that are absent in tumor cells but present in embryonic stem cells. Many important embryonic stem cell transcription factors are expressed in OvCSCs and have almost identical chromatin enhancer profiles. We identified over 7000 H1 hESC enhancers that have at least 95% overlap with OvCSC enhancers implying a shared regulatory network in these cell types. Motif analysis of nucleosome-free regions within enhancers reveals enrichment for many ESC transcription factors that are also expressed in OvCSCs. Our integrative analysis indicates that OvCSCs share common features of the ESC regulatory network and is beginning to provide novel insight on why CSCs have stem cell properties. We are continuing to build maps for additional histone modifications and genome-wide DNA methylation in OvCSCs to gain a comprehensive view of how the epigenome regulates the cancer stem cell state.

F-1095

CHROMATIN ORGANIZATION AND EPIGENETIC MODIFICATIONS ASSOCIATED WITH PLURIPOTENCY AND SELF-RENEWAL IN CANCER STEM CELLS

Biran, Alva Ada[1], Scaffidi, Paola[2], Meshorer, Eran[1]
[1]*The Hebrew University of Jerusalem, Jerusalem, Israel,* [2]*Cancer Research UK London Research Institute, London, United Kingdom*

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are characterized by distinct epigenetic features and an 'open' chromatin conformation. Tumor initiating cells, often referred to as cancer stem cells (CSCs), share several characteristics with pluripotent cells, including self-renewal and the potential to differentiate into different cell types, maintaining, in some tumors, hierarchical organization resembling that of tissues. To elucidate the global chromatin state of CSCs we analyzed the levels and distribution of a battery of histone modifications, as well as linker histone H1, in human CD133+ glioblastoma initiating cells (GICs) and in SSEA1+ in vitro-generated CSCs induced through oncogenic reprogramming of normal fibroblasts. In both cellular systems, we find that chromatin in

the CSC subpopulation resembles the characteristic open chromatin of pluripotent cells, compared to the non-CSCs. Importantly, we identified a strong CSC-specific downregulation of the differentiation-related linker histone variant H1.0 in both systems, serving as an indicator for their undifferentiated state. *In vivo* restoration of high H1.0 levels in the CSC subpopulation resulted in decreased CSCs frequency while H1.0 knockdown increased the proportion of the SSEA1+ self-renewing cells in the tumors. Genome-wide mapping of H1.0 by ChIP-seq revealed intronic depletion and exonic enrichment, particularly of constitutive exons, suggesting a role in exon recognition. Taken together, our results suggest that a subpopulation of cells within a tumor shares chromatin characteristics with pluripotent cells, and that the histone variant H1.0 might play a role in regulation of CSC function.

F-1096
MESENCHYMAL TRANSFORMATION OF GLIOMA STEM CELLS: NOVEL SIGNALING PATHWAYS AND THERAPEUTIC IMPLICATIONS

Brodie, Chaya[1], Giladi, Nis David[2], Lee, Hae Kyung[1], Ziv-Av, Amotz[2], Finniss, Susan[1], Cazacu, Simona[1], Xiang, Cunli[1], Mikkelsen, Tom[1], Poisson, Laila[1]

[1]Henry Ford Hospital, Detroit, MI, USA, [2]Bar-Ilan University, Ramat-Gan, Israel

Glioblastoma (GBM), the most aggressive primary brain tumors, exhibit increased invasiveness and resistance to anti-tumor treatments. These tumors contain a small population of glioma stem cells (GSCs) that are implicated in the increased migration, radio- and chemo-resistance of GBM and in tumor recurrence. GBM are categorized into proneural, neural, classical and mesenchymal subgroups, the latter being characterized by increased invasion and poor prognosis. The pathways that play a role in the mesenchymal transformation of GBM are just beginning to be understood. We recently identified RTVP-1 as a glioma-associated protein that regulates cell migration and invasion. In this study we explored the role of RTVP-1 in the mesenchymal transformation of GSCs and the molecular mechanisms that mediate its effect. Analysis of TCGA tumor specimens demonstrated that the expression of RTVP-1 was higher in mesenchymal GBM and predicted tumor recurrence and poor clinical outcome Using ChiP analysis, we found that the RTVP-1 promoter binds STAT3 and C/EBPbeta, the two master transcription factors that regulate mesenchymal transformation of GBM. Moreover, both STAT3 and C/EBPbeta regulated RTVP-1 expression in GSCs and mediated the induction of RTVP-1 by IL-6, a physiological activator of these transcription factors. RTVP-1 was expressed in different GSCs but not in human neural stem cells (NSCs). Silencing of RTVP-1 in GSCs decreased the mesenchymal phenotypes and transformation of these cells and increased their ability to differentiate into neural cells. RTVP-1 silencing also decreased the self-renewal of the cells, their stemness and migration. Moreover, RTVP-1 knockdown also decreased tumor volume of GSC-derived xenografts and increased xenografted mice survival. Overexpression of RTVP-1 in human NSCs inhibited their neural and induced their mesenchymal differentiation. To delineate the molecular mechanisms underlying the effects of RTVP-1, we performed gene array analysis of RTVP-1 silenced glioma cells and identified IL-6 and CXCR4 as major mediators of RTVP-1 effects on the mesenchymal transformation and self-renewal of GSCs. In addition, using a pull down assay with His-tagged RTVP-1 we identified HSP27, N-WASP and hnRNPK as novel interacting proteins of RTVP-1. Our data indicate that RTVP-1 induced self-renewal and migration of GSCs by the increased expression of IL-6 and CXCR4 and via its interaction with N-WASP, hnRNPK and HSP27. Moreover, the upregulation of IL-6 by RTVP-1 acts in a positive feedback loop to further increase RTVP-1 expression by activating the STAT3 pathway. Collectively, these results implicate RTVP-1 as a major determinant in the mesenchymal transformation of GBM and as a therapeutic target for the treatment of GBM and eradication of GSCs.

F-1097
CD99 IS A PROMISING THERAPEUTIC TARGET EXPRESSED ON DISEASE STEM CELLS IN MDS AND AML

Chung, Stephen Shiu-Wah[1], Devlin, Sean[2], Tavakkoli, Montreh[3], Klimek, Virginia[4], Park, Christopher Y.[5]

[1]Leukemia Service, Department of Medicine and Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA, [2]Department of Biostatistics and Epidemiology, Memorial Sloan-Kettering Cancer Center, New York, NY, USA, [3]Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA, [4]Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA, [5]Memorial Sloan-Kettering Cancer Center, New York, NY, USA

The myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are initiated and sustained by self-renewing stem cells. We performed transcriptomal profiling of purified MDS hematopoietic stem cells (HSC) and identified CD99 as a highly expressed cell surface transcript. Flow cytometry of MDS patient bone marrow (BM) samples (n=63) confirmed that CD99 is frequently increased on MDS HSCs (85%). Assessment of 78 paired diagnosis/relapse AML specimens revealed that CD99 is also frequently overexpressed at diagnosis (81%) and relapse (83%) in AML. CD99 is a potential leukemic stem cell (LSC) marker, as within the stem cell enriched CD34+CD38- fraction of AML, CD99 high cells exhibit a LMPP-like immunophenotype (CD90-CD45RA+), previously shown to be enriched for LSC activity, whereas CD99 low cells exhibit a CD90+/-CD45RA- phenotype resembling normal HSCs and MPPs. Providing additional support for CD99 as a LSC marker in AML, CD99 surface expression is significantly higher in the LSC-enriched CD34+CD38- fraction compared to bulk blasts (p=0.003), as well as in relapsed as compared to diagnostic specimens (p=0.007). To determine if CD99 is preferentially expressed on functional LSCs, we purified the top 10% and bottom 10% of CD99 expressing leukemic blasts from the LSC enriched LMPP-like fraction of a primary AML specimen. Limiting dilution xenotransplantation demonstrated an estimated LSC frequency of 1 in 24,401 in the top 10% of CD99 expressors, whereas transplantation of up to 360,000 of the bottom 10% of CD99 expressors did not lead to any engraftment. Thus, within the LSC-enriched LMPP-like compartment of AML, LSC activity appears to be restricted to high CD99 expressors. To determine the function of CD99 in AML, we stably transduced MOLM13 AML cells with a CD99 shRNA (8.0-fold knockdown) and xenografted them into NSG mice. Animals transplanted with these cells showed improved survival compared to vector controls (58d vs. 34d, p=0.02). Consistent with its described role in leukocyte trafficking, overexpression of CD99 in AML cell lines promoted transendothelial migration in transwell assays. In primary AML specimens, CD99 expression was higher on PB as compared with BM specimens (p=0.03). Together, these findings suggest that CD99 may promote AML aggressiveness by enhancing transendothelial migration and mobilization. To determine whether CD99 may be a relevant therapeutic target, we tested the ability of monoclonal antibodies (mAb) against CD99 to induce direct cytotoxicity in vitro. Anti-CD99 mAbs induced apoptosis in 17 AML and two MDS-derived cell lines, as well as in primary AML blasts (n=7) and MDS HSCs/CD34+ cells (n=3). These mAbs were cytotoxic in a CDC and ADCC independent manner, with relative sparing of normal cells expressing low levels of CD99 such as primary human HSCs and endothelial cells, suggesting a mechanism of cytotoxicity

unique to AML and MDS. Pre-coating of primary human LSCs with anti-CD99 mAb prior to transplantation into NSG mice led to impaired engraftment at eight weeks (20% vs. 67%, p=0.009) and improved survival (p=0.05). Anti-CD99 mAb treatment was associated with activation of Src-family kinases, and we propose that anti-CD99 mAbs may promote cytotoxicity by inducing oncogenic stress via dysregulated Src-family kinase activation. Our results establish CD99 as a cell surface marker expressed in AML and MDS stem cells, as a mediator of transendothelial migration, and as a promising therapeutic target for direct targeting by mAbs.

F-1098
A NOVEL DIAGNOSTIC ASSAY FOR DETECTION OF PRIMATE-SPECIFIC RNA EDITING EVENTS IN CANCER STEM CELLS

Crews, Leslie A.[1], Jiang, Qingfei[1], Zipeto, Maria A.[1], Lazzari, Elisa[1], Ali, Shawn[1], Court, Angela C.[1], Barrett, Christian L.[2], Marra, Marco[3], Frazer, Kelly A.[2], Jamieson, Catriona H.M.[1]
[1]Moores Cancer Center at University of California, San Diego and Sanford Consortium for Regenerative Medicine, La Jolla, CA, USA, [2]Pediatrics, Division of Genome Information Sciences at University of California, San Diego, La Jolla, CA, USA, [3]Canada's Michael Smith Genome Sciences Centre, Vancouver, BC, Canada

Introduction: The adenosine deaminase acting on dsRNA (ADAR) RNA editases have been linked to the pathogenesis of diverse malignancies, including leukemia, breast cancer and hepatocellular carcinoma. We previously showed that human chronic myeloid leukemia stem cells (CML LSC) harbor increased ADAR1 expression compared with normal and chronic phase progenitors. Whole transcriptome RNA sequencing (RNA-Seq) revealed increased adenosine to inosine (A-to-I) RNA editing during CML progression concentrated within primate specific Alu-containing transcripts. However, detection of RNA editing by RNA-Seq in rare cell populations can be technically challenging, costly and requires PCR validation. The objectives of this study were to 1) validate RNA editing of a subset of cancer-associated and stem cell relevant transcripts in the context of lentivirally enforced ADAR1 expression, 2) develop an RNA editing reporter assay in human leukemia cells, and 3) devise a qPCR-based diagnostic test to rapidly detect aberrant RNA editing in cancer stem cells (CSC). Methods: The BCR-ABL+ human leukemia cell line K562 was stably transduced with lentiviral human ADAR1 or vector. FACS-purified K562-ADAR1 cells were transfected with a luciferase-based reporter vector to confirm RNA editing activity. Highly differentially edited loci were selected from our previous RNA-Seq studies of CML progenitors (MDM2 and APOBEC3D), along with known RNA editing sites in stem cell functional genes (Gli1) and cancer-associated sites (AZIN1). Targeted sequencing was performed on high fidelity PCR products using primers flanking each editing site. RNA editing site-specific qPCR (termed RESSq-PCR) primers were designed for each site using an allele-specific strategy that detects cDNA containing either an A or G(I) representing an RNA editing event. RNA editing was detected by qRT-PCR in K562-ADAR1 and primary hematopoietic stem cells (HSC) lentivirally transduced with ADAR1. Results: Lentivirally enforced ADAR1 expression promoted RNA editing activity as measured by luciferase reporter activity. Increased A-to-I changes in MDM2, APOBEC3D, and Gli1 were confirmed by targeted sequencing. In independent experiments, RESSq-PCR accurately detected RNA editing in K562-ADAR1 cells (n=3) and in primary HSC overexpressing ADAR1 (n=4). Editing-specific primers distinguished G(I) bases at RNA editing sites in cDNA and as predicted gave no signal in gDNA. Relative A-to-I RNA editing ratios were increased by 2 to 3 fold in ADAR1-expressing cells at all sites, and detected low levels of AZIN1 RNA editing not measurable by direct sequencing. Conclusions: These results set the stage for development of primate-specific RNA editing as a novel diagnostic strategy for clinical CSC detection. These data shed new light on the mechanisms of ADAR1-mediated generation of malignant progenitors that drive therapeutic resistance, disease progression and relapse in CML and may be applicable to a variety of CSC-driven malignancies.

F-1100
MICRORNA PROFILE RELATED TO AUTOPHAGY AND APOPTOSIS IN COLON ADENOCANCER CELLS AFTER MTOR INHIBITION BY RAPAMYCIN

Ergin, Kemal[1], Aktaş, Safiye[2]
[1]Dokuz Eylul University, IZMIR, Turkey, [2]Department of Basic Oncology, Institute of Oncology, IZMIR, Turkey

Background: mTOR (mammalian target of rapamycin) is a serine-threonine kinase that regulate protein synthesis, cell growth and differentiation. In addition it plays an important role in cell skeletal and ribosome reorganisation, meiosis, translation, cell cycle, apoptotic and autophagic cell death. Recent studies indicate the role of mTOR in tumorigenesis. It was found that mTOR induced cell growth and proliferation in cancers. But its role in colon cancer still remain to be explained. Rapamycin is a macrolide antibiotic which blocks mTOR and is trialed in cancers included colon cancers. The aim of this study was to investigate the microRNA profile and related autophagic markers in a colon adenocarcinoma cell line (Caco-2) after inhibiton of mTOR by Rapamycin. Material and Methods: Two groups were designed for this study: a. Group I: Control group (Caco-2), b. Group II: Rapamycin (10 nM) incubated group. MicroRNA microarray was performed with Affymetrix system (Affymetrix GeneChip 2.0). After the bioinformatic analysis (Benjamini Hochberg corrected t-test p-value < 0.05) validation and targeted gene analysis (KEGG pathway analysis) was done for the expressed miRNA's. Then immunohistochemical/immunoflourescence (IHC/IF) analysis was performed with antibodies for apoptosis (TUNEL, bax, p53, caspase-3, caspase-8, Fas-L) and for autophagy (mTOR, beclin-1, ATG-12 and LC3BII) which were found in the pathway analysis. Results: It was found that 492 miRNA probesets had different expression pattern in the miRNA profile. It was shown that in group II 5 miRNA had an increased expression level while 487 miRNA had a reduced level compared to group I. Validation process has confirmed our microarray results. Pathway score for mTOR signalling pathway was 7.66, for apoptosis 1.92 and for regulation of autophagy 0.67. In the IHC/IF study reduced mTOR expression was seen in the rapamycin group as expected. Besides TUNEL, p53, ATG-12 and LC3BII was more intense and caspase-3 was moderate in the group II. Conclusion: It was found that the internal pathway of apoptosis related with the expressed microRNA's was much more important for the cell death in mTOR inhibited colon cancer cells. Future studies should aim to study the role of mTOR for the therapies in colon cancers in order to improve the outcome.

F-1101
MLL5 MEDIATES GLOBAL RESTRUCTURING OF NUCLEAR ARCHITECTURE IN GLIOBLASTOMA TUMOR-INITIATING CELLS

Gallo, Marco[1], Vanner, Robert[2], Coutinho, Fiona[2], Desai, Kinjal[3], Lupien, Mathieu[3], Bazett-Jones, David[1], Dirks, Peter B.[1]
[1]Hospital for Sick Children, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]Ontario Cancer Institute, Toronto, ON, Canada

Functional heterogeneity in cancer cell populations is hypothesized

to arise from the co-existence of different epigenetic states. We show that glioblastoma is characterized by extensive inter- and intra-tumoral epigenetic heterogeneity. We provide evidence that the Trithorax-related protein MLL5 contributes to maintaining epigenetic landscapes characteristic of the tumor-initiating cellular fraction in glioblastoma. Interestingly, we find that this protein suppresses post-translational modifications (PTMs) of nucleosomal H3, but not of other core histones. This effect was not observed in normal brain samples. Whereas it was found by others that H3 is mutated in a significant cohort of pediatric glioma patients, the repression of H3 PTMs we observed in adult glioblastoma is independent of mutational status and directly dependent on MLL5, as knockdown of MLL5 is sufficient to restore H3 PTMs. Electron spectroscopic imaging shows that global decrease in H3 PTMs caused by MLL5 results in the formation of extensive domains of compacted chromatin that contribute to a glioblastoma-specific epigenome. The degree of reorganization of nuclear structural architecture caused by MLL5 in glioblastoma has never been described in the literature and favours self-renewal of glioblastoma tumor-initiating cells. On the contrary, knockdown of MLL5 results in mature neural phenotypes. Consistent with these findings, MLL5 target genes are involved in positive regulation of cellular differentiation. Our work uncovered a novel cancer-specific epigenetic function for a Trithorax protein in acting as an epigenetic insulator to limit the spread of H3 PTMs. This function of MLL5 is required to maintain the self-renewal properties of tumor-initiating cells. Furthermore, we show that H3 dysregulation is an event common to pediatric and adult gliomas, although it is exquisitely regulated by epigenetic control in adults.

F-1102
BRAIN TUMOR INITIATING CELLS EXPRESS FUNCTIONAL COAGULATION FACTOR RECEPTORS AND RESPOND TO THEIR AGONISTS BY DIFFERENT PATTERNS OF VESICULATION, DEPENDING ON ONCOGENIC AND DIFFERENTIATION STATUS

Garnier, Delphine[1], Bentley, Victoria[1], Magnus, Nathalie[1], Meehan, Brian[1], Mehta, Shwetal[2], Stiles, Charles[3], Rak, Janusz[1]
[1]Pediatrics, Montreal Children's Hospital, Research Institute of McGill University Health Centre, Montreal, QC, Canada, [2]Barrow Neurological Institute, Phoenix, AZ, USA, [3]Dana-Farber Cancer Institute Div of Cellular and Molecular Biology, Boston, MA, USA

Brain tumor initiating (stem) cells (BTICs) exist within the vascular microenvironment (niche) that includes both blood vessels and blood. While the links between BTICs and angiogenic endothelium are well established, the role of blood coagulation and signalling in this regard has not been studied. To this end we screened different types of tumorigenic stem cells for the expression of receptors mediating cellular interactions with the coagulation system, namely tissue factor (TF) and protein activated receptors (PAR-1, 2, 3 and 4). TF acts as a receptor for coagulation factor VIIa, PAR-1, 3, 4 are activated by thrombin (IIa) and PAR-2 by TF/VIIa complex and factor Xa. We detected the expression of the aforementioned coagulation receptors in mouse embryonic stem (ES) cells, and mouse BTICs, harbouring p16 tumor suppressor (Ink4a-/-) deficiency and, in some cases, the activating mutation of the epidermal growth factor receptor (EGFRvIII). Interestingly the levels of coagulation receptor expression changed upon induction of cell differentiation, as manifested by decrease in levels of PAR-1 and PAR-3. Moreover, we found that mouse BTICs respond to stimulation of PARs by thrombin, as indicated by MAPK phosphorylation. In parallel we observed differences in patterns of vesiculation, as measured by Nanoparticle Tracking Technology (NTA/Nanosight). Extracellular vesicles (EVs) are membrane-derived organelles secreted by normal and cancer cells, and contain different multimolecular cargo. This process contributes to intercellular communication and tumorigenesis via the emission and uptake of oncogenic pro-coagulant and regulatory signals. While stimulation of BTICs with thrombin induced EGRvIII-related changes in vesiculation, differentiated BTICs exhibited a low and thrombin unresponsive profile of EV emission. Thus, contact with blood coagulation system may result in PARs activation by thrombin on the surface of BTICs, and this could alter the behavior of these cells or their progeny including a differential release of EVs. Exit from the BTIC pool changes the quantitative aspect of brain tumour cell vesiculation. Overall, we propose the existence of an unsuspected link between stemness/differentiation states in the BTIC population and the regulatory effects of blood-borne coagulation effector proteases, involving emission of EVs. Human glioblastoma (GBM) is associated with highly procoagulant microenvironment and our results suggest that BTICs driving progression of this disease may be influenced by these conditions. We postulate that agents able to control coagulation system in GBM may have therapeutic value.

F-1103
GENOMIC ANALYSES UNCOVER A NOVEL STAT3-OSMR FEED FORWARD SIGNALLING MECHANISM THAT REGULATES PATHOGENESIS OF GLIOBLASTOMA

Jahani-Asl, Arezu[1], Yin, Hang[2], Soleimani, Vahab[3], Chang, Natasha[1], Sincennes, Marie-Claude[1], Luchman, Artee[4], Puram, Sidharth[5], Ligon, Keith[5], Weiss, Samuel[6], Bonni, Azad[7], Rudnicki, Michael A.[1]
[1]Ottawa Hospital Research Institute, Ottawa, ON, Canada, [2]Yin lab / UGA, Athens, GA, USA, [3]McGill University, Montreal, QC, Canada, [4]U Calgary, Calgary, AB, Canada, [5]Harvard Medical School, Boston, MA, USA, [6] University of Calgary, Calgary, AB, Canada, [7]Washington University, St Louis, MO, USA

The transcription factor STAT3 plays an essential role in glioblastoma tumors that express the major oncogenic protein EGFRvIII, a constitutively active form of the epidermal growth factor receptor. However, the mechanisms by which STAT3 mediates EGFRvIII-induced glioblastoma tumorigenesis remain poorly understood. Here, using genome-wide analyses we identify the cytokine receptor OSMR as a critical component of EGFRvIII/STAT3 signaling in glioblastoma pathogenesis. STAT3 directly binds the promoter of the OSMR gene and induces its expression in human brain tumor stem cells (BTSCs) and mouse astrocytes. Knockdown of OSMR suppresses cell proliferation and tumor growth in vivo, and extends survival of mice bearing human EGFRvIII-expressing-BTSC xenografts. Strikingly, OSMR forms a receptor complex with EGFRvIII and thereby regulates STAT3 phosphorylation and its transcriptional output. Our findings define EGFRvIII, STAT3, and OSMR as components of a feed-forward signaling mechanism that drives glioblastoma tumorigenesis. OSMR-based therapies may prove valuable in the treatment of glioblastoma.

F-1104
RUNX1 AS AN EPIGENETIC REPROGRAMMER IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL)

Jenkins, Catherine E.[1], Bilenky, Misha[2], Shevchuk, Olena[1], Giambra, Vincenzo[1], Gusscott, Sam[1], Hirst, Martin J.[2], Weng, Andrew P.[1]
[1]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada, [2]Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada

T-cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive hematopoietic malignancy characterized by the outgrowth of developmentally arrested T-lymphoid blasts. Notch pathway activation

through mutation of *NOTCH1* or *FBW7* in over half of cases leads to hyperactive pathway activation through elevated transcription of target genes through NOTCH/CSL complex regulation. Recently, we found that RUNX1 binding was enriched near regions of dynamic NOTCH1/CSL binding at H3K27Ac-marked superenhancers which regulate cell identity as measured using ChIP-seq. We hypothesized that NOTCH1 and RUNX1 signaling might co-regulate target genes involved in the growth of leukemic clones by epigenetic reprogramming of the genome. In contrast, recent studies have identified *RUNX1* mutations in primary cases of T-ALL/ETP-ALL and have suggested that these mutations might function in a dominant-negative fashion, reducing RUNX1 activity and suggestive of tumor-suppressive function in this context. Notably, in cell lines harboring *RUNX1* mutations that are predicted to produced truncated RUNX1 protein, no truncated species was detected as measured by western blot. We next attempted to functionally characterize the role of RUNX1 in T-ALL cells by knocking down its expression using lentiviral shRNA hairpins in a broad panel of T-ALL cell lines. In contrast to data suggestive of a tumor-suppressive role, we found the vast majority of cells with reduced RUNX1 levels to show substantial growth defects as measured by an *in vitro* competitive growth assay. We confirmed this result in a subset of cell lines and xenograft-expanded patient samples through BrdU incorporation/DNA content assays and found cells to exhibit a consistent G_1 cell cycle arrest upon RUNX1 knockdown. Interestingly, there was no correlation between *RUNX1* mutation status and sensitivity to RUNX1 knockdown as measured by these assays. Due to the surprising level of co-occupancy of RUNX1 and NOTCH1 at dynamic Notch signaling targets, we have generated ChIP-seq libraries for the core histone marks: H3K4me1, H3K4me3, H3K9me3, H3K27me3, H3K27Ac, and H3K36me3 in T-ALL cells which have been either depleted of activated NOTCH1 or total RUNX1 protein. We will harness our existing libraries of steady-state of RUNX1 and NOTCH1 occupancy over the T-ALL genome to establish regions of epigenetic control. In order to explore potential pro-growth target genes that could be responsible for this proliferative phenotype, we examined our previous ChIP-seq data and found that NOTCH1/CSL bound to putative enhancers within the *IGF1R* and *IL7R* loci that were enriched in RUNX1 binding as confirmed by local ChIP-qPCR. Importantly, we find that RUNX1 and NOTCH1 co-regulate the cell surface expression of IGF1R and IL7R in an additive manner. The work from ourselves and others have demonstrated significant roles for IGF1R and IL7R in regulating not only bulk cell growth but also leukemia-initiating activity of T-ALL blasts, suggesting that *cis* co-regulation of target loci at superenhancers by RUNX and NOTCH factors plays an important role in the propagation of established T-ALL clones.

F-1105
MOLECULAR AND STRUCTURAL CHARACTERIZATION OF THE SH3 DOMAIN OF THE ONCOPROTEIN AHI-1 IN CHRONIC MYELOID LEUKEMIA

Liu, Xiaohu (Will)[1], Chen, Min[1], Rothe, Katharina[1], Lobo, Paolo[2], An, Jianghong[3], Cheng, SW. Grace[3], Moradian, Annie[3], Morin, Gregg B.[3], Van Petegem, Filip[1], Jiang, Xiaoyan[1]

[1]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada, [2]Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada, [3]Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada

Chronic myeloid leukemia (CML) is a clonal multilineage myeloproliferative disorder characterized by the presence of the oncoprotein BCR-ABL with increased tyrosine kinase activity. ABL tyrosine kinase inhibitors (TKIs) have been introduced into clinical practice with remarkable effects on chronic phase CML. However, early relapses, acquired drug resistance, and persistence of leukemic stem cells remain problematic. Improved treatment approaches to target other molecular elements active in CML stem/progenitor cells are needed. One candidate is AHI-1 (Abelson helper integration site 1), an oncogene that harbors two key domains, SH3 and WD40, and is highly deregulated in CML stem cells. We recently demonstrated that AHI-1 physically interacts with BCR-ABL and JAK2 in CML cells and that this interaction complex mediates transforming activity and TKI response/resistance of CML stem/progenitor cells. In this study, we have characterized the biological and structural functions of the SH3 domain of AHI-1. To determine biological roles of the SH3 domain, several mutant forms, including SH3 domain deletion (SH3Δ), were generated and stably transduced into BCR-ABL inducible cells. Using an apoptosis assay, we have shown that BCR-ABL+ cells transduced with the SH3Δ mutant displayed dramatically increased Annexin V+ cells, compared to cells with full-length AHI-1, in the presence of imatinib (57 vs. 26%), nilotinib (65 vs. 25%) and dasatinib (63 vs. 34%), suggesting that the SH3 domain of AHI-1 plays a role in the mediation of TKI resistance. The crystal structure of the AHI-1 SH3 domain revealed that the AHI-1 SH3 domain adopts a canonical SH3 folding, but with an unusual C-terminal α helix. There are three large negatively charged patches, which may be involved in binding selectivity and specificity. PD1R peptide, known to interact with the PI3K SH3 domain, was used to model the binding pattern between AHI-1 SH3 domain and its ligands and showed that an "Arg-Arg-Trp" stack may form within the binding interface, which could be a targeting site for designing specific drugs. Furthermore, using the AHI-1 SH3 domain as protein 'bait' in immunoprecipitation/mass spectrometry, Dynamin-2 (DNM2), a large GTPase mainly involved in endocytosis and vesicle trafficking, was identified as a potential interacting partner of AHI-1. Indeed, DNM2 expression was highly increased in CD34+ CML stem/progenitor cells compared to CD34+ normal bone marrow cells ($P<0.05$). In particular, transcript levels of Dynamin-2 were higher in CD34+ cells from IM-nonresponders (n=15) vs. IM-responders (n=11). Co-immunopreciptation with mutant forms of AHI-1 and DNM2 (HA-AHI-1, HA-AHI-1 SH3Δ, Myc-DNM2 and Myc-DNM2 PRDΔ) indicated that the PRD domain of DNM2 is mainly responsible for the interaction between AHI-1 and DMN2. Co-localization analysis using confocal microscopy further confirmed that the interaction between AHI-1 and DNM2 occurs in the cytoplasm. Moreover, AHI-1 was observed to co-localize with EEA-1 (early endosome marker) and LAMP-1 (late endosome marker), suggesting that the interaction between AHI-1 and DNM2 may be involved in endocytosis processes, which have not been shown to be deregulated in CML. In conclusion, investigation of the structure of AHI-1 SH3 domain and its interacting proteins will provide invaluable insight into identification of key interaction sites involved in the regulation of drug resistance, key targets for development of small molecule inhibitors for CML.

F-1106
PROBING HIPPO SIGNALING ACTIVITY IN HEMATOLOGICAL MALIGNANCY

Lorthongpanich, Chanchao, Jiamvoraphong, Nittaya, Wattanapanitch, Methichit, Laowtammathron, Chuti, Klincumhom, Nuttha, Issaragrisil, Surapol

Faculty of Medicine Siriraj Hospital, Mahidol University, Siriraj Center of Excellence for Stem Cell Research (SiSCR), Bangkok, Thailand

The Hippo pathway is an evolutionally conserved protein kinase cascade and known to be involved in an organ size control and cell contact inhibition. It has been demonstrated that deregulation of the Hippo pathway leads to cancer development. However, previous

studies were performed mainly in solid tumors and the role of Hippo signaling in non-solid tumors has not been fully elucidated. Here we aimed to investigate Hippo signaling activity in different types of hematological malignancy, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and chronic myeloid leukemia (CML). Similar to those solid tumors, Hippo signaling is inactive in all leukemic cell types. In agreement with this result, transcriptional analysis reveals a significant reduction of the Hippo core kinases in all leukemic cell types. However, the patterns of YAP and phosphorylated-YAP localization are different from those found in epithelial cells and cancers. Up-regulation of the Hippo core kinase activity was observed upon the treatment of demethylating agent in a dose-dependent manner. Collectively, our findings suggest that Hippo signaling pathway is inactive in hematological malignancy. However, further studies to characterize the Hippo signaling cascade in hematological malignancy need to be performed.

F-1107
PLURIPOTENT STEM CELL-DERIVED NEURAL PROGENITORS AS A SYSTEM FOR THE STUDY OF HUMAN GLIOMAS

Morozova, Olena[1], Olsen, Mari[1], Salama, Sofie[2], Haussler, David H.[2]
[1]University of California Santa Cruz, Santa Cruz, CA, USA, [2]University of California Santa Cruz; Howard Hughes Medical Institute, Santa Cruz, CA, USA

Malignant gliomas are cancers of the glial tissue of the central nervous system that are classified by the type of affected cells (most commonly astrocytomas, oligodendrogliomas, or oligoastrocytomas), by World Health Organization (WHO) grade (II-IV), and by their location within the brain. Gliomas account for over 70% of all malignant brain tumors diagnosed in the United States. High-grade (grade III and IV) gliomas are almost invariably fatal, with the median survival of 12-15 months and 2-5 years for grade IV and III gliomas, respectively. While patients with grade II gliomas can survive longer than 5 years, their disease is predominantly incurable and may transform into a secondary higher-grade tumor. Therefore, novel therapeutic targets are urgently needed for better management of malignant glioma patients. To study the full spectrum of molecular lesions in malignant gliomas, WHO grade II-IV neoplasms have been profiled as part of The Cancer Genome Atlas (TCGA), an initiative tasked with high resolution characterization of human cancers. The TCGA consortium and others revealed molecular subtypes of gliomas with similarities to different stages of neural development. While these studies provided important insight into the heterogeneity of malignant gliomas, the cellular origin of these diseases remains controversial due to the limited availability of normal human cells that would provide a reference for studying glioma development in a laboratory setting. Understanding the molecular subtypes comprehensively and with adequate normal comparators is essential for better patient stratification and personalized targeted therapies for malignant gliomas. Our preliminary studies, as part of the TCGA LGG AWG, revealed that a large proportion of LGG tumors harbored recurrent mutations in IDH and ATRX. In addition, clustering analysis of gene expression profiles showed that specific genetic changes were associated with different gene expression signatures, along with differences in patient survival. However, understanding the role these mutations play in tumor formation and progression is hampered by the lack of relevant cell lines and animal models. In this study, we evaluate the use of CRISPR-Cas9 system for the introduction of ATRX and IDH1 mutations into human embryonic stem cells to create neural progenitors carrying glioma-specific genetic changes. We have designed and tested RNA guides specifically targeting ATRX and IDH1 loci, and will use this system to study the effect of these changes on normal neural development. We will also investigate the potential for these mutations to act as cancer drivers. The establishment of a human neural progenitor system expressing ATRX and IDH1 mutations will serve as an invaluable resource for discovering and characterizing new therapeutic targets for malignant glioma patients.

F-1108
MN1 AND HOX GENE ACTIVATION ENFORCES AN IN VITRO GROWTH AND LEUKEMIC POTENTIAL UPON NORMAL HUMAN CORD BLOOD CELLS.

Norddahl, Gudmundur[1], Imren, Suzan[1], Gasparetto, Maura[2], Xiang, Ping[1], Heuser, Michael[3], Humphries, R. Keith[1]
[1]Terry Fox Laboratory, BC Cancer Agency Research Center, Vancouver, BC, Canada, [2]Division of Hematology, Hematologic Malignancies, University of Colorado, Aurora, CO, USA, [3]Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany

Great strides have been taken with whole genome sequencing of samples from AML patients. However, the diversity and scale of genomic and transcriptomic changes revealed pose a significant challenge to the clinical use of this information where there is a need to distinguish driver from passenger changes, and to identify shared pathways that might serve as Achilles heels. The development of new and more relevant strategies for creating forward genetic models of human AML would facilitate pinpointing key elements of the mechanisms of action of driver mutations, and provide a framework for testing new ways to target the LSC population. As an approach to the development of forward genetic models of aggressive human AML, we have focussed on engineered overexpression of members of the Hox transcription factor family and the oncogene MN1, both of which are strongly implicated in poor prognosis AML. In initial experiments we introduced the fusion gene NUP98HOXD13 (ND13) along with MN1 into normal human cord blood (CB) cells. Over-expression of ND13 alone and in combination with MN1 promoted proliferation of these cells in stroma-free cultures in the presence of myeloid supporting cytokines (IL-3, IL-6, SCF, FLT3L and TPO) for at least 75 days. Whereas, cord blood cells expressing MN1 alone had a significantly lower proliferative capacity in the same culture conditions. The majority (22/28) of NSG-3GS (NOD-SCID beta2 microglobulin knockout mice, transgenic for the human cytokines, IL-3, GM-CSF and SCF) mice transplanted with extensively cultured (33-65 days) MN1+ND13 cord blood cells succumbed to AML (median survival 65 days in all mice injected with 10^2-$5x10^6$ cells), and had splenomegaly (spleen weight=142±97mg). Whereas, only 4 out of 16 mice injected with ND13-expressing cells showed persistence of immature human myeloid cells (42±3%) as late as 250 days post-transplant and importantly, none of these mice developed AML. In stark contrast to this, MN1-expressing cells failed to proliferate in vivo. Upon limiting dilution transplantation, we revealed that the disease repopulating cell frequency was 800-fold higher in MN1+ND13- than in ND13-expressing cells, demonstrating a synergistic activity of MN1 and ND13 in human CB cells. Furthermore, GM-CSF alone supported proliferation of ND13- and MN1+ND13-expressing cells in vitro. Interestingly, MN1+ND13-expressing cells displayed increased JAK/STAT signaling, downstream of GM-CSF receptor, with high levels of STAT1 and STAT3 phosphorylation and decreased expression levels of SOCS1 and SOCS3, negative regulators of STAT signaling. Additionally, MN1+ND13-expressing cells showed increased resistance to all trans retinoic acid (ATRA) when compared to ND13 cells in agreement with the acquired ATRA resistance of AML patients with high levels of MN1 expression.

In conclusion, we have generated a new forward genetic model of transplantable human AML using human progenitor cells immortalized with a potent oncogene MN1 and an activated HOX gene, ND13. These cells are able to proliferate in stroma-free suspension cultures for an extended period of time and consistently induce AML in NSG-3GS mice. This model system for human AML should present a promising tool for in vitro screening approaches in the development of novel therapeutic strategies and in vivo validation of therapeutic targets.

F-1109
THERAPEUTIC TARGETING OF EZH2 AS A CANCER STEM CELL SPECIFIC ONCOGENE

Peng, Warner, Liu, Zhengian, Sanaat, Zohreh, Dolatkhah, Roya, Kimura, Kyle, Ellipeddi, Pavani, Esfandyari, Tuba, Farassati, Faris
University of Kansas Medical School, Kansas City, KS, USA

Oncolytic viruses are a novel family of anti-cancer agents capable of targeting cancer cells in a specific manner. Many earlier generation of oncolytic viruses are now in advanced phases of clinical trials. Ezh2 (enhancer of zeste homologue 2) or Ezh2, as the catalytically active component of the PRC2, is involved in transcriptional repression of certain genes by trimethylation of lysine 27 and, to a lesser extent, lysine 9 of histone H3. A range of human tumors show overexpression of Ezh2 where its overexpression is associated with poor prognosis. Ezh2 is involved in the biology of stem cells and is claimed to be a cancer stem cell specific oncogene. We have developed the first prototype of a mutated herpes virus that is programmed to specifically target and destroy Ezh2+ cells. We have tested this virus against a number of human malignancies including brain tumor, breast cancer, liver cancer and pancreatic cancer. Loss of viability and invasiveness were observed for all of these models. Additionally, inhibition of in vivo tumor growth was observed with administration of this virus against human cancer cells xenografts in immunocompromised mouse model. Growth of cancer stem cells in vivo was also significantly blocked by this virus. This work, therefore, explains a potential approach for targeting human malignancies on the basis of Ezh2 expression.

F-1110
DE-NOVO GENERATION AND REPROGRAMMING OF TUMOR PRODUCING CANCER STEM CELL-LIKE CELLS THROUGH DIFFERENT PATHS

Rossello, Ricardo A.[1], Rivera, Horacio Serrano[2]
[1]University of Puerto Rico, Medical Sciences Campus, San Juan, Puerto Rico, [2]Metropolitan University of Puerto Rico, San Juan, Puerto Rico

Recently, our group published findings that showed iPSC-like cells can be induced in vertebrate and invertebrate model organisms that span 550 million years from a common ancestor: in mammals, birds, fish, and fly. Parallel to this effort we noticed a large number of transformed colonies that were being formed. These cells were not stem cells, but, in some cases, had transformed characteristics. We examinee these cells for human, mouse, chicken. and noticed they had a variety of features. These cells were passed through a variety of experimental procedures to first determine their characteristics and, secondly, determine their tumor-generating prowess. RTPCR expression showed that asides from the stem cell genes (Oct-4, Sox-2, Klf-4, and C-myc) in the cassette, these cell also expressed SSEA1, ERAS, KLF-5. Proliferation assays (doubling time and MTT) showed divergent results. On the one hand, a sub set of cells (CSCL-1) showed high proliferative capacity, while another clone (CSCL-7) exhibited little to no proliferation, relative to control groups. These two cell types were labeled with GFP and injected into skid mice (nu/nu) to determine their tumor generation prowess. A distribution of 80% established cancer cell line (ATCC) and 20% of either CSCL-1 or CSCL-7. Extracted tumors were sectioned and immunostained to determine the contribution of the cells for each tumor. Surprisingly, the CSCL-7 clone, which had low proliferative capabilities in-vitro, contributed over 90% of the tumor growth, suggesting a quiescent state in-vitro, and activation in-vivo. Asides from low proliferation, these cells exhibited no telomerase activity and abnormalities were observed in chromosomal length. CSCL-1 exhibited large tumor contribution as well (55-60%), though much less than CSCL-7. In an effort to make a de-novo cell with cancer stem cell -like properties, we transduced cells with both the stem cell cassette and a new cassette containing, ERAS and SSEA1, and an inducible promoter (MTT). We were able to generate a cell line with similar properties to CSCL-1. RTPCR validated the expression, and the generation of tumors was similar to CSCL-1. These cells produced a 65% contribution to the generated tumor. Our final effort was to undo the reprogramming of the generated cancer cells, to determine if we could diminish any of the properties by controlling the expression of these genes. Because we used a Metallothionein inducible promoter for the expression of ERAS and SSEA1, we were able to control expression of the cassette. Results showed that both proliferation ($p<0.001$) and tumor contribution (6% vs 65%, $p<0.01$) were significantly diminished. Taken together, our results show that cancer stem cell properties can arise through a variety of paths, that some of these paths can be generated de-novo, and that the tumorigenic potential of these cells can be limited by controlling the expression of a subset of genes, suggesting a potential path to limit the damage of cancer stem cells.

F-1111
HUMAN BREAST CANCER STEM AND PROGENITOR CELL POPULATIONS DEMONSTRATE AGE-SPECIFIC EXPANSION AND CONTRACTION IN TUMORS WHICH IS NOT OBSERVED IN STEM AND PROGENITOR CELL POPULATIONS IN BENIGN BREAST TISSUE

Skinner, Amy M., Peckham, Jennifer L., Pommier, Rodney F., Muller, Patrick J., Schillace, Robynn V., Diggs, Brian, Hansen, Juliana E., Naik, Arpana M., Pommier, SuEllen
Surgery, Oregon Health and Science University, Portland, OR, USA

Background: Recent work employing lineage fate mapping in murine mammary ducts revealed that mammary stem cells (SCs) are bipotent and maintain a capacity to differentiate into luminal or myoepithelial cells. Immunophenotyping and transplantation experiments in mice demonstrate that SCs play unique ontogenic roles: clonal expansion occurs during puberty, differentiation takes place during lactation, and maintenance ensues during adulthood. We hypothesized that, as in mice, the percentage of SCs in human benign breast tissue remains relatively constant over time, while lineage-specific progenitor cell populations expand or contract during aging. Moreover, as breast cancers are thought to arise from a stem cell defect, we hypothesized that breast cancer stem and progenitor cells (BCSCs) exhibit unique expansion/ contraction characteristics during aging. Methods: Benign breast specimens were obtained from 49 women (age 22-74) following reduction mammoplasty, and invasive ductal carcinoma (IDC) specimens were collected from 60 women (age 27-89) following lumpectomy or mastectomy. SCs and BCSCs were sorted by multiparameter fluorescence-activated cell sorting to exclude lin- (CD31, CD45), and collect CD49f (integrin α6) and CD24 cells as $CD49f^{hi}CD24^{low}$ (PM), $CD49f^{hi}CD24^{hi}$ (PP), $CD49f^{low}CD24^{hi}$ (MP), and $CD49f^{low}CD24^{low}$ (MM) expressing cell subpopulations. Differences in distribution of stem and progenitor cell populations were determined for all women, separated into cohorts by age at diagnosis and grouped by decades (i.e. <35, 35-44, 45-54, 55-64, and >65 years old). Results: All 4 subpopulations of SCs were present in benign specimens and

tumors. When evaluated by decade, the frequency of stem and committed progenitor cells/total cells in benign tissue was 0.7%-19% in all age groups. The frequency of SC subpopulations varied with age. While MMs and MPs remained relatively constant, there were significant 2-fold and 3-fold increases in mean percentage of PMs and PPs, respectively, in benign tissues in the 35-44 age group, compared to the <35 age group. BCSCs subpopulations also varied with age, with distinct patterns that differed from benign tissues. The frequency of BCSCs/total tumor cells was 0.5%-18% in all age groups. BCSC subpopulations did not exhibit similar patterns of expansion in the corresponding benign age groups. The PP subpopulations exhibited a 5-fold expansion in tumors detected in the 45-54 age group ($p<0.05$). The MP subpopulation exhibited a 4-fold increase by the 55-64 decade ($p=0.02$). Among post-menopausal women, BCSC composition varied with hormonal status of tumors. ER- tumors expressed nearly 2-fold more PMs than ER+ tumors ($p=0.04$). Although the frequency of BCSCs was comparable between small (T1) and large (T3, T4) tumors, there were 5-fold more PPs and 3-fold more PMs in large tumors ($p<0.01$). Conclusions: The overall frequency and distribution of SC populations varied little with age in benign tissue. In contrast, tumors showed age-specific expansion/contraction patterns of cell populations that differed significantly from those observed in benign tissues. We have previously reported that mutations in BCSCs correlate with more aggressive disease. These data provide an explanation for the age-specific differences in biologic aggressiveness of breast cancers exhibited among women diagnosed in different decades of life.

F-1113

CHEMOTACTIC STIMULI FROM HEAD AND NECK SQUAMOUS CELL CARCINOMA CAUSES MIGRATION AND INVASION OF MESENCHYMAL STEM CELLS AND THEIR LOCALIZATION TO THE SURROUNDING TUMOR MICROENVIRONMENT

Watts, Tammara, Pinchuk, Irinia V., Cui, Ruwen, Powell, Don W.
University of Texas Medical Branch, Galveston, TX, USA

The overall survival rate for advanced head and neck squamous cell carcinoma (HNSCC) is quite poor and patients exhibit early disease recurrence resistant to chemotherapy and radiation. Annually ~55,000 new cases are diagnosed annually, 11,500 deaths reported, treatment costs exceed $3 billion. The clinical behavior of HNSCC requires innovative study, and I propose an emphasis on the role of the tumor microenvironment. Objective: We hypothesize that bone marrow derived mesenchymal stem cells (MSCs), by integrating into the cancer stem cell (CSC) niche and surrounding tumor microenviornment, directly influence tumor growth, progression and invasion. We hypothesize that MSCs are recruited into the HNSCC tumor microenvironment where they directly and indirectly contribute to cancer progression, propagation of the CSC niche and to radio- and chemo-resistance, resulting in the treatment failure common to HNSCC. Methods: MSC chemotaxis and invasion was measured using the Boyden chamber assay. Transwell filter supports were treated with matrigel to quantitate invasion. A triple label confocal approach was used to visualize MSCs within the tumor microenvironment of patients with HNSCC. To elucidate which chemotactic factor(s) are important for MSC homing, a multiplex magnetic bead assay approach was utilized. Western immunoblotting and PCR techniques were used to determine the presence of candidate chemotatic factors thought to be important in the homing of MSCs to the HNSCC tumor microenvironment. Results: We show here for the first time that HNSCC causes a nearly two-fold increase in MSC migration compared to serum free controls and 1.5 fold increase in MSC migration compared to conditioned media from oral kerotincyte controls ($p<0.005$). Likewise, the condition media from HNSCC cell lines caused a 1.5 fold higher rate of MSC invasion compared to the condition media of oral keratinocyte controls, and a 30-fold higher rate of invasion compared to serum media alone. Confocal microscopy revealed the co-localization of positive MSC biomarkers gremlin/CD105 and gremlin/CD90. No co-localization was appreciated with the hematopoietic stem cell marker CD 45 and alpha smooth muscle actin. Several cytokine signaling pathways have been shown to be important in the homing of MSCs to the tumor microenvironment. Using a multi-plex approach, we screened the CM from JHU-011 and JHU-019 HNSCC cells lines, MSCs and appropriate controls for 47 chemokines, cytokines, and growth factors previously shown to be important in the pathophysiology of cancer. A limited number of highly secreted candidate molecules were identified IL-6, IL-8, CCL5, VEGF, GRO-α, SCGF-b and MIF. MSCs secreted ~2,400 pg/ml of IL-6 compared to 150 pg/ml by CC7049 primary fibroblast controls. IL-6 was also highly secreted by HNSCC JHU-11 and JHU-019 cells, 2600 pg/ml and 3500 pg/ml, respectively. Similarly high levels of IL-8 were observed. Receptors for both IL-6 (IL-6R) and IL-8 (CXCR2) were present on the surface of the HNSCC cell lines JHU-011and -019 and MSC. Conculsions: These data, along with observations in other cancer models, suggest that paracrine signaling from HNSCC tumor cells promotes the homing of MSCs to the HNSCC tumor microenvironment, where they contribute to tumor growth, propagation of the CSC niche, and together with CSCs may confer the resistance to chemotherapy and radiation that is clinically observed in this patient population.

EMBRYONIC STEM CELL CLINICAL APPLICATION

F-1115

BIOPROCESS ECONOMICS OF MANUFACTURING PANCREATIC PROGENITORS FOR BETA CELL REPLACEMENT THERAPY

Pedroza, Rene Gonzalo[1], Wallner, Klemens[2], McCabe, Christopher[2], Piret, James[1]
[1]Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada, [2]Department of Emergency Medicine, University of Alberta, Edmonton, AB, Canada

The proliferative and differentiation capacity of stem cells makes them ideal candidates for large-scale production for beta cell replacement therapy, thereby overcoming the hurdle of donor shortage for islet transplantation. However traditional tissue culture technologies (e.g., clump passaging, planar adherent culture) make robust large-scale operations challenging. Modelling the cost of manufacturing with new tissue culture technologies early in the development process should help guide efforts to improve the cost-effectiveness of a candidate therapy prior to commercialization. Our case study was based on reported methods to manufacture pancreatic progenitors from single-cell cultures of human embryonic stem cells. We estimated the cost of goods for the expansion and differentiation stages of the process at different levels of cells per dose, doses per lot and demand, identifying the tissue culture vessel that minimize the manufacturing cost at every passage during expansion. The cost of the materials (i.e., media, tissue culture vessels) was the main component of the cost of goods. Additionally, a sensitivity analysis showed that changes in passage efficiency and differentiation could have a major impact on the cost of manufacturing. We aim to broaden this analysis by including the downstream component of the manufacturing process, and adding the facility and operating costs to the estimation of the cost of goods.

F-1116

ENSURING POST-GRAFTING SAFETY RIDDANCE OF STEM CELL THERAPY PRODUCTS FOR HUNTINGTON DISEASE WITH THE TK-SUICIDE GENE STRATEGY

Bugi, Aurore[1], Aron Badin, Romina[2], Nicoleau, Camille[3], Viegas, Pedro J. B.[4], Jan, Caroline[2], Lecourtois, Sophie[2], Guillermier, Martine[2], Helies, Jean-Marie[2], Peschanski, Marc[5], Hantraye, Philippe[2], **Perrier, Anselme**[1]

[1]I-Stem, Inserm/UEVE UMR861, INSERM, Evry, France, [2]MIRCen, URA CEA CNRS 2210, Fontenay-aux-Roses, France, [3]ISTEM, AFM - INSERM/UEVE UMR 861, Evry cedex, France, [4]I-STEM, Evry, France, [5]INSERM U861 I-Stem, Evry Cedex, France

Development of technologies that now provide unlimited access to therapeutically relevant human pluripotent stem cells (hPSC) derivatives has radically changed the outlook for using cell therapy to treat neurodegenerative disorders such as Huntington's disease (HD). An important safety concern for hPSC-derived cell therapy remains the potentially uncontrolled proliferation of grafted cells leading for example to teratoma/tumor formation or overgrowth. This is particularly true for hPSC-graft for HD as fetal ganglionic eminences-derived grafts, the current clinical standard transplanted in HD patients, are seen to proliferate after transplantation. To deal with possible adverse effects of graft derived from hPSCs we developed a safety system based upon genetic engineering of the grafted cells with the Herpes simplex type 1 thymidine kinase (TK) suicide gene. Materials and Methods: Transgenic human and Monkey PSC lines expressing TK, GFP and/or Neomycin were produced using lentivirus or plasmidic expression vectors. Sensitivity of these lines to GCV was first assessed in vitro on undifferentied PSCs, proliferative neural stem cells (NSC) and post-mitotic neurons derived from these lines. Efficacy of the TK-based safety system was then studied in vivo in an allo-transplantation context using hyper-proliferative immature neural grafts to challenge the system. 8 macaques (Macaca fascicularis) received bilateral intracerebral stereotaxic injections of quinolinic acid in the putamen, an established phenotypic model of HD. Two weeks after lesioning, animals received intrastriatal neural grafts expressing TK and GFP. All animals were lesioned and grafted in pairs using the same batch of cells. Magnetic resonance imaging scans were used to follow longitudinally graft volume at 21, 35, 52, 63 and 70 days post-grafting. At 35 days post-grafting one of the two animals grafted on the same day was randomly treated with val-GCV for 33 days. All animals were perfuse-fixed with PFA and all brains were recovered and processed for histology. Results and Conclusions: In vitro dose response analyses of GCV sensitivity of TK+ hPSC lines revealed that proliferative hPSC and hPSC-derived NSC are highly sensitive to GCV (IC50 in sub-micromolar range). In contrast, post-mitotic neurons generated from TK+ lines are insensitive to GCV. An MRI longitudinal follow-up was used to assess the implantation site, growth and survival of grafted cells in non-immunosuppressed macaques as well as to calculate their proliferation rate over time. Although the initial size of individual grafts was heterogeneous across the 8 primates, volumetric analysis suggests that GCV administration slows down or stops cell hyper-proliferation in treated animals compared to the untreated ESC-grafted animals. Histological characterization of graft composition and differentiation showed that neuronal content was not affected by val-GCV treatment. These results suggest hPSC allografts can survive and proliferate in the primate brain in the absence of peripheral immunosuppression in a primate model of HD and that the risk of overgrowth can be controlled using a TK-suicide gene system.

F-1117

STEM CELL CULTURING ON RECOMBINANT HUMAN LAMININ-521 FACILITATES SUPPLY AND PROPAGATION OF CLINICAL GRADE GENETICALLY STABLE HUMAN PLURIPOTENT STEM CELLS

Rodin, Sergey[1], Antonsson, Liselotte[2], Hovatta, Outi[2], Tryggvason, Karl[1]

[1]Karolinska Institute Department of Medical Biochemistry and Biophysics, Stockholm, Sweden, [2]Karolinska Institute/CLINTEC, Stockholm, Sweden

We have recently shown that extracellular matrix laminin-521 (LN-521) permits clonal derivation and long-term self-renewal of human embryonic stem (hES) cells (Rodin et al., Nat Commun, 2014). Culturing of dissociated hES and human iPS cells on LN-521 is as simple as culturing of fibroblasts or HEK-293 cells, and can be performed under xeno-free and chemically defined conditions. Clonal expansion of hES cells has been shown to be dependent on cell-cell contact, and clonal derivation of hES cells can be accomplished in vitro using a single cell biopsy from 8-cell human embryo (without destruction of it) and a culture dish coating consisting of LN-521 and E-cadherin in a 9:1 w/w ratio in xeno-free and chemically defined environment. This technique also facilitates clonal expansion of hES cells that are genetically manipulated e.g. for cell fate tracking and gene function analyses. Therefore, LN-521 based matrices provide a source and a simple method of propagation of clinical grade hES cells. Pluripotency of hES cells growing on LN-521 has been confirmed in both in vitro and in vivo experiments after up to 20 passages in culture. High-resolution DNA analysis has revealed low frequency of new genetic abnormality formation in the cells cultured on LN-521 cells. Normally, pluripotent hES cells undergo adaptive genetic changes during prolonged culturing in vitro, which resemble those observed in tumor cells. This trait may hamper the use of pluripotent hES cells in clinical applications. Although genetic instability has been attributed to the nature of pluripotent hES cells themselves, certain features of culturing procedures affect genetic stability of the cells. Thus, enzymatic passaging is supposed to affect number of aberrations in the hES cells. Since LN-521 allows both enzymatic and non-enzymatic passaging, passaging both in pieces and in single cell suspensions, as well as culturing of hES cells with and without ROCK inhibitors, we sought to identify the safest method regarding genetic stability of the cells. Pluripotent cells were cultured for 3 months on multi-well plates where they were subjected in parallel to the same conditions, but passaged using different procedures. One condition included treatment with ROCK inhibitor Y-27632. The passages were performed at the same time, but the ratios were different to keep similar density of the cultured cells. High-resolution DNA analysis using Illumina OmniExpress whole genome genotyping microarrays containing 750,000 probes for single nucleotide polymorphisms was performed to reveal small genetic aberration in the cultured cells. Analyses of 35 samples of five independent hES and human iPS cell lines have revealed the safest protocol for maintenance of genetic stability during extensive hES cell passaging. In addition, the efficacy of this matrix enables establishing large amount of cells at low passage numbers. The results may facilitate the use of pluripotent cells in clinical applications.

F-1118

THE STUDY FOR TUMORIGENESIS IN MOUSE EMBRYONIC STEM CELL BY SUBCUTANEOUS INJECTION.

Seok, Ji Hyeon, Jee, Joo Hyun, Choi, Hye Kyung, Yun, Ji hyun, Cho, Saet byul, Roh, Hang Sik, Lee, Jong Kwon, Kim, Tae Sung, Jo, Young Hee, Seong, Won Keun, Jeong, Ja Young

Toxicological Research Division, National Institute of Food and Drug Safety Evaluation, Chungcheongbuk-do, Republic of Korea

Clinical applications using pluripotent stem cells are expected to become a realistic option for treating a variety of incurable diseases in the near future. Especially for stem cell therapy, tumorigenesis is the main critical issue. However there is insufficient guideline for find out of tumorigenic in stem cell. Therefore, the purpose of our study evaluates tumorigenesis in undifferentiated embryonic stem cell that is origin of all kind of stem cells. We injected undifferentiated mouse embryonic stem cells in nude mice, by subcutaneous each per cell concentration. And we investigated of the tumor which is body weight, tumor size, tumor formation rate and the origin of histopathology as time goes on. As a results, a large number of injected cell group was generate to the more increase of tumor volume and the more fast appear in tumor formation. These results will contribute to the pre-clinical tumorigenesis data in subcutaneous injection by undifferentiated embryonic stem cells.

F-1120

GENETICALLY MODIFIED HUMAN EMBRYONIC STEM CELLS DERIVED ERYTHOCYTES ADAPTABLE TO RECIPIENTS OF MOST BLOOD TYPES

Wu, Zhao

SiDanSai Biotechnology Company, Shanghai, China

Erythocytes are the most common blood cell type playing a critical role of carrying oxygen and collecting carbon dioxide. Transfusion of blood cells save lives but there is always a colossal gap between the supply of blood product and the requirement from recipients. Meanwhile, the principles of compatibility for blood transfusions have been generating shortage of blood in certain types. Different blood types are determined by specific antigens secreted by and located in erythrocyte such as ABO, Rh, Kell, and etc, and blood transfusion between individuals from different blood types can cause severe outcomes such as hemolysis. In this work, we have created genetically modified human embryonic stem cells (hESCs) by nullifying those antigens on the genomic level by utilizing TALEN (transcription activator-like effector nuclease), a specific and robust genome editing tool. And then differentiate those engineered hESCs into erythrocytes. Those erythrocytes do not express essential antigens for specific blood types and enable the transfusion adaptability of the corresponding blood product to recipients from different blood types. This will be the genuine replacement of conventional blood product. In addition to meeting the requirements of war and war preparedness, the genuine universal blood would also be largely used in ambulance and disaster areas such as fire, earthquake, traffic accident, and work injury. Transfusion of this universal blood has major advantages compared with the conventional methods: 1. It does not require blood type matching, which can save time in extreme emergency such as earthquake, fire, and first aid. Saving time is equal to saving life in such situation. 2. The stem cell-derived erythocytes eliminates the risk of cross-infection by Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV). 3. The unlimited proliferation and differentiation characteristics of human embryonic stem cell can solve the blood product resource issue fundamentally.

F-1121

HUMAN ES CELL-DERIVED MSCS OUTPERFORM BONE MARROW MSCS IN TREATING AN EAE MODEL OF MULTIPLE SCLEROSIS

Wang, Xiaofang[1], Kimbrel, Erin[2], Lu, Shi-Jiang[2], Pachter, Joel S.[3], Crocker, Stephen[4], Lanza, Robert[2], **Xu, Ren-He**[5]

[1]ImStem Biotechnology, Inc., Farmington, CT, USA, [2]Advanced Cell Technology, Marlborough, MA, USA, [3]Cell Biology, University of Connecticut Hlth Ctr, Farmington, CT, USA, [4]Neuroscience, University of Connecticut Hlth Ctr, Farmington, CT, USA, [5] University of Connecticut Hlth Ctr, Farmington, CT, USA

Current therapies for multiple sclerosis (MS) are largely palliative, not curative. Mesenchymal stem cells (MSC) harbor regenerative and immunosuppressive functions that may impact MS severity and progression, yet variability and low potency of MSC from adult sources hinder their therapeutic potential. Here we present a unique method for generating large quantities of MSC from human embryonic stem (hES) cells. We describe the first study showing hES-MSC dramatically reduce clinical symptoms (disease score ~3 [paraplegia] to ≤1 [near normal]) and prevent demyelination in an EAE model of MS. The disease-modifying effect of hES-MSC was significantly greater than that of bone-marrow (BM)-MSC and, mechanistically, may relate to their lower expression of IL-6. Indeed, an anti-hIL-6 neutralizing antibody improved the disease-modifying effect of BM-MSC. A distinct ability to extravasate and migrate into inflamed CNS tissues also likely contributes to the robust therapeutic effects of hES-MSC in EAE.

GERMLINE CELLS

F-2001

OVER-EXPRESSION OF GERM CELL SPECIFIC GENES IN HUMAN EMBRYONIC STEM CELLS INDUCES DIFFERENCES IN GENE EXPRESSION, CELL CYCLE AND CELL MORPHOLOGY PARAMETERS

Panula, Sarita[1], Ramathal, Cyril[2], Sukhwani, Meena[3], Stukenborg, Jan-Bernd[4], Takahashi, Kazutoshi[5], Nakamura, Michiko[5], Söder, Olle[4], Orwig, Kyle E.[3], Yamanaka, Shinya[5], Reijo Pera, Renee A.[2], Hovatta, Outi[1]

[1]CLINTEC, Karolinska Institute, Stockholm, Sweden, [2]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, USA, [3]Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA, USA, [4]Department of Women's and Children's Health, Karolinska Institute, Stockholm, Sweden, [5]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Infertility affects 10-15 % of couples, most commonly due to a reduced number or lack of germ cells. In the adult, sperm or oocytes originate from a small group of cells that are specified early on in embryonic development. Defects at this early event can have lifelong consequences. However, very little is known about early germ cell specification and differentiation in humans. It has been shown, however, that human embryonic stem cells (hESCs) share a remarkably similar gene expression profile with early germ cells and that they can be differentiated to germ cells in vitro. Our aim was to utilize the germ cell properties of hESCs for studying the function of germ cell specific genes, DDX4, NANOS3 and DAZL through over-expression. We hypothesize that effects of over-expression can be observed in hESC culture conditions and within a germ cell niche through xenotransplantation into the seminiferous tubules of mouse testes. HESCs were transfected by lipofection with either DDX4, NANOS3

or DAZL expression vectors with PiggyBac transposase. The over-expression was confirmed at both gene and protein level and it remained stable through several passages. Gene expression changes in over-expressed cells were analyzed by Biomark 48.48 dynamic array. Induced expression of germ cell genes PLZF and CXCR4 were observed in DAZL over-expressed cells. Surprisingly, also NODAL expression was induced in DAZL over-expressed cells. NODAL expression has been connected with meiotic inhibition, where as DAZL is an intrinsic factor for meiotic initiation. Interestingly, the gene expression of DAZL was down regulated in NANOS3 over-expressed cells. All over-expressed cells had an increased expression of PAX6, an early ectodermal marker, although the morphology of these cells was similar to undifferentiated hESCs. However, morphological outliers were observed separate from colonies, in DAZL and NANOS3 over-expressed cells. These cells are positive for DAZL by immunostaining, and preliminary time-lapse imaging (Cell-IQ) indicates that they are highly motile in culture. All over-expressed cell lines exhibited typical hESC cell cycle structure, however, NANOS3 and DDX4 transfected cells had more cells in G2/M phase relative to control. Our preliminary results indicate a transcriptional interaction between DAZL and NANOS3 expression and a similar change in morphology with over-expression of these genes. We also found indications of cells attempting to compensate the meiosis driving properties of DAZL over-expression through upregulation of meiosis inhibitory genes. This study demonstrates that over-expression of a single germ cell specific gene in hESCs, is sufficient to alter gene expression, cell morphology, cell behavior, and cell cycle. The in vitro analysis of germ cell genes in hESCs provides a valuable tool to study the germ cell pathway, and together with the results from xenotransplantation to a germ cell environment can contribute to the understanding of human germ cell specification and differentiation.

F-2002
GERMLINE STEM CELL DIFFERENTIATION IS DEPENDENT ON THE FORMATION OF A DIFFUSION BARRIER BY THE SOMA IN THE DROSOPHILA TESTES

Fairchild, Michael John, Tanentzapf, Guy
Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada

When stem cells exit their niche they undergo differentiation, this process is dependent on stem cells shutting down signaling pathways that favour maintenance over differentiation. In the Drosophila testes, when germline stem cells exit their niche and differentiate they become encapsulated by somatic cells. Here we show that encapsulation establishes a diffusion barrier which renders them inaccessible to maintenance cues. Failure to establish this diffusion barrier leads to defective differentiation of the germ line. The Drosophila testes house a stem cell niche containing two stem cell populations, the germline stem cells, which develop into spermatozoa, and the somatic stem cells, which wrap around and encapsulate the germline to support spermatogenesis. While most studies have focused on the signaling pathways involved in soma-germline interactions few investigations have addressed how the somatic cells structurally set up the different microenvironments required by the germline. To investigate these processes we undertook a forward genetic screen of all cytoskeletal genes in the fly using tissue specific RNAi knockdown in the soma. Using this screen we identified Chickadee, the Drosophila homologue of the actin polymerizing protein Profilin, as being essential in the soma for diffusion barrier formation and germline differentiation. When Profilin was knocked down in the soma, the germline formed large tumors that heterogeneously expressed markers characteristic of germline stem cells, including some consistent with signaling pathways usually active only in the niche. To investigate whether or not structural changes in the germline environment led to the formation of these germline tumors we employed extracellular dyes to trace germline encapsulation. We found that in wildtype testes there was an 'open' compartment that was permeable to dye, consisting of both populations of stem cells as well as their early daughter cells up to 2-cell spermatogonial cysts, and a 'restricted'/encapsulated compartment that was significantly less permeable to dye, that typically began in the 4-cell spermatogonial stages. When we observed testes with profilin knocked down in the soma we saw that the tumorous germline was also a completely open environment similar to germline stem cells, and in addition that the open compartment of the tumors were often seen to be connected to the open compartment around the niche. We also saw that when later germline cysts from the restricted compartment lost encapsulation, they took up dye from the extracellular space, a sign of failing membrane integrity. This led to a model where encapsulation at the 2-4 cell spermatogonial stages shields the germline from the stem cell maintenance factors in the niche, allowing differentiation to occur.

F-2003
EPIGENETIC PROFILING OF POLAR BODY ACCURATELY REFLECTS ITS SIBLING OOCYTE

Sha, Hongying[1], Wang, Tian[2], Zhu, Jianhong[3]
[1]State Key Laboratory for Medical Neurobiology, Shanghai, China, [2]State Key Lab for Medical Neurobiology, Fudan University, Shanghai, China, [3]Fudan University Huanshan Hospital and State Key Laboratory for Medical Neurobiology, Shanghai, China

DNA methylation and histone modification is essential to gene expression during mammalian development. Elucidating the epigenetic profile during oogenesis is important to understand genomic imprinting and reprogramming. In human oocytes, improved methods are needed to reliably predict oocyte developmental outcomes. The first and second polar body (PB1 and PB2) are germ cells extruded from oocyte during maturation and fertilization. To test the hypothesis that the epigenetic profiling of polar body (PB) is representative of its sibling oocyte, we performed polar body biopsy to identify important epigenetic modifications for early development, i.e. acetylation of H3K9, H3K18, H4K5, and H4K12, and methylation of H3K4 and H3K9 and gene-specific establishment of maternal methylation imprints (Snrpn, Igf 2r, Peg1 and Peg3) in a mouse model. Our results indicate the epigenetic profiling of PB1 accurately reflects its sibling oocyte and PB2 accurately reflects the female pronucleus of its sibling zygote . This would allow for preimplantational genetic diagnosis of genomic imprinting-related rare genetic diseases such as Beckwith-Wiedemann syndrome, Silver-Russell syndrome, Angelman syndrome, etc. This could also pave the way for oocyte ranking and selection based on the epigenetic profiles of polar body to access oocyte quality in IVF applications.

F-2004
THE ROLE OF WNT SIGNALING IN THE REGULATION OF SPERMATOGONIAL STEM CELLS

Takase, Hinako M.[1], Nusse, Roeland[2]
[1]Department of Developmental Biology, Stanford University, Stanford, CA, USA, [2]Howard Hughes Medical Institute, Department of Developmental Biology, Stanford, CA, USA

Spermatogenesis is a well-organized and continuous process of male germ cell proliferation and differentiation. Spermatogonial stem cells (SSCs) are undifferentiated germ cells that act as precursor cells for spermatogenesis. It is largely unknown how SSCs are maintained and which signals are involved in SSC proliferation. Wnt signaling is implicated in stem cell self-renewal and maintenance in various tissues,

but the role of Wnt signaling in spermatogenesis has yet to be studied. To determine if spermatogonial stem cells respond to Wnt signals, we used a new genetic model, Axin2-CreERT2 mice, which can trace the fates of Wnt-responsive cells when crossed with reporter strains. Initial short-term tracing studies show that the Axin2 gene is expressed in the SSC population. Subsequent long-term lineage-tracing experiments demonstrate that Axin2-positive SSCs continuously produce germ line cells at all stages of differentiation, including mature spermatozoa. We observed that several Wnt ligands have unique patterns of expression in the adult testis. These results suggest a functional role for Wnt in SSC regulation and maintenance.

F-2005
DEFECTS IN RETROTRANSPOSONS SILENCING PATHWAY IN AZOOSPERMIA TESTES

Tseng, Huan-Chin[1], Cheng, Luca C.-W.[2], Lin, Sheng-Hsiang[1], Lin, Shau-Ping[2], Hwu, Yuh-Ming[1]
[1]Department of Medical Research, Mackay Memorial Hospital, New Taipei City, Taiwan, [2]Institute of Biotechnology, College of Bio-Resources and Agriculture, National Taiwan University, Taipei, Taiwan

Infertility is a worldwide reproductive health problem, which affects 20%-25% of couples. Half of the cases are due to male factors, and about 60-75% of male infertility cases are idiopathic, since the molecular mechanisms underlying the defects remains unknown. Recent studies indicated that loss of PIWI protein (piRNA interacting proteins) genes in mice might be linked to lost of methylation on retrotransposon sequences and thus de-repressed retrotransposon activities associated with impaired spermatogenesis. To what extent this PiRNA mediated retrotransposon regulation pathway contributes to human spermatogenesis defects are not well studied. To this end, we collected 31 testes biopsy samples from non-obstructive azospermia(NOA) patients with confirmed defects in spermatogenesis. Several testis biopsy samples from obstructive azospermia(OA) patients were used as control group of normal spermatogenesis. We compare the retrotransposon gene expression level by quantitative PCR.The expression level of retrotransposon gene (Alu, Line-1) in NOA testes increased significantly than OA testes. This result indicates that activation of retrotransposon gene may disrupt spermatogenesis process. It is noteworthy that NOA testes, which expressed higher level of retrotransposon gene, expressed relatively low level of PIWI gene. Taken together, these analyses may reveal essential function of retrotransposons silencing, and also the PIWI protein family genes in spermatogenesis, and therefore contribute to the understanding of another etiology for non-obstructive azospermia patients.

F-2006
POLAR BODY GENOME TRANSFER TO PREVENT THE TRANSMISSION OF INHERITED MITOCHONDRIAL DNA DISEASES

Wang, Tian[1], Sha, Hongying[2], Zhu, Jianhong[3]
[1]State Key Laboratory for Medical Neurobiology, Fudan University, Shanghai, China, [2]State Key Laboratory for Medical Neurobiology, Shanghai, China, [3]Fudan University Huanshan Hospital, Shanghai, China

Inherited mitochondrial DNA (mtDNA) diseases transmit maternally and cause severe phenotypes. Since no effective treatment or reliable genetic screening is available, nuclear genome transfer between patients' and healthy eggs to replace mutant mtDNAs (Mitochondria Replacement, MR) holds promises. The first and second polar body (PB1 and PB2) are germ cells divided from their sibling oocyte and zygote during oogenesis. Since polar body contains very few mitochondria and share same genomic scale as oocyte, here we perform polar body transfer to prevent the transmission of inherited mtDNA variants. We compare the value of different germline genome transfer, i.e., spindle-chromosome transfer (ST), first polar body transfer (PB1T), pronuclear transfer (PNT), and second polar body transfer (PB2T), to exchange mtDNA genotype in a mouse model. Reconstructed embryos support normal fertilization and produce live offspring. Strikingly, genetic analysis via high throughput pyrosequencing confirms polar body generated-offspring possesses minimal donor mtDNA carry-over compared with spindle-chromosome (low/medium carry-over) and pronuclear (medium/high carry-over) transfer. All PB1T offspring contains undetectable mtDNA heteroplasmy level (0%, n=6), which is significantly lower than ST offspring(5.53%±1.43%, mean±s.d., n=8) (p=0.0007). PB2T infants possessed 1.7%±2.8% (n=6) mtDNA carry-over on average, which is significantly lower than PNT infants (23.7%± 11.1%, n=7) (p=0.0012). Then, when MR-treated female offspring (F1 generation) reached puberty and mated with males, the second generation of MR-progeny (F2 generation) was accessed, and all F2 PB1T progeny still harbors undectable heteroplasmy level. Additionally, polar body transfer extends donor genome sources and significantly increases the efficiency when performed together with oocyte chromosome transfer. Our preclinical model demonstrates polar body transfer, especially PB1 transfer, which circumvent the possibility of mtDNA heteroplasmy carry-over in offspring, holds great potential in preventing the transmission of inherited mtDNA diseases.

F-2007
NICOTINAMIDE PROMOTES THE FOLLICLES DEVELOPMENT AND IMPROVES THE OVARY FUNCTION OF POF MODEL MOUSE

Wang, Shufang
301 Hospital, Beijing, China

The oocytes are store in the ovary from fetal stage. Most of follicles are arrest for a long time. There are lots of genes controlling the development of follicles. We found that vitamin PP also named nicotinamide can promote the granulosa cells proliferation and oocyte growth in vitro. And next we examined whether VPP can rescue the premature ovary failure. Female mice were injected intraperitoneally with 50 mg/kg cyclophosphamide (CTX). After 15 consecutive days of injection, 0.01 g VPP was injected to the POF model mouse. Ovarian function was evaluated by ovulation; the number of follicles were counted observed them through hematoxylin and eosin staining. We found that the VPP can rescue the damaged ovary. Apoptosis of the granulosa cells (GC) was analyzed by TUNEL staining. We found VPP can cause inhibit the apoptosis in the ovary and Ddx4 a marker of germ-line cells increased in the ovary. We detected the apoptosis in the new born mice ovary of control group and VPP treated group. We found after VPP treated the apoptosis was sharply decreased in the new born ovary. And through the RNA ARRAY analysis we found VPP can decrease the expression of pro-apoptosis genes for example: Atm; Casp8; Casp12; Prkar1b; Bnip3; Capn1; bcl3; Il1r1; lrrk2; Cell death proteins Tgfb1; Tnfsf12; Hip1; Tgfb2; Bnip3 expression are inhibited too. And also lots of proteins of negative regulation of cell proliferation including Timp2;Timp2;Tgf b2;Tgfb3;Cdh5;Gata3;Ptges;Ptgs2;Ifitm3 are inhibited by VPP. Nicotinamide is involved in regulated forkhead transcription factors, sirtuins, protein kinase B (Akt), Bad, caspases, and poly (ADP-ribose) polymerase, we found its new function in oocyte development and give a new way to help infertility women and premature ovary failure women.

TOTIPOTENT / EARLY EMBRYO CELLS

F-2009
GENE REGULATION NETWORKS IN THE DEVELOPING PIG EMBRYO AT THE PRE-IMPLANTATION STAGE

Cao, Suying[1], Han, Jianyong[1], Wu, Jun[2], Li, Ning[1]
[1]State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing, China, [2]Novogene Bioinformatics Institute, Beijing, China

Pig is an ideal model and has great potential for either providing pork, animal breeding or regenerative medicine. Recent studies reported porcine pre-implantation embryonic development (PED) are insufficient for expositing the specific molecular mechanism and gene regulatory network, which has hindered the derivation and application of porcine embryonic stem cells and porcine induced pluripotent stem cells. The present study assessed differential gene expression during embryonic development at different preimplantation stages in mouse and pig, which includes mouse *in vivo*-derived pre-implantation embryos as well as porcine *in vivo*-derived pre-implantation embryos and somatic cell nuclear transfer (SCNT) embryos. Through genome-wide transcriptome analysis, we found that there are several unique molecular gene regulation networks which regulate ZGA, lineage segregation and embryonic metabolism during pig PED. It doesn't show obvious differences between mouse and pig *in vivo*-embryos from 4-cell stage, however there are significant differences in 4-cell and blastocyst stage. The hierarchical order between pig *in vivo*-derived embryos and SCNT embryos also reveals identical results. We compare a series of gene regulation networks which are important to maternal deposition and zygotic genome activation (ZGA) between pig and mouse suggesting that the zygotic genome activation of pig normal embryos occurs at the 4-cell stage, while activation appears to be postponed one cell cycle in pig SCNT embryos. Especially, the genes associated with demethylation changed their expression levels after micromanipulation in SCNT embryos. Then we split the porcine blastocyst into two parts: putative inner cell mass (ICM) and the trophectoderm (TE). Through analysis of specific gene expressed in putative ICM and TE, we find that pig and mouse pre-implantation embryos share common regulatory networks during the first lineage segregation, although some interestingly different genes within the same pathways are activated in mouse and pig embryos. However regulation of second lineage segregation events during PED shows different between mice and pigs. In addition, we also find that there is a significant difference in the heat map patterns for marker genes such as Oct4, Cdx2, Gata6, Sox2 and Nanog etc., which suggests that regulation of ICM development among pig, mouse and the human are more conserved than those of TE development. Finally we observed an enrichment in the pathways related to fatty acid metabolism during ZGA and lineage segregation in pig embryos. Thus, fatty acids whose biosynthesis and elongation in pre-implantation embryos may be play an important role in early embryonic development. In summary, our data could be perspective for optimizing porcine pluripotent stem cell cultural system and grasp of porcine early embryonic development.

F-2010
META-ANALYSIS OF THE TRANSCRIPTIONAL ARCHITECTURE BETWEEN NAÏVE AND PRIMED EMBRYONIC STEM CELLS WITH EARLY EMBRYOS

Huang, Kevin, Maruyama, Toru, Fan, Guoping
UCLA, Los Angeles, CA, USA

Embryonic stem cells (ESCs) cultured in the presence of combinatorial signaling pathway modulators are postulated to represent a ground (or "naïve") state of pluripotency. Recently, the naïve ESCs has been derived in both mouse and human models. However, whether the expression profile of naïve ESCs also resembles transcriptomes of pre-implantation embryos has not been carefully studied. Using stringent systems biology approaches, we identified reproducible gene co-expression networks characterizing the naïve and primed pluripotent state in both mouse and human models. Comparisons with pre-implantation embryos showed that mammalian naïve networks share significant similarities with the inner cell mass. These include a core set of conserved hub genes such as IDH1, TFB2M, and ERSBBA involved in metabolic processes. Furthermore, cross-species network comparisons revealed the naïve state is partially conserved between human and mouse ESCs, but the transcriptional organization of primed hESCs is clearly different from primed mESCs. Our results demonstrate that the naïve embryonic stem cell state exhibits transcriptional and metabolic features resembling the inner cell mass. This transcriptional state appears to be potentially orchestrated by a core set of hub genes preserved between human and mouse.

F-2011
MOUSE EMBRYONIC STEM CELLS DERIVED BY SCNT INTO INTERPHASE 2 CELL EMBRYOS

Kang, Eunju[1], Wu, Guangming[2], Ma, Hong[1], Li, Ying[1], Hedges-Tippner, Rebecca[1], Van Dyken, Crystal[1], Wolf, Don P.[1], Scholer, Hans R.[2], Mitalipov, Shoukhrat M.[1]
[1]Oregon Health and Science University, Beaverton, OR, USA, [2]Max Planck Institute for Molecular Medicine, Munster, Germany

Until now, successful reprogramming by somatic cell nuclear transfer (SCNT) has been demonstrated only when the recipient cytoplasm from metaphase-arrested oocytes or zygotes is utilized. This supports the conclusion that critical reprogramming factors are present in metaphase but not interphase cytoplasm. We revisited this issue and show that several maternal and embryonic factors, thought to be critical for reprogramming, are evenly distributed in both metaphase and interphase cytoplasm of mouse oocytes and early embryos. Next, we conducted SCNT into enucleated, interphase 2-cell embryos, carefully matching the cell cycle stages of the somatic nucleus and embryonic cytoplasm. Such SCNT embryos reconstructed with fetal fibroblasts or adult cumulus cells developed to blastocysts that supported the derivation of embryonic stem cell lines (ntESCs) at high rates. When injected into host diploid or tetraploid embryos, novel ntESCs contributed to germline or all-ESC chimeras. Our results demonstrate that interphase cytoplasm of early mouse embryos is also capable of reprogramming somatic cells to pluripotency. These findings provide insights into the basic mechanisms underlying SCNT-based reprogramming and are relevant to the production of human patient-specific ESCs.

F-2012

HIRA-MEDIATED H3.3 INCORPORATION IS REQUIRED FOR DNA REPLICATION AND RIBOSOMAL RNA TRANSCRIPTION IN THE MOUSE ZYGOTE

Lin, Chih-Jen[1], Koh, Fong Ming[2], Wong, Priscilla[1], Conti, Marco[1], Ramalho-Santos, Miguel[2]

[1]Center for Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA, [2]University of California San Francisco, San Francisco, CA, USA

Extensive chromatin reprogramming occurs at fertilization and is thought to be under the control of maternal factors, but the underlying mechanisms remain poorly understood. We report here that maternal Hira, a chaperone for the histone variant H3.3, is strictly required for mouse development past the zygote stage. Oocyte-specific deletion of Hira leads to zygotes in which the protamines are evicted but nucleosomes, including H3.3, fail to assemble in the sperm genome, which remains condensed and does not form the male pronucleus. Although the female pronucleus forms, parthenogenetic mutants also arrest at the zygote stage. Both paternal and maternal genomes show highly reduced levels of DNA replication and nascent transcription in the mutants. It has long been thought that the low levels of transcription that occur in the zygote are not required for the first cleavage. Surprisingly, we found that there is significant transcription of ribosomal RNA mediated by RNA Polymerase I in both pronuclei in the zygote, and that this transcription is entirely Hira-dependent. Inhibition of RNA Polymerase I in wild-type embryos abolishes ribosomal RNA transcription and leads to an arrest at the zygote stage. These results reveal that Hira-mediated H3.3 incorporation is essential for reprogramming the chromatin of both gametic genomes in order to allow DNA replication and transcription. Our findings further reveal a novel role for RNA Polymerase I-mediated transcription in developmental competence of the mammalian zygote, and warrant a re-definition of zygotic gene activation in mammals.

EMBRYONIC STEM CELL DIFFERENTIATION

F-2015

ESTABLISHING SCALABLE SUSPENSION PRODUCTION OF NEURAL STEM CELL FROM HUMAN EMBRYONIC STEM CELL

Huang, Patricia[1], Lin, Chih-Min[1], Couture, Sylvana M.[2], Gutierrez, John[2], Hsu, David[2], Couture, Larry[3]

[1]Center for BioMedicine and Genetics, Beckman Research Institute, City of Hope, Duarte, CA, USA, [2]Beckman Research Institute, Center for BioMedicine and Genetics, City of Hope, Duarte, CA, USA, [3]CATD, Beckman Research Institute, City of Hope, Duarte, CA, USA

The ability to scale up human embryonic stem cell (hESC) culture, maintain its pluripotency, and induce differentiation into multiple lineages, particularly neural lineages, offer novel cell sources for the usage in the field of biomedicine. To this end, a robust and scalable suspension culture system was established at Center for Biomedicine and Genetics (CBG) to grow hESC in suspension as aggregates. Taking advantage of the hESC cells are maintained as aggregates similar to the EB, we are able to establish a process to generate NSC from the suspension culture directly without the EB formation step required by the traditional NSC differentiation process. In this study, we developed a scalable and time/cost effective process to produce neural stem cell (NSC) from hESC in suspension culture. First, a hESC line, H14, was adapted and expanded in suspension culture using the procedure established at CBG. Three hESC resource banks with normal karyotype and pluripotency markers expression were generated for this study. We were able to significantly improve the efficiency of NSC differentiation process by synchronizing hESC at G1 phase of the replication cycle with DMSO for 48 hours before differentiation. When the synchronized hESCs were differentiated into ectodermal lineage in suspension culture using BMP antagonist, Noggin and FGF2, we were able to generate culture enriched with NSCs. Rosette formation within the aggregates become visible two days after the induction step suggesting the differentiation process is effective. This is further confirmed by the FACS analysis which showed more than 80% of cells were Pax6 positive and the expansion fold increased more than 5 times within 7days of induction. The kinetics of gene expression, including Pax6 and Sox1 for NSC markers and Oct4 and Rex1 for pluripotency markers, was analyzed during the differentiation process by RT-qPCR. As expected, Pax6 and Sox1 gene expression increased in a time-dependent manner whereas Oct4 and Rex1 expression decreased over time during the differentiation process. The results demonstrated we have established a scalable and cost effective NSC differentiation process in the suspension culture system for NSC.

F-2016

AF9 PROMOTES HESC NEURAL DIFFERENTIATION THROUGH RECRUITING TET2 TO NEURODEVELOPMENTAL GENE LOCI FOR METHYLCYTOSINE HYDROXYLATION

Qiao, Yunbo[1], Jing, Naihe[2]

[1]Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China, [2]Shanghai Institute of Biochemistry and Cell Biology, Shanghai, China

AF9 mutations are implicated in human neurodevelopmental diseases and murine AF9 mediates H3K79 dimethylation of Tbr1 during cortical neuron generation. However, AF9 function remains unknown in human neurodevelopment. Here human ESC (hESC) neural differentiation was used for a human neurodevelopment model. AF9 overexpression promoted neural differentiation and neurodevelopmental gene expression. The 5-methylcytosine (5mC) dioxygenase TET2, identified in AF9-conjugated protein complex, physically interacted with AF9 and both factors were colocalized with 5-hydroxymethylcytosine (5hmC)-positive neural cells and were required for appropriate hESC neural differentiation. AF9 and TET2 co-occupied in multiple key neurodevelopmental gene loci and TET2 occupancy depends on AF9 recruitment. Consequently, AF9 and TET2 cooperate to direct 5mC-5hmC conversion and sequential neural-target gene activation. These findings define AF9 and TET2 as a regulatory complex for modulating human neural development and reveal a novel mechanism by which the AF9 DNA binding specificity and TET2 hydroxylation activity cooperate to control neurodevelopmental gene activation.

F-2017

HUMAN EMBRYONIC STEM CELL-DERIVED INSULIN+ NKX6.1+ PDX-1+ CELLS SHOW ACCELERATED IN VIVO MATURATION AS COMPARED TO PANCREATIC PROGENITORS

Rezania, Alireza[1], Arora, Payal[1], Asadi, Ali[2], Kieffer, Timothy[2]

[1]BetaLogics, Janssen, Raritan, NJ, USA, [2]Cellular and Physiological Sciences, University of British Columbia, Vancouver, BC, Canada

Recent advancements in the generation of functional pancreatic beta cells from embryonic stem (ES) cells has resulted in the generation of fetal-like pancreatic precursor cells capable of reversing hyperglycemia in diabetic mice following an extensive in vivo maturation period.

Further in vitro maturation of human ES-derived pancreatic precursor cells has generally resulted in generation of non-glucose responsive polyhormonal cells lacking key beta cell specific transcription factors, such as NKX6.1, PDX-1, and MAFA. We previously showed that polyhormonal cells are shunted towards an alpha cell lineage rather than mature beta cells. We hypothesized that in vitro generation of single hormonal, insulin+ NKX6.1+ cells will result in accelerated in vivo maturation to functional beta cells as compared to pancreatic progenitors (PDX-1+ NKX6.1+). Building on our previous differentiation recipes, we developed a six stage protocol resulting in the in vitro generation of cells that are insulin+ NKX6.1+ PDX-1+ but that remain glucose unresponsive. Transplantation of this population of cells into diabetic and non-diabetic immunodeficient mice and rats demonstrated significantly enhanced maturation along with significantly higher levels of circulating human C-peptide as compared to ES-derived pancreatic precursor cells. Moreover, the cells were able to reverse hyperglycemia in a much shorter time frame as compared to pancreatic precursor cells. These results demonstrate that in vitro generated insulin+ NKX6.1+ cells show significantly enhanced in vivo maturation and efficacy as compared to pancreatic precursor cells.

F-2018
ARID3A MODULATES THE FIRST CELL FATE DECISION BY DIRECT REGULATION OF BOTH EMBRYONIC AND EXTRAEMBRYONIC GENE EXPRESSION

Rhee, Catherine, Beck, Sam, Lee, Bum-Kyu, Anjum, Azeen, Tucker, Haley, Kim, Jonghwan
Molecular Biosciences, The University of Texas at Austin, Austin, TX, USA

The decision of the first cell fate commitment in mammalian embryos to either the placenta-directed trophectoderm (TE) or the embryo-destined inner cell mass (ICM) is controlled by antagonistic actions of two transcription factors, Cdx2 and Oct4. Cdx2 represses pluripotency-associated genes, such as Oct4 and Nanog in TE, whereas Oct4 represses TE-specific genes including Cdx2 in ICM. Intensive studies in embryonic stem (ES) cells discovered numerous key regulators involved in the establishment and/or maintenance of the ICM lineage. However, besides Cdx2, only a few factors have been reported as regulators of the TE lineage, which limits our understanding of the regulatory mechanisms in the first cell fate decision. Arid3a, a transcription factor well-known for its requirement in B-lymphocyte development, has recently been identified as a member of the ES cell pluripotency network. We found that Arid3a is moderately expressed in ES cells, while its expression is significantly increased upon differentiation. In particular, Arid3a is highly expressed in the extraembryonic tissues which eventually give rise to the placenta, suggesting its putative role in placenta development. Consistent with our observation, prior loss-of-function studies of Arid3a in mice showed the lethal phenotype of null embryos before E11.5. All these results strongly suggest that Arid3a may play important roles in ES cell differentiation and early development of embryos. However, until today, the detailed functions and mechanisms of Arid3a in ES cell context remain largely unknown. Here we report that Arid3a is a central, direct regulator of both TE-specific and pluripotency-associated gene expression during ES cell differentiation. Knockdown of Arid3a in ES cells delays differentiation while it is dispensable for self-renewal. Ectopic expression of Arid3a allows ES cells to exit self-renewal and initiate differentiation, specifically toward TE lineage. Moreover, overexpression of Arid3a in ES cells maintained in TE culture media is sufficient to generate functional trophoblast stem-like cells. Our genome-wide expression profiling and target mapping of Arid3a in ES cells revealed that Arid3a directly activates TE-specific genes, such as Cdx2 and Gata3, while directly repressing core pluripotency-associated genes, including Oct4 and Nanog. We further show that the repression of pluripotency-associated genes by Arid3a is mediated by histone deacetylases (HDACs). Our results demonstrate that Arid3a is a critical novel regulator in TE lineage specification, and provide insights into the molecular nature of the segregation between the TE and ICM lineages during early embryo development.

F-2019
HIGHLY EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS MAINTAINED IN TESR™-E8™ TO DEFINITIVE ENDODERM

Riedel, Michael J., Luu, Yvonne, Lam, Stephanie, Eaves, Allen C., Thomas, Terry E., Louis, Sharon A.
STEMCELL Technologies Inc., Vancouver, BC, Canada

Human pluripotent stem cells (hPSCs) can be maintained in culture indefinitely or directed to differentiate using appropriate differentiation media and protocols. Translating laboratory protocols to clinically useful cell therapies will require more defined culture systems for maintaining and differentiating hPSCs. TeSR™-E8™, a xeno-free, low-protein hPSC maintenance medium provides a simplified medium for culturing undifferentiated hPSCs. However, our studies indicate that definitive endoderm (DE) differentiation is less efficient in hPSCs cultured in TeSR™-E8™ compared to the same hPSCs maintained in mTeSR™1 when using STEMdiff™ Definitive Endoderm. Here, we describe STEMdiff™ Definitive Endoderm (TeSR™-E8™ Optimized), a DE differentiation kit optimized for hPSCs maintained in TeSR™-E8™. Human embryonic stem cell (hESC; H1, H9) and induced pluripotent stem cell (hiPSC; WLS-4D1, STiPS-M001) lines were maintained in either mTeSR™1 or TeSR™-E8™ on Matrigel® for at least 5 passages. In control conditions, hPSCs that were ready for passaging (70% confluent cultures with colonies containing dense centers; 4-6 days after seeding) were differentiated to DE using STEMdiff™ Definitive Endoderm. Alternatively, hPSCs cultured in TeSR™-E8™ were incubated in TeSR™-E8™ with or without our novel pre-differentiation supplement for at least 24 hours prior to passaging and during the overnight culture of single cells prior to initiation of DE differentiation. The culture medium was replaced daily using this supplemented TeSR™-E8™ until passaging, at which time DE differentiation was performed using the STEMdiff™ Definitive Endoderm Kit with standard protocol. In all experiments, DE differentiation efficiency was assessed by flow cytometric analysis of CXCR4 and SOX17 co-expression. As previously observed, DE differentiation of hPSCs cultured in mTeSR™1 using STEMdiff™ Definitive Endoderm was consistent and highly efficient [92 ± 6% CXCR4+/SOX17+ (range: 78.4% - 98.5%); mean ± SD, n = 20 (3-8 per cell line)] across all cell lines tested. However, these same cell lines maintained in TeSR™-E8™ differentiated more poorly [52 ± 26% (range: 15.0% - 92.1%), n = 20 (3-8 per cell line)]. Using STEMdiff™ Definitive Endoderm (TeSR™-E8™ Optimized), hPSCs maintained in TeSR™-E8™ differentiated to DE with high efficiency and low variability [92 ± 7% (range: 76.5% - 98.9%), n = 20 (3-8 per cell line)], showing no significant difference compared to hPSCs maintained in mTeSR™1 and differentiated using the standard STEMdiff™ Definitive Endoderm protocol (p=0.99 vs. mTeSR™1 by paired t-test). The improvement in efficiency was time-dependent, with cells cultured for at least 40 hours in the pre-differentiation medium (24 hours as aggregates and 16-24 hours as single cells) exhibiting highly efficient differentiation. Extended time (between 40 hours and 7 days) in pre-differentiation medium did not adversely affect DE differentiation efficiency. We further tested this optimized protocol under entirely xeno-free conditions by using hPSCs (H1)

cultured in TeSR™-E8™ cells on Vitronectin-XF™ (developed and manufactured by Primorigen Biosciences) and observed similarly efficient DE differentiation compared to hPSCs cultured on Matrigel®. In summary, the lower efficiency DE differentiation of hPSCs maintained in TeSR™-E8™ or other low protein hPSC maintenance media can be eliminated with the use of STEMdiff™ Definitive Endoderm (TeSR™-E8™ Optimized).

F-2020

HUMAN CRANIAL PLACODAL LINEAGES FROM EMBRYONIC STEM CELLS CULTURED IN 3D AGGREGATES

Ronaghi, Mohammad, Nasr, Marjan, Heller, Stefan
Otolaryngology - Head and Neck Surgery, Stanford University, Stanford, CA, USA

Cranial placodes generate the anterior pituitary, lens, olfactory epithelium, cranial ganglia, and almost all cell types of the inner ear. Research from various animal models has provided good insight into how competence to adopt placodal lineage identity is initially specified from non-neural head ectoderm. The pre-placodal region becomes subsequently spatially restricted to form the cranial placodes. Placodes adopt different identities along the anterior-posterior axis and this patterning requires the integration of multiple local signaling pathways. Although not all signaling pathways that instruct placodal patterning during development have been completely elucidated, we hypothesize that already existing knowledge can be applied to manipulate the formation of human placodal lineages in three dimensionally grown aggregates of human embryonic stem cells. Our strategy initially aims to initially generate non-neural ectoderm that is competent to respond to different anteriorizing and posteriorizing signaling pathways. Here, we present the first findings where we identified a strategy to generate mixed aggregates that harbor multiple placodal lineages. This strategy was devised by systematic exploration of inhibitory and inductive compounds and monitoring multiple markers for early cell fates including non-neural ectoderm with qRT-PCR. Specifically, we generate aggregates that display placodal cells that express markers indicative of olfactory, lens and trigeminal, as well as otic lineages. We further show that manipulation of anteriorizing and posteriorizing signaling pathways alters the composition of 3D aggregates that display placodal lineages. Using qRT-PCR and immunostaining and imaging, we quantitatively characterized the initial formation of the olfactory, lens and trigeminal, as well as otic lineages. Protracted cultures of the differently enriched aggregates led to differentiation of mature cell types of the different placodal lineages. In conclusion, we report that different human cranial placodal lineages can be generated with an optimized three dimensional aggregate culture system.

F-2021

WASKO HUMAN EMBRYONIC STEM CELLS AS MODEL FOR WISKOTT-ALDRICH SYNDROME: A NEW TOOL TO STUDY THE EFFICACY OF GENE THERAPY STRATEGIES

Sánchez, Almudena[1], Toscano, Miguel G.[2], Muñoz, Pilar[1], Cobo, Marién[1], Gregory, Philip[3], Holmes, Michael C.[3], Martín, Francisco[1]
[1]Departament of Genomic Variability, GENYO. Centre for Genomics and Oncological Research: Pfizer-University of Granada-Andalusian Regional Goverment, Granada, Spain, [2]Amarna Biotech, Cabimer, Sevilla, Spain, [3]Sangamo BioSciences Inc, Richmond, CA, USA

Wiskott-Aldrich syndrome (WAS) is an X-linked primary immunodeficiency characterized by eczema, immunodeficiency and thrombocytopenia and caused by mutations in the *WAS gene*. The immunological defects in WAS are well characterized, however the mechanisms that lead to the thrombocytopenia and leukemia remain contradictory. Nowadays, bone marrow transplant is the only treatment, however not always is available and can have severe secondary effects. It is therefore necessary to develop alternative therapies. Gene therapy is becoming a very promising therapeutic alternative for several primary immunodeficiencies, including WAS. In spite of the initial success, several secondary effects (leukemia, silencing of the transgene) appeared in the human clinical trials that were not observed in the animal models. Using Zinc finger nucleases targeting the first intron of the WAS locus, we have developed two human embryonic stem cells (hESC) lines deficient for WASP protein (AND-1 WASKO c1.1 and AND-1 WASKO c1.2). We showed that megakaryocytes (MK) and platelets derived from AND-1 WASKO cells mimic several aspect of the defects found in MK and platelets from WAS patients (lower responses to ADP and thrombin, lower levels of CD43 antigen). Interestingly, we have also observed differences during the hematopoietic- and megakaryocytic- differentiation protocols; WASKO cells consistently generated higher numbers of hematopoietic progenitors, MK and platelet when compared to wild type AND-1 cells. We next generated AND-1 WASKO c1.1 lines transduced with lentiviral vectors expressing WASP. Most of the phenotypic alterations observed in AND-1 WASKO c1.1 cells were alleviated upon expression of WAS following lentiviral transduccion. The reduced response to ADP and thrombin of MK and platelets derived from AND-1 WASKO was restored upon LV expression of WASP. Additionally, the differentiation kinetics of AND-1 WASKO transduced with the lentiviral vectors were closer to AND-1 than to AND-1 WASKO c1.1. We propose AND-1 WASKO cells as model to study the role of WASP at early stages of hematopoietic differentiation as well as a human model to study different therapeutic strategies for WAS.

F-2022

ENDOCRINE SPECIFICATION AND MATURATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED PANCREATIC PROGENITORS

Sackett, Sara Dutton, Li, Grace, Tremmel, Dan, O'Brien, Cori, Nair, Gopika, Xu, Xiaofang, Odorico, Jon S.
Surgery, University of Wisconsin, Madison, WI, USA

Stem cell based therapies, such as the differentiation of ß-cells from human embryonic stem cells (hESCs) hold great potential for the treatment of Type I diabetes. However, a majority of published protocols produce an immature population of cells that are marginally responsive to glucose in addition to requiring a long *in vivo* differentiation period; potentially making it difficult to translate this work to the clinic. The objective_of our study is to improve upon current differentiation protocols through manipulation of media components as well as cell culture platforms and substrates to advance the *in vitro* differentiation and the *in vivo* maturation. We hypothesize_that the differentiation block in deriving functional endocrine cells from human pluripotent stem cells *in vitro* can be overcome through optimization of the protocol and by incorporating tissue-specific ECM into the *in vitro* system. In addition, by transplanting cells at earlier stages in the differentiation process and altering the *in vivo* differentiation environments, we aim to shorten the *in vivo* maturation period required to cure diabetic animals. Using a 4 stage 18 day protocol beginning with bFGF, Activin A and BMP4, we can show H1 cells express key stage specific gene signatures and pancreatic transcription factors (*Sox17, Foxa2, Pdx1, Ngn3, Nkx6.1, Gcg, Ins*) as cells differentiate through the protocol as characterized by immunocytochemical (ICC), QPCR and *in vivo* analyses. We postulate that the incomplete differentiation and maturation observed *in vitro* may be due to incomplete or improper extracellular signals. Therefore, we have incorporated extracellular

matrix (ECM) from decellularized human pancreata as a scaffold on Transwell (TW) inserts into the culture protocol to enhance the differentiation of hES into functional ß-cells. ICC analysis of cells co-cultured with matrix on TW shows expression of stage appropriate proteins and incorporation of the differentiating cells into the matrix (Laminin). We are monitoring several cohorts of mice to determine the effect of pancreas ECM on the *in vivo* maturation period. SCID/Beige mice were transplanted under the kidney subcapsule with 2-5x10^6 cells from several protocols and at multiple stages in the endocrine lineage specification with and without matrix to determine if these pancreatic progenitors are capable of differentiating into a homogenous population of mature, functional ß-cells *in vivo*. We find that while many of the cells are polyhormonal and express both insulin and glucagon at the end of the *in vitro* differentiation period, many of these cells become monohormonal during *in vivo* differentiation as shown by ICC staining, whereas with other protocols they remain polyhormonal; and some protocols produce few hormone positive cells while generating teratomas with a relatively high frequency. ELISA analyses show detectable levels of c-peptide (>120 pM by 20 weeks) in transplanted mice, while others do not. Similarly in some cases glucose tolerance tests show improved glucose clearance beginning as early as 12 weeks and increased c-peptide following glucose stimulation. Although the precise mechanism of the *in vivo* maturation remains undefined, it is clear that some *in vitro* derived cell populations have significantly greater potential for differentiating into insulin-secreting ß-cells than others. The incorporation of ECM into our differentiation protocols holds great promise for a crucial advance for the ß-cell replacement field.

F-2023
COMPARATIVE ANALYSIS OF HUMAN AND RHESUS MACAQUE EMBRYONIC STEM CELL CORTICAL NEURON DIFFERENTIATION IDENTIFIES NOTCH2NL AS A CANDIDATE GENE FOR BRAIN DEVELOPMENT DEFECTS ASSOCIATED WITH COPY NUMBER ALTERATIONS AT CHROMOSOME 1Q21.1

Salama, Sofie R.[1], Jacobs, Frank[2], Ewing, Adam D.[2], Novak, Adam M.[2], Roberts, Jacqueline M.[3], Rosenkrantz, Jimi L.[1], Kuhn, Robert M.[2], Diekhans, Mark[2], Raney, Brian J.[2], van Lodewijk, Gerrald[4], Field, Andrew[2], Haeussler, Maximilian[2], Clawson, Hiram[2], Belfiore, Marco[5], Addo, Marie-Claude[6], Uhrig, Sabine[7], Zwolinski, Simon[8], Katzman, Sol[2], Chen, Bin[3], Haussler, David H.[1]

[1]Center for Biomolecular Science and Engineering, HHMI - UC Santa Cruz, Santa Cruz, CA, USA, [2]Center for Biomolecular Science and Engineering, University of California Santa Cruz, Santa Cruz, CA, USA, [3]Molecular, Cell and Developmental Biology, University of California Santa Cruz, Santa Cruz, CA, USA, [4]Rudolf Magnus Institute, University of Utrecht, Utrecht, Netherlands, [5]Service de génétique médicale, Lausanne, Switzerland, [6]Service de génétique médicale, lausanne, Switzerland, [7]Institut für Humangenetik, Graz, Austria, [8]Department of Cytogenetics, Newcastle upon Tyne, United Kingdom

In addition to providing a resource for regenerative medicine, directed differentiation from embryonic stem cells provides a powerful tool for molecular analysis of early developmental processes in humans and primates where tissue availability is limited and experimental manipulation is not practical. Comparative analysis of differentiation between humans and primates can reveal the molecular basis for phenotypic differences between us. We have focused on cortical neuron differentiation in hopes of identifying the molecular mechanisms that underlie the prolonged and expanded cortical neurogenesis leading to our large and complex cerebral cortex. We have developed a common protocol for cortical neuron generation from human, chimpanzee and rhesus pluripotent stem cells that recapitulates early events in cortical development and enables us to do a comparative molecular analysis of this process. RNA-Seq based transcriptome analysis of samples collected at matched time points throughout human and macaque cortical neurosphere generation revealed that relative to macaque, human embryonic stem cell derived neurospheres displayed elevated and prolonged expression of Notch signaling pathway genes as well as genes involved in cell cycle progression and control of neurogenesis. Most dramatically, human cortical neurospheres have robust expression of NOTCH2NL, a truncated, secreted version of NOTCH2 that arose in the human lineage after divergence from old world monkeys like rhesus macaque. NOTCH2NL resides on chromosome 1q21.1 a region noted for copy number variations and implicated in neural-developmental diseases. We performed a detailed analysis of the evolutionary history of NOTCH2NL using a recently revised assembly of chromosome 1 and short read sequencing data derived from a variety of primates. We find that NOTCH2NL first arose in the common ancestor of human, chimpanzee and gorilla, but that subsequent segmental duplications and gene conversion events after our divergence from chimpanzee created four NOTCH2NL genes in total, two of which are expressed during cortical development and also exhibit copy number variation associated with aberrant brain size in 1q21.1 locus-linked micro- and macrocephaly patients. This establishes NOTCH2NL-paralogs as new candidates for 1q21.1-associated neurological disorders and suggests that the creation and expansion of NOTCH2NL may have contributed to the evolution of the larger hominin neocortex.

F-2024
ANALYSIS OF MOUSE ES CELL-DERIVED VENTRAL MIDBRAIN CELLS TO IDENTIFY A SPECIFIC CELL SURFACE MARKER

Samata, Bumpei, Doi, Daisuke, Takahashi, Jun

Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Objective: Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells represent a promising source of midbrain dopaminergic (DA) neurons to treat Parkinson's disease (PD). Although ES and iPS cells can be induced in vitro, there is a problem that the cells contain a mixture of different cell subtypes, and some of them may lead to tumor formation after transplantation. For a safe and efficient transplantation, midbrain DA neurons need to be purified as donor cells. Therefore, this study is aimed to identify a specific cell surface marker to sort midbrain DA neurons. Methods: We induced neural progenitor cells from mouse ES cells (EB5) by modified serum-free floating culture of embryoid body-like aggregates (SFEBq) method. To identify ventral midbrain cells, we used Lmx1a, a midbrain marker, and Corin, a floor plate marker. The sorted Lmx1a+Corin+ cells were evaluated by immunostaining, and analyzed by employing a microarray experiments. Results: Midbrain DA neurons, expressing Foxa2, Lmx1a, Nurr1 and Th, were induced by the modified SFEBq method. On day 9, 30.5 ± 5.3% of total cells were positive for Lmx1a and Corin. The sorted Lmx1a+Corin+ cells generated high percentage of TH+ (tyrosine hydroxylase, a DA neuron marker) cells. Furthermore, the Lmx1a+Corin+ cells did not contain undifferentiated or non-neural cells. When the cells were grafted in the brains of 6-OHDA lesioned rats, the grafts contained a large number of TH+ cells without overgrowth. By comparing gene expressions between mouse ES cell-derived Lmx1a+Corin+ and Lmx1a+Corin- cells, we have selected candidate molecules preferentially expressed in Lmx1a+Corin+ cells. Conclusion: Lmx1a and Corin double-selection strategy may lead to purification of DA progenitor cells, which contain no unwanted

cells such as undifferentiated cells and non-neural cells. To identify a specific marker of DA progenitor cells, we need to undertake further analyses of candidate genes.

F-2025
HUMAN EMBRYONIC STEM CELL-LINE CARRYING RAT TYROSINE HYDROXYLASE-EGFP TRANSGENE TO VISUALIZE DOPAMINERGIC NEURONAL DIFFERENTIATION

Sarma, Sailendra Nath, Kohda, Masanobu, Ohsako, Seiichiroh
The University of Tokyo, Faculty of Medicine, CDBIM, Tokyo, Japan

The present toxicological assessment systems are mainly based on animals or in vitro-cultured animal-derived cells and do not or not sufficiently mirror the situation in humans. Human embryonic stem cells (hESC) have unique attributes that can be applied in drug discovery, from initial target ideas to clinical trials. This study tried i) to generate human pluripotent stem cell lines carrying tyrosine hydroxylase (TH) gene-driven EGFP as a reporter, by which live-cell imaging can be employed to monitor the specific cell type numbers and morphology, as well as ii) to establish an effective alternative developmental toxicity test system. We have successfully selected stable hESC lines by transfection using 10-kb rat TH promoter connected with EGFP into KhES1. The expression of EGFP only appeared during the neuronal cell differentiation culture process of hESC. The intensity of the EGFP increased day by day during the dendrite formation. Using this hESC cell, we tried to investigate the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in neuronal cell developmental process in vitro, because it has been reported that, fetal brain functions are affected by the TCDD exposure in rodent studies. The expression of EGFP might be able to monitor the real time dopaminergic neuronal differentiation during neuronal developmental process. These cells will provide a qualitative and quantitative advantage to investigators in human neuro-developmental research. The research was supported by Grant-in-aid from the Ministry of Health, Labour and Welfare of Japan.

F-2026
THE EFFECTS OF CARBAMAZEPINE AND VALPROIC ACID ON GENE EXPRESSION IN A HUMAN EMBRYONIC STEM CELL BASED ASSAY FOR NEURO-DEVELOPMENTAL TOXICITY

Schulpen, Sjors, de la Fonteyne, Liset J.J., de Klerk, Arja, Piersma, Aldert H.
Center for Health Protection, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands

There is a high need for in vitro methods to reduce animal use in developmental toxicity testing. The application of embryonic stem cells provides a valuable starting point, since they can differentiate into different cell lineages in culture in such a way that in vivo differentiation pathways are mimicked. The use of mouse embryonic stem cells (mESC) within the embryonic stem cell test (EST) has been shown to function as a useful method to determine the developmental toxicity of compounds. The read-out is based on the effect of a compound in a dose depended manner on differentiation into contracting myocardial cell foci. However, the extrapolation of data to in vivo and human hazard is challenging. The use of human embryonic stem cells (hESC) avoids the extrapolation between species. We developed a standardized protocol in which hESC (H9-cells) differentiate into neural cells. Within 10 days, hESC lost their pluripotent capacity and in parallel, microscopically observable neuronal structures occurred. This was monitored by immune staining and gene expression analysis using Q-PCR with stem cell- and neuronal specific markers. We studied the effect on gene expression of increasing concentrations of Carbamazepine and Valproic acid at different time points within the differentiation period. The performance comparison between mESC and hESC based neural differentiation assays will have to show the added value in practice of the latter test system for (neuro-) developmental toxicity testing.

F-2027
INHIBITION OF GSK3BETA ENHANCES EMBRYONIC STEM CELL-DERIVED SKELETAL MYOGENESIS

Shelton, Michael[1], Metz, Jeff[1], Carpenedo, Richard[2], Demers, Simon-Pierre[3], Stanford, William L.[4], **Skerjanc, Ilona**[1]
[1]University of Ottawa, Ottawa, ON, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]University of Sheffield, Sheffield, United Kingdom, [4]Ottawa Hospital Research Institute, Ottawa, ON, Canada

Cell therapies for treating muscular dystrophy require an adequate quantity of muscle progenitor cells (MPC) not yet attainable from adult donors. Here, we treat human embryonic stem cells (ESCs) with the GSK3-inhibitor CHIR99021, creating cultures with ~90% skeletal myogenic identity. Gene expression analysis identified progressive expression of mesoderm, somite, dermomyotome, and myotome markers, following patterns of embryonic myogenesis. CHIR99021 markedly enhanced transcript levels of the pan-mesoderm gene T and paraxial-mesoderm genes MSGN1 and TBX6. Immunofluorescence confirmed that 90% of cells expressed BrachyuryT immediately following treatment. After one week, ~60% of cells expressed the muscle progenitor protein, PAX3. By 7 weeks, 50% of cells were myosin heavy chain+ve myocytes/myotubes surrounded by a 40% population of PAX7+ve MPCs, indicating ~90% of cells had achieved myogenic identity. Importantly, the PAX7+ve MPCs persisted during terminal differentiation, reminiscent of embryonic satellite cells. These studies establish a foundation for serum-free and chemically-defined skeletal myogenesis of ESCs.

F-2028
NOX4-MEDIATED GENERATION OF REACTIVE OXYGEN SPECIES INDUCES THE QUIESCENCE OF VASCULAR PROGENITORS DERIVED FROM HUMAN EMBRYONIC STEM CELLS

Song, Sun-Hwa, **Suh, Wonhee**
College of Pharmacy, Ajou University, Suwon, Republic of Korea

Reactive oxygen species (ROS) are involved in a wide range of cellular processes. However, few studies have examined the generation and function of ROS in human embryonic vascular development. In this study, the sources of ROS and their roles in the vascular differentiation of human embryonic stem cells (hESCs) were investigated. During vascular differentiation of hESCs, CD34+ cells had quiescence-related gene expression profiles and a large fraction of these cells were in G0 phase. In addition, levels of ROS, which were primarily generated through NOX4, were substantially higher in hESC-derived CD34+ cells than in hESC-derived CD34- cells. To determine whether excess levels of ROS induces quiescence of hESC-derived CD34+ cells, ROS levels were moderately reduced using selenium to enhances antioxidant activities of thioredoxin reductase and glutathione peroxidase. In comparison to untreated CD34+ cells, selenium-treated CD34+ cells exhibited changes in gene expression that favored cell cycle progression, and had a greater proliferation and a smaller fraction of cells in G0 phase. Thus, selenium treatment increased the number of hESC-derived CD34+ cells, thereby enhancing the efficiency with which hESCs differentiated into vascular endothelial and smooth muscle cells. This study implicates NOX4 generates ROS in CD34+

vascular progenitors during hESC differentiation, and shows that modulation of ROS levels using antioxidants such as selenium may be a novel approach to increase the vascular differentiation efficiency of hESCs.

F-2029
SELECTION OF STABLE HOUSEKEEPING GENES IN DIFFERENTIATING HUMAN PLURIPOTENT STEM CELLS

Synnergren, Jane[1], Holmgren, Gustav[1], Sartipy, Peter[2]
[1]*Systems Biology Research Centre, School of Bioscience, Skövde, Sweden,* [2]*Cellectis AB, Gothenburg, Sweden*

Housekeeping genes (HKGs) are involved in basic functions needed for the sustenance of the cell, and are assumed to be constitutively expressed at a constant level. Based on these features, HKGs are frequently used for normalization of gene expression data. However, studies have shown that there is a large variability of stable housekeeping genes in various cell types. In a previous study employing human embryonic stem cells (hESCs) we observed that the expression of commonly used HKG varies in stem cells to a degree that rendered them inappropriate as reference genes. Here we present an extended study of HKGs in human pluripotent stem cells, including nine global gene expression datasets from both hESC and human induced pluripotent stem cells (hiPSCs), obtained from studies of differentiation towards endoderm, mesoderm, and ectoderm. Sets of stably expressed genes during each of these differentiation regimes were identified and a handful of genes were identified as generally applicable HKGs. We validated the stability of a selection of genes from this novel set of HKGs in independent, publicly available gene expression data from hPSCs and observed substantial similarities with our results. The gene expression profiles were confirmed by real-time QPCR analysis. Taken together, these results suggest that differentiating hPSCs have a distinct HKG signature which is different from somatic cell types, and underscore the necessity to validate the expression profiles of putative reference genes before using them for normalization. In addition, these novel putative HKGs can preferentially be used as reference genes in expression analyses of differentiating hPSCs.

F-2030
DIRECTING HUMAN EMBRYONIC STEM CELL DIFFERENTIATION TOWARDS A RENAL LINEAGE GENERATES A SELF-ORGANIZING KIDNEY

Takasato, Minoru[1], Er, Pei X.[1], Becroft, Melissa[1], Vanslambrouck, Jessica M.[1], Stanley, Ed[2], Elefanty, Andrew G.[2], Little, Melissa H.[1]
[1]*Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia,* [2]*Murdoch Childrens Research Institute, Parkville VIC, Australia*

With the prevalence of end-stage renal disease rising 8% per annum globally, there is an urgent need for renal regenerative strategies. The kidney is a mesodermal organ that differentiates from the intermediate mesoderm (IM) through the formation of a ureteric bud (UB) and the interaction between this bud and the adjacent IM-derived metanephric mesenchyme (MM). The IM itself is derived from the posterior primitive streak. Although the developmental origin of the kidney is well understood, nephron formation in the human kidney is completed before birth. Hence, there is no postnatal stem cell able to replace lost nephrons. The goal of our project was to direct differentiation of pluripotent human ES cells (hESCs) towards kidney in a stepwise manner. To achieve this, we developed fully chemically-defined monolayer culture conditions using growth factors expressed during normal embryogenesis. For the induction of posterior primitive streak (PPS), concentrations of BMP4, Activin A and WNT agonist CHIR99021 were tested. It was determined that 2-3 days of high BMP4 / low Activin A or high CHIR99021 alone was sufficient to induce PPS with over 90% efficiency. For the induction of IM, we used FGFs that are expressed in the IM *in vivo*. Without FGFs, the cells were differentiated only to *OSR1* positive trunk mesoderm. However, the addition of FGF2 or FGF9 induced *OSR1* together with the additional IM markers, *PAX2* and *LHX1* by day 6 of differentiation. In order to assess the commitment of these cells to renal fate, induced IM were cultured up to day 18. Timecourse RT-PCR from day 0 to day 18 showed that gene expression changed in a stepwise manner from primitive streak to IM followed by simultaneous induction of MM and ureteric epithelium (UE) gene expression. Importantly, *HOXD11* expression indicated that differentiation was directed to the metanephric region of embryogenesis but not to the mesonephric one. By day 14 of differentiation, the protocol induced the synchronous induction of elongating epithelial structures of $PAX2^+/GATA3^+/ECAD^+$ UE together with a surrounding mesenchymal $PAX2^+/SIX2^+/WT1^+$ MM. Within the dish, these populations together formed a self-organising structure reminiscent of the embryonic kidney, including the formation of renal vesicles, the first phase of nephron formation. When these hESC-derived kidney progenitor cells were aggregated with cells from dissociated mouse embryonic kidney cells and grown as an organoid at an air-media interface, hESC-derived components integrated into mouse-derived kidney structures, demonstrating the broad renal potential of induced human kidney progenitors *ex vivo*. To further assess the self-organizing potential of our hESC-derived kidney progenitors, aggregations were generated using hESC-derived cells only. These aggregates contained self-organizing events, including combinations of MM and UE, renal vesicles, proximal tubules and collecting ducts. In summary, we report the successful differentiation of human pluripotent cells to a self-organizing kidney. The coordinated induction of cells from the various key cellular populations involved in kidney development demonstrates the requirement for interacting niches for the creation of complex morphogenetic structures. The capacity for such populations to undergo self-organization *in vitro* bodes well for the future of tissue/organ bioengineering and the potential for pluripotent-stem-cell-based renal regeneration.

F-2031
TRACTION FORCES ARE NECESSARY FOR EARLY ENDODERM SPECIFICATION

Taylor-Weiner, Hermes A., Ravi, Neeraja, Engler, Adam
Bioengineering, University of California, San Diego, La Jolla, CA, USA

Morphogenic events during early development, including gastrulation, require embryonic stem cell (ESC) force-mediated motility and coincide with initial fate specification, resulting in the formation of the definitive endoderm (DE) and mesoderm layers of the trilaminar embryo. Moreover, ESCs in vitro inhibit cell traction forces when maintained in a pluripotent state, suggesting a switch in cell contractility that is initiated upon differentiation. However, it is not well understood how traction forces become activated during differentiation and whether integrin-signaling regulates fate choices. Typically traction forces are measured on hydrogels, but to more closely mirror the ESC niche, we measured tractions in ESCs bound to 3D fibrillar fibronectin (FN) matrices using a unique FRET probe. To address the extent to which tractions forces are required for ESC differentiation, we examined mouse ESCs grown in DE inductive medium and found that inhibiting cell tractions via the addition of blebbistatin, an inhibitor of non-muscle myosin, prevented expression of SOX17, an early marker of DE. When blebbistatin was removed, DE induction medium was sufficient to activate ESC traction forces, resulting in a decrease in the observed fibronectin FRET intensity ratio. By contrast ESCs grown in

pluripotency medium did not exert significant tractions against the FN matrix. Laminin isoforms have been reported to improve DE induction efficiency, but it is not clear whether traction forces mediate this effect. Mouse ESCs grown in DE inductive medium on fibrillar matrices with varying amounts of laminin-111 decreased their fibronectin traction forces in a laminin-dependent manner, suggesting preferential binding to laminin over fibronectin. The effects of laminin on DE traction forces and differentiation were reversed only when the laminin-binding alpha3 integrin was blocked, suggesting integrin-specific signaling regulates DE induction. These data imply that traction forces and integrin-signaling are important regulators of early fate decisions in ESCs.

F-2032
THE EXPRESSION AND ROLE OF ARYL HYDROCARBON RECEPTOR IN EARLY DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

Teino, Indrek, Pook, Martin, Tiido, Tarmo, Maimets, Tovio
University of Tartu, Tartu, Estonia

Aryl hydrocarbon receptor (AHR) is a liganddependent transcription factor belonging to bHLH/PAS family that upon activation translocates to the nucleus and controls the expression of a diverse set of genes. The role of AHR in various cell types was established in its importance as a major mediator of toxicity of different environmental contaminants. Following studies significantly broadened the role of AHR in cancer as well as in normophysiology, including regulation of cell cycle, importance in female reproduction etc. Previous studies on the role of AHR in the regulation of human embryonic stem (hES) cell pluripotency are limited. However, there are data showing the importance of AHR in regulation of development and functioning of cells with high developmental potency (e.g. neural, liver, hematopoietic and breast cancer stem cells/progenitors). Micro-RNAs (miRNAs) have been shown to be novel regulators of pluripotency and important regulators of stem cell differentiation during early mammalian development. A cluster of miRNAs, miR-302, including miR-302b/c/a/d and miR-367 has been shown to be highly expressed in embryonic stem cells and they have been successfully used to reprogram somatic cells into pluripotency. A previous study has shown that AHR along with pluripotency markers Oct4, Nanog and Sox2 directly bind to and activate the expression of miR-302. Importantly, the expression of AHR was elevated after transduction of pluripotency factors to mouse embryonic fibroblast (MEF) cells subsequently resulting in lower expression after reprogramming. Our preliminary results indicate that AHR is expressed, altough at low levels, in hES cells. Moreover the prototypical ligand and an environmental contaminant TCDD did not appear to have an effect on the pluripotency of hES cells. A recent study in murine embryonic stem (mES) cells revealed that AHR expression was repressed by pluripotency factors and polycomb group proteins and its expression was elevated as non-directed differentiation proceeded. Our preliminary data on human embryonic stem cells however indicate that in early directed differentiation with retinoic acid the expression of AHR is maintained at low levels. Taken together there are data indicating the importance of low AHR levels in pluripotent ES cells and its positive effect on cell reprogramming. Whether the upregulation of AHR during differentiation, as seen in mES cells, is species-specific or is it valid in hES cells is currently under investigation.

F-2033
GENERATION OF RESPIRATORY EPITHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS - NEW THERAPEUTIC APPROACH FOR (GENETIC) LUNG DISEASES

Ulrich, Saskia, Haller, Ralf, Merkert, Sylvia, Weinreich, Sandra, Menke, Sandra, Mauritz, Christina, Martin, Ulrich
Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover Medical School, Hannover, Germany

The *in vitro* production of respiratory epithelial (progenitor) cells from human pluripotent stem cells (hPSCs) offers promising new options for the treatment of respiratory diseases. Importantly, efficient technologies for targeted gene correction, based on e.g. zinc finger nucleases (ZFNs) or transcription activator like effector nucleases (TALENs), makes the hPSC-based treatment of genetic lung diseases like cystic fibrosis (CF) and surfactant deficiencies feasible. A prerequisite for such approaches is an efficient and robust differentiation strategy for the *in vitro* generation of the desired respiratory epithelial cell types. We therefore aim at the differentiation of human embryonic (hESCs) as well as human induced pluripotent stem cells (hiPSCs) into respiratory epithelial cell types. To evaluate the earliest respiratory differentiation steps, we make use of a hESC (hES3) reporter cell line (kindly provided by A. Elefanty) expressing eGFP under the endogenous promoter of NK2 homeobox 1 transcription factor (NKX2.1), known as the earliest marker in lung development. Moreover, we established a NKX2.1-eGFP hiPSC reporter line as well as non-corrected and gene-corrected CF patient-derived hiPSCs. With our current serum-free monolayer-based differentiation strategy we were able to generate highly enriched $FOXA2^+/SOX17^+$ and $CXCR4^+/C\text{-}KIT^+$ definitive endoderm. Via subsequent anteriorization, $FOXA2^+/SOX2^+$ anterior foregut endoderm was induced which then gave rise to NKX2.1-$eGFP^+$ cells. Coexpression of NKX2.1-eGFP with the endodermal marker FOXA2 as well as qRT-PCR analysis of the purified NKX2.1-$eGFP^+$ population indicated a respiratory phenotype of the NKX2.1-$eGFP^+$ cells, excluding the neuronal or thyroidal cell fate. Additionally, a subset of the NKX2.1-$eGFP^+$ cells coexpressed SOX2 demonstrating specification towards a proximal airway progenitor cell phenotype. Moreover and in view of a hPSC-based therapeutic approach for CF, we were able to generate Cystic fibrosis transmembrane conductance regulator $(CFTR)^+$ cells, a cell type affected by CF, from wildtype hPSCs as well as from non-corrected and gene-corrected CF-hiPSCs. In summary, with the use of hPSC reporter cell lines we are able to demonstrate $NKX2.1^+$ respiratory epithelial progenitor formation. This progenitor pool will be the basis for maturation strategies to finally provide mature respiratory cells types for cell replacement therapies as well as disease modeling, drug screening and toxicity tests *in vitro.* A first step towards a hPSC-based ex-vivo gene therapy for CF was achieved by the generation of $CFTR^+$ cells from gene-corrected CF-hiPSCs.

F-2034
A TIME-COURSE STUDY TO DECIPHER THE TRANSCRIPTIONAL DYNAMICS AT A SINGLE-CELL LEVEL IN BMP MEDIATED DIFFERENTIATION

V, Sivakamasundari, Robson, Paul
Stem Cell and Developmental Biology, Genome Institute of Singapore, Singapore

Transcriptomic analyses of bulk samples obscure the identity of the true direct targets of a differentiation stimulus. BMP signaling is known to direct differentiation of human Embryonic Stem Cells (hESCs) towards extra-embryonic lineage. Using this as a model system, we have

employed C1 microfluidic single-cell RNA-sequencing technology to identify the first responders of BMP signal induction in hESCs. A time-course study on more than 150 individual cells has enabled us to capture the earliest dynamic transcriptional states involved in BMP mediated differentiation, at a single-cell level.

F-2035

GAP JUNCTIONAL INTERCELLULAR COMMUNICATION IS ESSENTIAL FOR PRIMITIVE ENDODERM FORMATION IN EMBRYOID BODIES

Wörsdörfer, Philipp, Edenhofer, Frank
Institute of Anatomie and Cell Biology, University of Würzburg, Würzburg, Germany

Connexins (Cx) are expressed during pregastrulation development, form gap junctions and contribute to the establishment of defined communication compartments in the early conceptus. A critical function of gap junctions in early development, however, could not be yet clearly demonstrated. The connexin isoforms Cx43 and Cx45 are coexpressed during that developmental stage. Here we describe, the generation of Cx43/Cx45 double deficient mouse embryonic stem cells (mESCs). They were differentiated into embryoid bodies (EBs), an in vitro model for pregastrulation and early gastrulation. While Cx deficient mESCs did not show any obvious phenotype regarding cell proliferation or apoptosis, we observed that the expression of Cx43 and Cx45 is required for the establishment of primitive endoderm (PE) in EBs. Lentiviral overexpression of either Cx43 or Cx45 rescues the observed phenotype in Cx43/45 deficient mESCs, indicating a redundant function of these isoforms during the process. Defective PE formation leads to a block in subsequent differentiation events e.g. germ layer specification. Viral overexpression of the Occulodentodigital Dysplasia (ODDD) associated mutant Cx43 G138R, that is not able to form functional channels, does not rescue the differentiation defect. Our study demonstrates that Cx-deficient ESCs represent a new and straightforward model to analyze the role of connexins in the early conceptus and suggests an essential role of gap junctional intercellular communication during embryonic development.

F-2036

IDENTIFICATION OF MOLECULAR SIGNATURES RELEVANT TO AMYOTROPHIC LATERAL SCLEROSIS USING HUMAN STEM CELL-DERIVED MOTOR NEURONS

Williams, Luis A., Davis-Dusenbery, Brandi N., Sandoe, Jackson, Eggan, Kevin Carl
HHMI, Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

Amyotrophic Lateral Sclerosis (ALS) is a fatal neuromuscular disorder characterized by the selective degeneration of cortical and spinal motor neurons. Mutations in several genes, including SOD1, TARDBP, FUS, UBQLN2, VCP and C9ORF72 can cause ALS. It is not known how alterations in these genes with diverse cellular functions converge in the death of motor neurons. TARDBP and FUS encode RNA binding proteins involved in RNA metabolism, implicating dysregulation in RNA pathways as a contributor to ALS pathogenesis. Since it is not possible to retrieve and culture viable motor neurons from ALS post-mortem samples or living patients, the directed differentiation of spinal motor neurons from human pluripotent stem cells represents a great alternative for the generation of human cellular models for the study of ALS and other neurological disorders. In order to identify transcriptional changes potentially relevant to ALS, we have carried out RNA-Seq of differentiated spinal motor neurons expressing either reduced levels of ALS-linked genes or the variants of these genes associated with disease. Downregulation of gene expression levels was accomplished using an siRNA approach. Samples expressing ALS-linked mutant variants were generated using genome-editing technology to either introduce a single base pair change in the endogenous locus, or to target an expression cassette into the AAVS1 "safe harbour" locus. We anticipate that RNA-Seq analyses will help define a set of ALS-relevant molecular signatures by comparing the overlap (if any) of changes generated by alterations in the different genes linked to ALS, which in turn will provide insights into potential mechanisms underlying motor neuron death in ALS.

F-2037

MODULAR SURFACES DELIVER INSOLUBLE CUES TO PROMOTE MESENDODERM DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS.

Wrighton, Paul[1], Klim, Joseph[2], Hernandez, Brandon A.[3], Kiessling, Laura L.[4]
[1]University of Wisconsin Madison, Madison, WI, USA, [2]Harvard University, Cambridge, MA, USA, [3]Biochemistry, University of Wisconsin Madison, Madison, WI, USA, [4]Chemistry and Biochemistry, University of Wisconsin Madison, Madison, WI, USA

Human pluripotent stem (hPS) cells possess the extraordinary capacity to self-renew indefinitely and differentiate into specialized cell types. Soluble growth factors and small molecules are powerful means of influencing hPS cell differentiation. Protocols for differentiation typically rely on these cues while employing substrata consisting of complex mixtures of biomolecules, such as Matrigel. Such surfaces interact with myriad cellular receptors, obscuring the influence of specific insoluble signals on hPS cell fate. We demonstrate that modular peptide-modified surfaces can deliver insoluble signals that help guide differentiation. Surfaces that specifically engage glycosaminoglycans are superior to Matrigel in promoting hPS cell differentiation to definitive endoderm, mesodermal progenitors or cardiomyocytes. The superiority of the modular surfaces rests in their ability to modulate signaling pathways. Specifically, we show integrins and integrin-linked kinase activate the Akt signaling pathway, which is antagonistic to mesendoderm differentiation. The ability to attribute cellular responses to specific interactions between the cell and the substrate can advance the development of strategies to optimally activate signaling pathways governing cell fate.

F-2038

THE DIRECTED DIFFERENTIATION OF SPINAL V3 INTERNEURONS FROM MOUSE EMBRYONIC STEM CELLS

Xu, Hao, Sakiyama-Elbert, Shelly E.
Biomedical Engineering, Washington University, St Louis, MO, USA

Spinal cord injury affects approximately 265,000 Americans with 12,000 new injuries occurring each year. Disrupted connectivity along the spinal cord leads to disrupted function caudal to the injury site. Recent studies in rodent spinal cord injury models have observed functional recovery after spontaneous reorganization around the lesion site. Better understanding of spinal cord circuitry could be critical to developing therapeutic strategies to treat spinal cord injury. V3 interneurons (INs) have been identified as an integral component of left-right rhythmic locomotion. This commissurally-projecting glutamatergic neuron population can synapse onto motoneurons (MNs) and other INs. Their long projection distance combined with the ability for their axons to cross the midline make them an important cell population in understanding local rewiring after spinal cord injury. Although V3 INs are vital to locomotion, they comprise only about 10% of the ventral spinal cord. The scarcity of this population drives the need for an induction protocol to

differentiate V3 INs from mouse embryonic stem cells (ESCs). During development, V3 INs arise from the p3 progenitor domain which is adjacent and ventral to the MN domain. Directed differentiation of spinal MNs from mouse ESCs can be achieved by aggregation of ESCs into embryoid bodies (EBs) for two days and then exposing them to smoothened agonist (SAG, a sonic hedgehog agonist) and retinoic acid (RA) for four days (2-/4+). Literature has shown that increasing Shh concentration and exposure duration drives more ventral fates. We investigated the effects of varying SAG concentration and SAG exposure time on V3 IN induction while holding RA concentration at 2 μM. RA concentration was then varied with the best working SAG conditions. At the end of induction, V3 IN progenitor marker Nkx2.2 and V3 IN post-mitotic marker Sim1 expression were assessed via qRT-PCR and either immunocytochemistry or in situ hybridization. Compared to the uninduced controls of the same time point, qRT-PCR showed a significant increase in Sim1 expression (nearly 100 fold) with 0.5 μM SAG and 10 nM RA after 6 days of exposure (2-/6+). Additionally, Nkx2.2 showed nearly 40 fold increase compared to the same control. Since there is not a good Sim1 antibody, in situ hybridization was used to visualize the presence of Sim1 in sectioned EBs. Sim1 positive cells were observed in EBs induced with 0.5 μM SAG and 10 nM RA at 2-/6+. When the sectioned EBs were stained post in situ with Nkx2.2 antibody, colocalization of Sim1 and Nkx2.2 expression was observed. Sim1 expression has been observed in both V3 INs of the spinal cord and neurons of the hypothalamus. RAX, a hypothalamus marker, was used to verify the nature of the induced Sim1 positive cells. Hypothalamus samples from mouse brains at embryonic day 13 to 14 were collected and mRNA extracted for qRT-PCR. Little to no RAX expression was observed via qRT-PCR after 2-/6+ with 0.5 μM SAG and 10 nM RA (Ct values > 33) while the hypothalamus samples had significant amounts of RAX mRNA (Ct values in mid 20s). These results indicate that with the new induction protocol, Nkx2.2 and Sim1 positive V3 INs are generated. This protocol provides a faster, higher throughput method to generate V3 INs than isolation from embryonic mouse tissue. The resulting cells can potentially be used to study locomotion circuitry and as potential therapeutics for spinal cord injury.

F-2039
SOX9 ACCELERATES ESC DIFFERENTIATION BY REPRESSING SOX2 EXPRESSION THROUGH P21 (WAF1/CIP1)

Yamamizu, Kohei, Schlessinger, David, Ko, Minoru
National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

Cell identity during development is altered by transcription factors (TFs) that function as molecular switches to activate or repress specific genes. The mechanisms responsible for the silencing of pluripotent genes and the activation of program genes in early differentiation remain unclear. Previously, we have generated global gene expression profiles obtained by overexpressing single TFs using the NIA mouse embryonic stem cell (ESC) bank, which comprises 137 mouse ESC lines. To identify master TFs for lineage-specific differentiation, we established a correlation matrix between the global gene expression responses to the induction of single TFs and the global gene expression profiles of a variety of tissues and organs in vivo. Based on the correlation matrix, we successfully identified 21 master and ancillary TFs for differentiation of ESCs into various lineages, including myocytes, blood cells, hepatocytes, and neurons. In this study, we focus on TFs that accelerate early differentiation of ESCs into one or more of the three germ layers. For 36 TFs that showed extensive effects on expression profiles when overexpressed in ESC clones, we assessed the extent of expression of differentiation toward each germ layer by FACS with markers of FLK1 for mesoderm, FOXA2 for endoderm, and PSA-NCAM for ectoderm. Overexpression of *Sox9* was exceptional in showing efficient and rapid activation of all 3 germ layer markers correlated with early disappearance of pluripotent markers. Furthermore, *Sox9* enhanced differentiation of ESCs into endothelial cells, hepatocytes, or neurons in appropriate lineage-specific media. The decrease in cell proliferation accompanying early differentiation was at least partially mediated by increases in *p21* (Waf1/Cip1) expression. Interestingly, *p21* represses *Sox2* expression through direct binding of the SRR2 enhancer of *Sox2* gene and knockdown of *p21* with siRNA completely abolishes the *Sox9*-elicited inhibition of *Sox2* and acceleration of differentiation and. By contrast, knockout of *Sox2* after 2 days of differentiation accelerated differentiation of all 3 germ layers from ESCs. This molecular cascade, Sox9-p21-Sox2, could be an early general programming steps for ESC differentiation.

F-2040
CHARACTERIZATION OF CARDIOMYOCYTES DIFFERENTIATED FROM PLURIPOTENT HUMAN EMBRYONIC STEM CELLS USING DEFINED, XENO-FREE MUSCLE-SPECIFIC LAMININ MATRICES

Yap, Lynn[1], Ohman, Miina Karelia[1], Chai, Xiaoran[1], Sun, Yi[2], Cai, Zuhua[1], Chong, Liyen[1], Sigmundsson, Kristmundur[1], Cook, Stuart[1], Ghosh, Sujoy[1], Tryggvason, Karl[1]
[1]Cardiovascular and Metabolic Disorders Program, Duke-National University of Singapore Graduate Medical School, Singapore, [2]BioLamina, Solna, Sweden

Cardiomyocytes (CM) undergo cell cycle arrest and their proliferation ceases after birth. Therefore cardiac muscle injury is often permanent and results in high mortality rates in myocardial infarction patients. Recent progress in stem cell research has opened up possibilities for new cell therapy approaches for the treatment of cardiac muscle injury. However, significant limitations remain concerning the reproducibility, efficiency and maturity of these CM differentiation protocols. We have demonstrated using transcriptome analysis that laminin-221 (LN-221) is the most abundant laminin isoform in the human adult left ventricular heart chamber, which also contains LN-521 and LN-211. We produced LN-221 and mimicked the natural in vivo matrix environment of the heart by using a human recombinant LN-521 and LN-221 matrix for differentiation of human embryonic stem (hES) cells. This defined matrix is able to robustly support differentiation of hES cells into CM using chemically defined and xeno-free methodology. In this study, we characterize the cells during differentiation of hES cells to CMs using RNA sequencing, immunocytochemistry (ICC) and flow cytometry. Gene expression studies show a downregulation of pluripotent genes (NANOG, POU5F1 and SOX2) and suppression of ectodermal gene (PAX6). On the other hand, increase expression of mesendodermal genes (T, MESP2, SOX17) are indicative of cardiac lineage specification. Cardiac progenitors were detected at day 5 as confirmed by expression of NKX2-5, ISL and C-KIT. These progenitors continue to differentiate into spontaneously beating CM. The expression of CM-specific genes such as cardiac troponin T (TNNT2), α-actin (ACTC1), α-tropomyosin (TPM1) and myosin light chain (MYL7) were measured after day 9 and remain highly expressed till day 30. Cardiac-specific proteins were measured by flow cytometry which showed expression of > 80 % positive for TNNT2. Furthermore, the CMs exhibit organized sarcomeric striations, have large rectangular cell morphology with reduced proliferation rate, which are typical phenotype for mature CM. To determine the level of maturity, functional assessment such as single cell patch clamp, calcium imaging and multi electrode array will be utilized.

F-2041
FOXA2 UP-REGULATES GENE EXPRESSION FOR A9 DOPAMINERGIC NEURONAL PHENOTYPE WITH NURR1 VIA EPIGENETIC MODIFICATION

Yi, Sang Hoon[1], Rhee, Yong-Hee[2], Lee, Sang-Hun[3]
[1]Hanyang University, Seoul, Republic of Korea, [2]Graduate School of Biomedical Science and Engineering, Seoul, Republic of Korea, [3]Department of Biochemistry and Molecular Biology, College of Medicine, Hangyang University, Seoul, Republic of Korea

Understanding how dopamine (DA) phenotypes are acquired in midbrain DA (mDA) neuron development is important for bioassays and cell replacement therapy for Parkinson disease. We investigate a feed-forward mechanism for Nurr1 and Foxa2 in mDA neuron development. Nurr1 acts as a transcription factor for DA phenotype gene expression. However, Nurr1-mediated DA gene expression was inactivated by forming a protein complex with CoREST, and then recruiting HDAC1, an enzyme catalyzing histone deacetylation, to DA gene promoters. Co-expressions of Nurr1 and Foxa2 were established in mDA neuron precursor cells by a positive cross-regulatory loop. In the presence of Foxa2, the Nurr1-CoREST interaction was diminished, and CoREST/HDAC1 proteins were less enriched in DA gene promoters. Consequently, histone 3 acetylation, which is marker for open chromatin structures, was strikingly increased at DA phenotype gene promoters, such as tyrosine hydroxylase (TH) and dopamine transporter (DAT). These data establish the interplay of Nurr1and Foxa2 as the crucial determinant for DA phenotype acquisition during mDA neuron development. This work was supported by grants from the Bio and Medical Technology Development Program (2010-0020232), and the Medical Research Center (2008-0062190), funded by the National Research Foundation of Korea (NRF) of the Ministry of Education, Science and Technology (MEST), Republic of Korea.

F-2042
INDUCTION OF INNER EAR HAIR CELL LIKE CELLS FROM TRANSCRIPTION FACTOR MATH1-TRANSFECTED MOUSE EMBRYONIC STEM CELLS

Yoshikawa, Masahide, Ouji, Yukiteru, Nakamura-Uchiyama, Fukumi, Wanaka, Akio
Nara Medical University, Kashihara, Japan

Background and Aim: Math1, a basic helix-loop-helix transcription factor homolog of the Drosophila atonal gene, is considered to be a key factor for induction of sensory hair cells (HCs) during development of the organ of Corti or cochlea. Although embryonic stem (ES) cells are able to produce HC-like cells, the role of Math1 in induction of those cells has not been thoroughly elucidated. In the present study, we performed introduction of Math1 into ES cells in order to achieve efficient generation of HC-like cells. Methods: ES cells carrying Tet-inducible Math1, Math1-ES cells, were generated using a Tet-On gene expression system. Embryoid bodies (EBs) formed in the absence of doxycycline (Dox) for 4 days were allowed to grow for an additional 14 days in the dishes in the presence of 400 ug/ml of Dox. Results: At the end of those 14-day cultures, approximately 10% of the cells in EB outgrowths expressed the HC-related markers myosin6, myosin7a, calretinin, a9AChR, and Brn3c (also known as Pou4f3), and showed formation of stereocilia-like structures, whereas few cells in EB outgrowths grown without Dox showed those markers. Conclusion: Math1-ES cells are considered to be an efficient source of ES-derived HC-like cells and Math1 may be an important factor for induction of HC-like cells from differentiating ES cells.

F-2043
PI3K AKT INHIBITS ACTIVIN INDUCED DEFINITIVE ENDODERM SPECIFICATION VIA THE REGULATION OF PHOSPHO SMAD2 DEGRADATION

Yu, Jason Shu Lim[1], Ramasamy, Thamil S.[2], Cui, Wei[3]
[1]Imperial College London, London, United Kingdom, [2]Molecular Medicine, University of Malaya, Malaysia, Malaysia, [3]Surgery and Cancer, Imperial College London, London, United Kingdom

Interplay and cross-regulation between different signaling pathways play a major role in the development of multicellular organisms, yet it remains unclear as to how these different pathways coordinate to specify the multiple cellular subtypes during organogenesis. Recent findings have shown that coordination between PI3K/Akt inhibition and Activin/Smad2 induction is critical in regulating the differentiation of hESCs towards the definitive endoderm (DE), which gives rise to several important organs such as the pancreas and liver. However, the molecular details underpinning this relationship remain unclear. In light of this, we have developed a chemically defined differentiation protocol through which we are able to robustly differentiate hESCs to the DE, thereby allowing us to interrogate the signalling mechanisms involved in this initial stage of specification. Using this system, we found that PI3K/Akt antagonizes Activin-induced DE differentiation by attenuating the half-life of Activin-induced phospho-Smad2, which results in the attrition of Smad2 transcriptional activity. PI3K/Akt antagonism of Smad2 is achieved via the phosphorylation of Smad2-T220 linker residue, which induces the binding of the E3 ubiquitin ligase Nedd4L to Smad2 resulting in its degradation. As such, inhibition of PI3K/Akt signaling reduced the phosphorylation of Smad2-T220, thereby extending the duration and transcriptional activity of Activin-induced activated Smad2, consequently promoting a more robust mesendoderm and endoderm differentiation. Our results identify a direct role for PI3K/Akt in regulating Smad2-T220 phosphorylation, representing a new and novel relationship between these two pathways which interact to regulate the fate determination of hESCs.

F-2044
THE ROLE OF SUBSTRATE ELASTICITY IN INFLUENCING THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO NEURONS

Zaltsman, Yefim[1], Musah, Samira[2], Wrighton, Paul[1], Zhong, Xiaofen[3], Chang, Qiang[3], Kiessling, Laura L.[1]
[1]University of Wisconsin - Madison, WI, USA, [2]The Wyss Institute for Biologically Inspired Engineering, Boston, MA, USA, [3]Waisman Center, University of Wisconsin - Madison, WI, USA

Human pluripotent stem (hPS) cells possess the remarkable capacity to self-renew indefinitely and differentiate into virtually all cell types. HPS cells thus represent an unlimited source of cells with potentially transformative applications such as modeling of human developmental and disease processes, cell-based regenerative medicine, and drug discovery and toxicity testing. All of these applications, however, require efficient and reproducible methods for propagating hPS cells and directing their differentiation to desired cell types. To date, focus has been on how soluble factors such as growth factors and small molecules influence these pathways. In physiological settings, however, stem cells receive both soluble and insoluble signals. Yet the role of insoluble signals, such as substrate elasticity, in influencing hPS cell fate decisions is less clear. We found that, even in the presence of soluble factors that promote pluripotency, compliant substrata, with elasticity similar to human brain tissue, override these signals to induce efficient hPS cell differentiation to neurons. The molecular mechanism

is through modulation of the localization of the transcriptional coactivator YAP. Nuclear exclusion of YAP in cells cultured on compliant substrata or the depletion of YAP by RNA interference effects neuronal differentiation. The neurons derived by substrate induction alone_in absence of neurogenic factors_express mature markers and possess action potentials. Our findings indicate that mechanical cues can override soluble signals, suggesting that their contributions to early human development and in vitro differentiation are profound. Current hPS cell differentiation protocols are almost exclusively carried out on tissue culture polystyrene, a substrate which is orders of magnitude stiffer than biological tissues. Therefore, we anticipate that utilizing substrates with more biologically relevant mechanical properties will increase the efficiency of existing differentiation protocols and perhaps give access to currently elusive cell types.

F-2045
DERIVATION OF PUTATIVE HUMAN PERICYTES FROM EMBRYONIC STEM CELLS WITH GMP COMPATIBLE CHEMICALLY DEFINED SERUM FREE MEDIUM

Yang, Jay (Jiwei), Hassanipour, Mohammad, West, Michael D., Larocca, David
BioTime, Inc., Alameda, CA, USA

Pericytes are endothelium-associated cells that play an important role in normal vascular structure and function, including maintenance of the blood brain barrier, tissue repair and regeneration. Improper functioning of pericytes can result in abnormal vasculature and contribute to a variety of diseases including cardiovascular ischemia, diabetes-associated retinopathy, kidney damage, and hepatic fibrosis. Replacement of pericytes using cell therapy may be useful for treating a variety of vascular diseases. Here we report the successful development of processes for deriving pericytes from human embryonic stem cells. The putative pericytes have high proliferative capacity and stability and can be expanded from 1x10e6 to 1x10e12 cells in as little as 6 passages. Tra-1-60 and Oct-4 were undetectable indicating lack of contaminating pluripotent stem cells. The cells appeared to be morphologically homogeneous and like early-passage primary bone marrow derived mesenchymal stromal/stem cells (BM-MSCs), displayed cell surface markers CD73+(90%)/CD105+(90%)/CD31-/CD45-. However, unlike BM-MSCs, they also expressed CD146 (90%), a surface marker for pericytes, and low expression of CD90, a common marker for BM-MSCs. The cells were initially positive for 2 additional pericyte markers, PDGFRβ (40-50%) and NG-2 (10-20%) at early (< p3) passage, but lost expression of both markers with continued passage in culture in a manner similar to primary pericytes. The hES derived pericytes differentiated under standard culture conditions promoting osteogenesis and chondrogenesis as demonstrated by alkaline phosphatase and Alcian blue staining. The differentiation potential was maintained through long-term passage, as both early (p1) and late (p21) passage pericytes differentiated toward bone and cartilage cells. Adipogenic media used to differentiate BM-MSCs to fat cells, however, did not result in differentiation of hES derived pericytes, primary brain or placental pericytes. Our preliminary results also showed hES derived pericytes aligned with endothelial cells to form tubular structures on Matrigel in an in vitro test for angiogenesis. hES-derived pericytes expressed podoplanin (>90%) throughout the long-term culture, similar to what we observed for primary placental pericytes. We are currently investigating additional structural and functional characteristics of the cells and their potential applications for vascular research, drug development, and cell therapy.

EMBRYONIC STEM CELL PLURIPOTENCY

F-2046
STEMNESS AND EXPANSION OF PLURIPOTENT STEM CELLS IS INDEPENDENT OF CX43 INTERACTIONS BETWEEN PLURIPOTENT STEM CELLS AND FEEDER CELLS

Kim, Jin-Su, Hwang, Seung-Taeh, Kwon, Dae-Kee, Park, Kwang-sook, Arai, Yoshie, Moon, Bo Kyung, Lee, Soo-Hong
CHA University, Seoul, Republic of Korea

Gap junctional intercellular communication (GJIC) is thought to play an important role in survival and differentiation of human embryonic stem cells (hESC) and even induced pluripotent stem cells (iPSC). Connexin 43 (Cx43, one of the major gap junction protein) among GJIC is crucial in maintaining the stemness of pluripotent stem cells. However, the roles of GJIC between hESC and feeder cell has rarely been studied. In this study, we examined whether direct interaction through gap junction affects on the maintenance of hESC stemness that were evaluated thorough RT-PCR, Western blotting, histological staining and so on. Cx43 down-regulation by small interfering RNA (siRNA) caused a profound inhibitory effect on GJIC. hESC cultured on Cx43 down-regulated feeder cell group was not effect on pluripotent marker (such as, hESC proliferation, AP activity, Oct4, Sox2, Nanog expression). These results demonstrate that Cx43-mediated GJIC between hESCs and feeder cells is not a critical factor for the conservation of hESC stemness and expansion.

F-2047
COMPARISON OF TELOMERE AND GENE EXPRESSION PROFILES OF HUMAN EMBRYONIC STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS

Lee, Jeoung Eun, Back, Ami, Lee, Jin Il, Shim, Myung Sun, Lee, Sung-Geum, Yi, Min-Jeong, Kwon, So-Jung, Lee, Dong Ryul
CHA Stem Cell Institute, CHA University, Seoul, Republic of Korea

Human Embryonic Stem Cells (hESCs) and induced pluripotent stem cells (hiPSCs) have self-renewal ability and differentiation ability to all kinds of cell type in the body, so it seems that they are mostly closer to clinical application and regenerative medicine in the near future. Many studies have compared the characteristics including genetic and epigenetic profiles of hiPSCs to hESCs, but the results are unclear and under debating yet. Telomeres play an important role in maintaining chromosome stability and cell proliferation, and telomere length is maintained by telomerase. It is known that functional telomeres are essential for the pluripotancy of ESCs and iPSCs. In this study, we compared the telomere length and telomerase activity of hiPSC to hESC, and then compared the gene expression profiles of hiPSC to hESC including cell proliferation and self-renewal related genes. We used CHA-hES 15 (from CHA Stem Cell Institute) for hESCs, and iPS-Foreskin-1 (from WiCell) for hiPSCs, and FS-6 (primarily cultured foreskin fibroblast cells, from CHA Stem Cell Institute) for somatic cells. Compared with FS-6, iPS-Foreskin-1 and CHA-hES 15 showed long telomere length and strong telomerase activity, but CHA-hES 15 showed longer telomere length and stronger telomere activity than iPS-Foreskin-1. When we compared gene expression profiles between CHA-hES 15 and iPS-Foreskin-1 using microarray analysis, the correlation value was 97.36% and about 400 genes were differentially expressed (more than two fold over- or down-expression). From the results, it suggests that hESC might have more functional telomeres than hiPSC although both of them have pluripotent stem cell properties.

F-2048

ACETATE LINKS GLYCOLYSIS TO HISTONE ACETYLATION AND PLURIPOTENCY IN HUMAN EMBRYONIC STEM CELLS

Aberdam, Daniel[1], Moussaieff, Arieh[1], Shen-Orr, Shai[2], Amit, Michal[3], Elena-Herrmann, Benedicte[4], Meshorer, Eran[5], Itskovitz-Eldor, Joseph[6], Nahmias, Yaakov[7]

[1]INSERM, Paris, France, [2]TECHNION, Haifa, Israel, [3]Technion, Haifa, Israel, [4]CNRS, Lyon, France, [5]The Hebrew University of Jerusalem Institute of Life Sciences, Jerusalem, Israel, [6]Rambam Health Care Campus, Haifa, Israel, [7]Hebrew University of Jerusalem, Jerusalem, Israel

Differentiation of pluripotent stem cells is a slow process, marked by the gradual loss of pluripotency factors over days in culture. While the first few days of differentiation show minor changes in the cellular transcriptome, intracellular signaling pathways remain largely unknown. Recently, several groups demonstrated that the metabolism of pluripotent mouse and human cells is different from that of somatic cells, showing a marked increase in glycolysis previously identified in cancer as the Warburg effect. Here, we sought to identify the earliest metabolic changes induced at the first hours of differentiation. High-resolution NMR analysis identified 35 metabolites and a distinct, gradual transition in metabolism during early differentiation. Metabolic and transcriptional analyses showed the induction of glycolysis toward acetate in pluripotent cells, and an increase in cholesterol biosynthesis during early differentiation. Importantly, this metabolic pathway regulated differentiation of human and mouse embryonic stem cells. Acetate delayed differentiation preventing differentiation-induced histone de-acetylation in a dose-dependent manner. Glycolytic inhibitors upstream of acetate caused differentiation of pluripotent cells, while those downstream delayed differentiation. Our data suggests that a rapid loss of glycolysis in early differentiation down-regulates acetate production, causing a loss of histone acetylation and concomitant loss of pluripotency. It highlights the important role metabolism plays in pluripotency and early differentiation of stem cells.

F-2049

DNA REPLICATION STRESS IN MOUSE EMBRYONIC STEM CELLS

Ahuja, Akshay Kumar[1], Herrador, Raquel[1], Mendez, Juan[2], Lopes, Massimo[1]

[1]Institute of Molecular Cancer Research, Zurich, Switzerland, [2]Centro Nacional de Investigaciones Oncológicas, Madrid, Spain

Embryonic stem cells (ESCs) have the ability to self-renew and to differentiate into multiple cell types. Conditional inactivation of ATR - the central factor activated by DNA replication stress - rapidly leads to stem cell depletion, suggesting in principle that stem cells experience replication stress during proliferation. Indeed, ESCs exhibit high levels of endogenous γH2AX foci, a marker for replication stress and DNA damage. However, as ESCs lack 53BP1 foci, a specific marker for DNA double strand breaks, γH2AX has been suggested to mark peculiar chromatin structure, rather than DNA damage, generally considered counterintuitive in cells crucial for development and tissue homeostasis. We now show, both in cultured ESCs and in vivo in early embryos, that γH2AX is accompanied by strong staining for RPA and Rad51 - both single stranded (ss)DNA-binding proteins recruited to chromatin upon replication stress. Accordingly, replication forks in ESCs travel markedly slower than in differentiated cells as visualized by DNA fiber analysis. Visualization of ESC replication intermediates by electron microscopy confirms massive accumulation of ssDNA gaps and reveals accumulation of reversed replication forks, a clear mark of replication stress recently associated with cancer onset and therapy. Furthermore, as in differentiated cells experiencing exogenous genotoxic stress, inactivation of poly ADP ribose polymerase (PARP) in ESCs rapidly leads to extensive breakage of replicating chromosomes. Upon induction of differentiation, all these hallmarks of replication stress disappear concomitantly with loss of Oct4, well before cells stop proliferating and undergo terminal differentiation. Thus, stemness - and not hypreproliferation - in ESCs is inherently associated with genotoxic stress. We will report our latest results of our current investigations, aiming to identify the source of the abundant DNA breaks observed in ESCs and the molecular mechanisms evolved by these cells to tolerate them during DNA replication. Pilot experiments reveal that similar phenomena may be associated with induced hyper-proliferation of adult stem cells, emphasizing the importance of our surprising observations in ESCs.

F-2051

LENGTHENING THE CELL CYCLE OF ES CELLS CAUSES ES CELLS TO DIFFERENTIATE WITHOUT A CHANGE IN PROLIFERATIVE COMPETENCY OR CELL SURVIVAL

Alexson, Tania, van der Kooy, Derek J.

University of Toronto, Toronto, ON, Canada

Stem cells are defined by two hallmark characteristics: multi-potentiality and self-renewal. Conventionally, embryonic stem (ES) cells self-renew by expansionary symmetric division (ESD) whilst having a relatively unregulated, short cell cycle. We hypothesize that lengthening the cell cycle time (CCT) of ES cells can modify ES cell self-renewal either by acting on existing weak points (low expression of pluripotency factors) within the cell or by allowing for the reception of a differentiation signal. Interestingly, Oct4 expression was dynamic during mitosis: 94% of prophase cells were Oct4+, only 8% in metaphase, while expression was partially (53%) restored in telophase. This illumiates a weak point during the cell cycle in which ES cells are primed to differentiate. Subsequently, we utilized drugs that have been conventionally used to arrest cell cycling to ascertain whether the fate of ES cells can be altered by looking at the ES to neural transition. All drugs tested thus far have failed to elicit a phase-specific arrest. Importantly, the lowest effective dose of nocodazole (Noc) causing microtubule depolymerization did not halt the cells in metaphase, but rather resulted in endoreduplication and increased CCT from ~10 to 24 hrs. Pre-treating ES cells with Noc for 12 hrs drastically compromised their ability to form either new clonal ES cell colonies or clonal neurospheres once Noc had been removed relative to controls. The reduction in new ES cell colony formation was not due to cells that had failed to proliferate as the number of persisting single cells was substantially dimished compared to controls. Moreover, the colonies_albeit few_that did form post-Noc exposure were quite comparable (80%) in size relative to controls. Together these data are inconsistent with Noc simply impairing proliferation. Alternatively, Noc exposure could impose a survival challenge upon ES cells. There are, however, no data to indicate such a challenge: the effect of Noc is phase-specific, cell death genes were not upregulated, and generating polyploid ES cells without also tremendously increasing the CCT by cytocholasin B exposure does not dramatically reduce ES cell colony formation. These data both rule out general cytotoxicity and highlight the importance of lengthening the ES CCT in compromising ES cell colony formation. Finally, two ES cell lines mutant for cell death genes_apoptosis inducing factor apoptosis inducing factor (AIF) or apoptotic peptidase activating factor1 (Apaf1)_were unable to rescue the reduction in ES cell colony. In contrast, Apaf1-/- ES cells paritally resuced primitive neurosphere formation relative to controls (50%). Moreover, Noc-exposure increased the Oct4lo/off population which is in accordance with the neural resuce and a fate switch. Thus, the

reduction in ES cell colony formation_and hence ES cell fate_is due, we argue, to ES cells differentiating into the primitive neural stem cell fate; the neural cells subsequetly undergo a survival challenge that is Apaf1 mediated. This switch in fate did not occur at the expense of the cell's overall proliferative competency as colony formation_in this case neural_was rescued. Therefore, there appears to be two types of decision a stem cell can make, and these are dissociable: whether or not to retain proliferation competency, and whether or not to retain potency. Modifications to the CCT of ES cells appear to regulate the potency versus differentiation (and not the proliferative) decision.

F-2052
DELINEATING THE MECHANISMS AND CONSEQUENCES OF HUMAN EMBRYONIC STEM CELL HETEROGENEITY

Allison, Tom[1], Andrews, Peter W.[2], Smith, Andrew[3], Barbaric, Ivana[2], Sloane-Stanley, Jackie[4]
[1]University of Sheffield, Sheffield, United Kingdom, [2]University of Sheffield, Sheffield, United Kingdom, [3]University of Edinburgh, Edinburgh, United Kingdom, [4]Weatherall Institute of Molecular Medicine, Oxford, United Kingdom

The use of human embryonic stem cells (hESCs) in clinical applications has been delayed due to the lack of understanding of fundamental decision-making with respect to self-renewal and differentiation. Accumulating evidence suggests that the states in which hESCs exist may be far more dynamic than previously thought, raising the question as to whether these different sub-states can bias the outcome of cell fate decision -. We are particularly interested in a bias towards the endodermal lineage. To test these hypotheses, we made a GFP knock-in reporter line into the GATA6 translational ATG initiation codon, by zinc finger nuclease mediated gene targeting. Using these reporter cells we have found that a fraction of SSEA3 and GATA6 co-expressing cells do make stem cell colonies and are capable of making clonal cell lines indicating that they are still within the stem cell compartment. By qPCR we have found an increase in the expression of other endoderm makers within the GATA6+ stem cell population, but no increase in mesodermal or ectodermal markers, suggesting that they may indeed exhibit an endodermal lineage bias. We have begun to assess this bias using a robust embryoid body system to track individual cell organisation as well as a monolayer assay to assess functional bias. Finally, upon reprogramming of our human ESC line to a more naïve state, we see a loss of heterogeneity within our cells, demonstrating a more primitive state within the stem cell compartment. Using specific compounds we are performing a screen to isolate a combination of chemical inhibitors to push cells into different states within the stem cell compartment. Our expectation is that this will lead to optimised conditions for endodermal biased cells that can increase the efficiency of differentiation protocols.

F-2053
THE EVALUATION OF LET7 FAMILY EXPRESSION IN HUMAN EMBRYONIC STEM CELLS IN COMPARISION TO MESENCHYMAL AND UNRESTRICTED SOMATIC STEM CELL

Arefian, Ehsan[1], Kiani, Jafar[2], Soleimani, Masoud[3], Aghaee-Bakhtiari, Seyed Hamid[4], Naderi, Mahmood[4], Atashi, Amir[4], Gheisari, Yousof[5], Ahmadbeigi, Naser[6], Shariati, S. Ali M.[7], Faridani, Omid R.[8]
[1]Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran, [2]Department of Molecular Biology and Genetic Engineering, Stem Cell Technology Research Center, Tehran, Iran, [3]Tarbiat Modares University, Hematology Dept, Tehran, Iran, [4]Stem Cell Technology Research Center, Tehran, Iran, [5]Isfahan University of Medical Sciences, Isfahan, Iran, [6]Tehran University of Medical Sciences, Tehran, Iran, [7]Stanford University, Stanford, CA, USA, [8]Ludwig Institute for Cancer Research, Stockholm, Sweden

Each type of stem cell illustrates a unique profile of microRNAs expression according to the origin of their derivation and biological pathways. Therefore, microRNAs could be used as biomarkers of stem cell characterization. Let7 family members including let7-a, let7-b, let7-c, let7-d, let7-f, let7-g, let7-I and hsa-mir-98 are very critical in regulation of stemness, proliferation and differentiation in stem cells. The profile of expression of this family in cancer cells and differentiated cells are different and, therefore, their profiling could be used as an effective procedure to characterize different states of cells. In this study, we evaluate let7 family microRNAs' expression by SL-PCR technique in three different stem cells including human embryonic Stem cells (hESC), human bone marrow derived stem cells (hMSC) and unrestricted somatic stem cells (USSC). In brief, the three characterized stem cells were separately cultured and subjected to total microRNA extraction. Synthesis of cDNA was carried out with incubation of 100 ng of total RNA with 0.01 µM of each microRNA designed SL-PCR probe in 10 µl ligation reaction at 42 °C for 2 hours. After ligation inactivation by temperature, the cDNAs were subjected to PCR by universal primers. The U6 gene was used as the internal control. Finally the PCR products were fractionated using 16% polyacrylamide/TBE gel electrophoresis. The quantitative analysis of results showed that hESC pattern of let7 family microRNAs' expression is significantly different from hMSC and USSC expression pattern. The expression of let7-c and let7-b were significantly reduced in hESC in comparison with hMSC and USSC cells.

F-2054
ENRICHMENT OF HUMAN PLURIPOTENT STEM CELLS POPULATIONS IN PARTICULAR CELL CYCLE STAGE BY SYNCHRONIZATION REVEALS NOVEL G2/M-SPECIFIC CDK2-CYCLINB1 INTERACTION

Barta, Tomas[1], Hampl, Ales[2]
[1]International Clinical Research Center, St. Anne's University Hospital, Brno, Czech Republic, [2]Department of Histology and Embryology, Masaryk University, Brno, Czech Republic

Cell cycle is not only the series of events leading to the duplication of cells, but it also plays an important role in maintenance of stemness and pluripotency in pluripotent stem cells (PSCs). PSCs within the blastocyst as well as in culture undergo rapid successive symmetrical cell divisions to provide sufficient number of equivalent progeny. However, continuous proliferation without differentiation demands unique gene networks regulation and cell cycle control. The rapid proliferation is achieved by unique cell cycle structure, lacking fully developed gap phases and containing rounds of DNA synthesis (S phase) and chromosomome/cell division (M phase).

Molecules and signaling pathways governing self-renewal and/or differentiation of PSCs still remain not to be fully elucidated. Cyclin-dependent kinases (CDKs), key molecular drivers of cell cycle, exhibit sustained high activity throughout the cell cycle of pluripotent stem cells as a consequence of lack or weak expression of CDK inhibitors and hyperphosphorylation of Rb protein. Several reports suggest that CDKs provide a link between pluripotency and self-renewal of human PSCs (hPSCs). Here we investigated in detail the activities of CDK1 and CDK2, and the expression of their partnering cyclins during the progression of cell cycle in both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). Most importantly, we demonstrate that there is unexpected physical interaction of CDK2 and cyclinB1 in these hPSCs, which is not detectable in human somatic cells, here represented by human foreskin fibroblasts. This CDK2-cyclin B1 association exhibits cell cycle-dependent dynamics, being more pronounced in G2/M phase compared to other phases of cell cycle. Correspondingly, when measured by in vitro assay using histone H1 and Rb fragment as substrates, kinase activity of CDK2-cyclin B1 complex is maximal during G2/M phase of cell cycle. In hESCs, simultaneous down-regulation of CDK2 and cyclin B1 mediated by siRNAs results in their accumulation in G2/M phase. Taken together, our data indicate that in hPCSs some unknown processes take place in G2/M phase of their cell cycle, execution of which requires the activity of novel complex containing S-phase specific CDK2 and G2/M-specific cyclin B1.

F-2055
CPG ISLAND-MEDIATED GLOBAL GENE REGULATORY MODES IN MOUSE EMBRYONIC STEM CELLS

Beck, Sam[1], Lee, Bum-Kyu[1], Rhee, Catherine[1], Song, Jawon[1], Woo, Andrew[2], Kim, Jonghwan[1]
[1]University of Texas at Austin, Austin, TX, USA, [2]University of Western Australia and Royal Perth Hospital/WAIMR, Perth, Australia

Unique characteristics of embryonic stem (ES) cells are precisely maintained by multi-layers of regulatory mechanisms including transcriptional and epigenetic regulations. Recent explosions of data on the various DNA binding proteins (DBPs) occupancies as well as the distributions of histone marks in ES cells have advanced our understanding about the transcriptional regulation underlying self-renewal and pluripotency. However, detailed mechanisms of DBP mediated transcriptional regulation in collaboration with epigenetic regulations in ES cells are still not well understood. In order to elucidate how DBPs interact with cis-regulatory elements to precisely control gene expression programs in ES cells, we performed a compendium analysis using public data sets of various DBP occupancies, histone modification signatures, chromatin accessibility, and DNA methylations. Based on the target co-occupancies of 105 DBPs, we first defined four major classes (Core, PRC, Myc, and CTCF) and two minor classes (p53 and Rest) of DBPs. Each class of DBPs binds to the distinct cis-regulatory elements marked by distinct histone modifications. Moreover, binding sites for each of these DBP classes are different in their distribution of distance from their target genes, reflecting their unique roles in transcriptional regulations. Interestingly, we find CpG island (CGI) context in promoters renders two distinctive global regulatory modes in ES cells. Myc and PRC class DBPs reciprocally regulate CGI-containing genes in proximal promoter regions, while have little effects on CGI-less genes. We also show that this CGI-dependent transcriptional regulatory mechanism is tightly linked to the divergence in global gene expression patterns, 'generally on' and 'fluctuation' of CGI-containing gene activities or 'by-default silence' and 'tissue specific activation' of CGI-less genes. We also found that co-occupancy of Core class DBPs selectively defines active enhancers in ES cells. Although Core class DBPs bind to the enhancers of both CGI-containing and CGI-less genes, these play more critical roles in tissue-specific gene expression of CGI-less genes than CGI-containing genes. Moreover, we show that similar to Core class DBPs in ES cells, each cell type has its own enhancer-binding master regulators, which confers the cell type specific functionality by activation of tissue specific CGI-less gene expressions. Taken together, our analyses provide novel insights into previously unknown CGI dependent global gene regulatory modes in ES cells - general regulation of CGI-containing promoters by Myc and PRC classes, and tissue-specific regulation of CGI-less genes by enhancer binding Core class DBPs.

F-2056
IN UTERO INJECTION OF EMBRYONIC STEM CELLS TO IDENTIFY THE FETAL STEM CELL NICHE

Bertin, Enrica[1], Piccoli, Martina[2], Franzin, Chiara[2], Spiro, Giovanna[2], Braghetta, Paola[3], Donà, Silvia[3], Dedja, Arben[4], Schiavi, Francesca[5], Bonaldo, Paolo[3], De Coppi, Paolo[6], Pozzobon, Michela[2]
[1]Department of Woman and Child Health, University of Padova, Padova, Italy, [2]Fondazione Istituto di Ricerca Pediatrica Città della Speranza, Padova, Italy, [3]Department of Histology, Microbiology, and Medical Biotechnologies, University of Padova, Padova, Italy, [4]Department of Vascular and Cardiothoracic Science, University of Padova, Padova, Italy, [5]Familial Cancer Clinic and Oncoendocrinology, Veneto Institute of Oncology, Padova, Italy, [6]UCL Institute of Child Health, London, United Kingdom

Background: The stem cell niche is the microenvironment where stem cells reside. Different elements define the niche and regulate stem cell characteristics, like stromal support cells, gap junctions, soluble factors, extracellular matrix proteins, blood vessels, and neural inputs. Stem cells have been identified in human and murine amniotic fluid; both cell type are selected for the marker c-Kit and are called amniotic fluid stem (AFS) cells. AFS cells are characterized by the expression of pluripotency markers and differentiate in culture into all the three embryonic lineages. Aim: The aim of this study was to investigate the role of amnion (AM) and amniotic fluid (AF) as stem cell niche. Methods: Through in utero transplantation (IUT), we injected YFP+ mouse embryonic stem cells (YFP+ ESC) into the AF of E13.5 wild type fetuses (C57BL6/J), and 4 days after IUT we evaluated their pluripotency by immunofluorescence (IF), qRT-PCR, single cells multiplex PCR and teratoma assay. We assessed the proliferative and apoptotic status by EdU and TUNEL assay. We analysed the foetuses using haematoxylin and eosin staining, IF and PCR for detection of YFP. We performed ELISA assay for detection of stem cell factor (SCF), vascular-endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and insulin growth factor (IGF-1) in the AF both before and after YFP+ ESC injection Results and Conclusion: YFP+ ESC injected through IUT didn't integrate into foetuses. Only few YFP+ ESC resulted EdU+ in the AF and AM, whereas no apoptotic cells where identified. YFP+ ESC isolated from the AF and the AM at E17.5 maintained the expression of pluripotency markers (Oct4, Sox2, Nanog, c-Myc, Klf4) both at molecular and protein level. YFP+ ESC isolated from the AM were more similar to ESC in culture, in respect to YFP+ ESC isolated from the AF, and only YFP+ ESC isolated from AM were able to form teratoma. Moreover, cytokine analyses and oxygen concentration revealed the presence of fundamental niche characteristic factors in the foetal microenvironment constituted by AF and AM. This is the first indication that ESC may reside in the AM without differentiating, as it occurs in stem cell niches. The interplay between AM and ESC creates the dynamic system necessary for sustaining the undifferentiated state of the cells themselves.

F-2057

CHARACTERIZING THE ROLE OF REX1 IN HUMAN PLURIPOTENT CELLS AND THE FUNCTIONAL DOMAINS ASSOCIATED WITH IT

Bhatia, Sonam[1], Sethi, Mohit[1], Draper, Jonathan S.[2]
[1]McMaster University, Hamilton, ON, Canada, [2]McMaster SCCRI, Hamilton, ON, Canada

REX1 (Reduced Expression 1, ZFP42) is a pluripotency associated transcription factor that is often used as a marker for characterizing naïve pluripotent cells or fully reprogrammed pluripotent cells. The role of REX1 and it's involvement in pluripotent cells is unclear, as in murine embryonic stem cells (ESCs) Rex1 null cells are fully pluripotent in their abilities to form chimeras. We have previously characterized the expression of REX1 in human ESCs (hESCs) and have shown that REX1 subdivides the human pluripotent compartment into stable REX1+ and REX1- pluripotent cells, which are not mutually inter-convertible. This was in contrast to the mouse Rex1 expression where the Rex1+ and Rex1- cells exist in a state of metastable equilibrium. This contrast in regulation of murine and human Rex1 might stem from a difference in the role of Rex1 in the two species. In order to address this hypothesis we are investigating the putative roles of REX1 in maintaining or regulating pluripotency in hESCs. We have created hESC lines stably expressing N-terminus fluorescent and C- and N-terminus 3XFlag tagged versions of REX1 under the expression of a conditional doxycycline inducible system. The tagged REX1 expressing lines will be used to investigate the function of REX1 in maintaining self-renewal and differentiation capabilities of hESCs. The tagged versions of the protein will be used in techniques such as co-immunoprecipitation, and chromatin-immunoprecipitation (ChIP) to reveal it's functional associations. Some preliminary data suggests the involvement of REX1 in regulating the cell division/cycle in pluripotent stem cells. Additionally, we have created REX1 truncation mutants based on evolutionary conserved domains to reveal the regions most important for mediating its functions.

F-2058

DEPROGRAMMING REPROGRAMMING: UNCOVERING MECHANISMS AND INDUCERS OF PLURIPOTENCY VIA COMBINATORIAL ANTIBODY SCREENING

Blanchard, Joel W., Xie, Jia, Lerner, Richard A., Baldwin, Kristin K.
The Scripps Research Institute, La Jolla, CA, USA

Development and differentiation proceed by a cascade of contingent events initiated by cell surface signaling. These cell surface events result in signal transduction and ultimately epigenetic modification of the genome. Recently, much progress has been made by using combinations of transcription factors or microRNAs to reprogram cells into induced pluripotent stem cells (iPSCs). Thus, we know much about ways to regulate fates by interceding in downstream signaling that primarily acts directly on the genome. However, little attention has been paid to the possibility of initiating reprogramming at the cell surface. We hypothesize that enhancing or replacing nuclear reprogramming factors with antibody based perturbations of cell surface signaling cascades will reveal previously unknown routes to reprogramming, and produce iPSCs with less unwanted heterogeneity than exists in current lines. To test this hypothesis developed a lentiviral antibody screening platform that enables rapid and efficient screening of greater than 10^9 unique antibodies that can act as agonists, antagonists or blockers. Antibody targets can rapidly be identified upon discovering a phenotype by extracting the antibody genotype, purifying the target via immunopercipitation, and subsequent sequencing by mass spectrometry. To demonstrate these methods can be employed as an unbiased approach for uncovering cell surfaces mechanisms and signaling cascades that initiate reprogramming, we first performed combinatorial screens to replace Sox2, Oct4 and Klf4 during the reprogramming of mouse fibroblasts to pluripotency. After screening more than 100 million antibodies targeted to extracellular domains we identified more than 150 hit antibodies that can substitute for each of the factors during reprogramming to pluripotency. Induced pluripotent cells derived using the identified antibodies appropriately express all characteristic stem cell markers, can be directed to differentiate *in vitro*, contribute to the inner cell mass of a blastocyst *in vivo* and yield live chimeric offspring. These results demonstrate that perturbing cell surface signaling events can reprogram somatic cells to pluripotency in the absence of individual reprogramming factors. We are currently identifying and verifying the targets and mechanisms of action of antibodies as well as combining focused libraries for each factor to develop methods for complete antibody reprogramming.

F-2059

BCL3 BRIDGES LIF-STAT3 SIGNALING TO OCT4 TRANSCRIPTION AND DNA BINDING ACTIVITY IN THE MAINTENANCE OF NAÏVE PLURIPOTENCY

Chen, Chen-Yun[1], Lee, Desy S[2], Yan, Yu-Ting[3], Shen, Chia-Ning[4], Hwang, Shiaw-Min[5], Lee, Sho Tone[3], Hsieh, Patrick C.H.[3]
[1]Program in Molecular Medicine, National Yang-Ming University and Academia Sinica, Taipei, Taiwan, [2]Institute of Clinical Medicine, National Cheng Kung University and Hospital, Tainan, Taiwan, [3]Institute of Biomedical Science, Academia Sinica, Taipei, Taiwan, [4]Genomics Research Center, Academia Sinica, Taipei, Taiwan, [5]Bioresource Collection and Research Ctr, Hsinchu, Taiwan

Leukemia inhibitory factor (LIF) regulates mouse embryonic stem cell (mESC) pluripotency through STAT3 activation, but the downstream signaling remains largely unelucidated. Using cDNA microarrays, first we verified B cell leukemia/lymphoma 3 (Bcl3) as the most significantly downregulated factor following LIF withdrawal in mESCs. Using chromatin-immunoprecipitation of STAT3, Bcl3 was found to be a direct target of LIF-STAT3 signaling. Further, Bcl3 knockdown altered mESC morphology and reduced expression of pluripotency genes including Oct4. Conversely, Bcl3 overexpression promoted Oct4 and Nanog promoter activities. Bcl3 was also found to regulate Oct4 transcription through association with Oct4 and beta-catenin, and through regulation of DNA binding of Oct4, acetylated histone 3, and RNA polymerase II on the Oct4 promoter. These results establish Bcl3 as a direct target of the LIF-STAT3 pathway for regulating Oct4 transcription and DNA binding, and thus shed light on the mechanism of Bcl3 as a downstream molecule of LIF/STAT3 signalling in pluripotency maintenance.

F-2060

THE IGF2BP FAMILY OF RNA BINDING PROTEINS REGULATE AN RNA NETWORK IN PLURIPOTENT STEM CELLS TO MAINTAIN CELL SURVIVAL

Conway, Anne E.[1], Wilbert, Melissa L.[1], Jones, Leanne[2], Yeo, Gene[1]
[1]Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA, USA, [2]University of California, Los Angeles, CA, USA

The regulation of gene expression by transcription factors in human pluripotent stem cells (hPSCs) has been extensively studied; however, regulation of the transcriptome by RNA-binding proteins (RBPs) is equally important, but not well understood. Here, we have characterized a family of highly conserved RNA-binding proteins, the insulin-like growth factor 2 mRNA binding proteins (IGF2BPs) that are expressed in human pluripotent stem cells and appear to regulate many pathways

including cell survival networks. IGF2BP1 (CRD-BP/ IMP1), IGF2BP2 and IGF2BP3 are widely expressed during embryogenesis; however, expression is restricted to the germ line postnatally. Interestingly, IGF2BPs often are found upregulated in a number of cancers. To characterize the mechanisms by which IGF2BP1 directly regulates its target RNAs in hESCs, we performed CLIP-seq (Cross-linking and immunoprecipitation followed by high throughput sequencing) and RNA-seq in H9 hESCs. The results of CLIP-seq revealed that IGF2BP1 bound the majority of its putative targets within coding sequences or 3'UTRs. However, IGF2BP1 also bound intronic sequences, adding insight into its proposed co-transcription role in controlling RNA processing. Depletion of IGF2BP1 in these cells using shRNA resulted in cell death, smaller colonies and smaller Embryoid bodies (EBs) from differentiation of these cells. Interestingly, cells in which IGF2BP1 levels were reduced retained pluripotency, as assayed by marker expression. These cells did exhibit a significant up-regulation of pro-apoptotic factors and loss of cell adhesion markers, as well as disruption of the cytoskeleton. Based on these data, we propose that IGF2BP1 acts to promote cell survival by maintaining the stability and localization of its target RNAs.

F-2061
REPROGRAMMING BY PLURIPOTENT CYTOPLASM

Daughtry, Brittany Landis[1], Kang, Eunju[2], Ma, Hong[2], Mitalipov, Shoukhrat M.[2]
[1]Cell and Developmental Biology, Oregon Health and Science University, Portland, OR, USA, [2]Division of Reproductive and Developmental Sciences, Oregon National Primate Research Center, Oregon Health and Science University, Beaverton, OR, USA

Somatic cell nuclei can be reprogrammed to pluripotency in hybrids generated by whole cell fusion with embryonic stem cells (ESCs). However, the tetraploid nature of resultant hybrid cells limits their applications in regenerative medicine. It remains unclear if ESC nuclear material is essential for reprogramming in a hybrid system. To investigate reprogramming activity of isolated ESC cytoplasm, we enucleated mouse ESCs by gradient centrifugation and subsequently fused with fetal fibroblast (FF) nuclei carrying Oct4-GFP. Resulting cytoplasmic hybrids or "cybrids" contained exclusively FF nuclei but mixture of FF and ESC cytoplasm. Re-expression of GFP was evident in tetraploid ESC-FF hybrids but not in cybrids indicating lack of reprogramming. We measured mitochondrial genome (mtDNA) heteroplasmy as indicator of cytoplasmic mixture and determined that hybrids contain greater than 69% of ESC mtDNA while cybrids comprise less that 22% of ESC mtDNA. We conclude that generation of cybrid cells containing ESC cytoplasm and somatic cell nuclei is feasible and may serve as a model for studying cytoplasmic reprogramming in pluripotent cells. Future studies will determine if increase in ESC cytoplasmic component in cybrids can enhance reprogramming.

F-2062
REGULATION OF HUMAN EMBRYONIC STEM CELL PLURIPOTENCY AND SELF-RENEWAL BY A NOVEL RNA BINDING PROTEIN L1TD1

Emani, Maheswara Reddy[1], Närvä, Elisa[1], Rahkonen, Nelly[1], Nousiainen, Kari[2], Viitala, Miro[1], Lähdesmäki, Harri[2], Lund, Riikka Johanna[1], Lahesmaa, Riitta[1]
[1]Turku Centre for Biotechnology, University of Turku and Abo Akademi University, Turku, Finland, [2]Department of Information and Computer Science, Aalto University, Espoo, Helsinki, Finland

Human pluripotent stem cells (hPCSs), including embryonic and induced pluripotent stem cells (hESCs and hiPSCs), offer a wide range of opportunities for cell therapy and disease modeling. The detailed understanding of the molecular mechanisms regulating the stem cell status is still elusive. We have identified a novel RNA binding protein, L1TD1, with previously unknown function that is highly expressed in hESCs and iPSC, but not in normal somatic cells. Here we demonstrate that L1TD1 regulates self-renewal and is a novel marker for hESCs. L1TD1 is highly expressed in hESCs and rapidly down-regulated during differentiation. Moreover, our results show that silencing of L1TD1 induces downregulation of known regulators of pluripotency OCT4, NANOG and SOX2 leading to differentiation of the cells. L1TD1 is also highly expressed in testicular and colorectal cancers. We have further demonstrated that L1TD1 interacts indirectly (via RNA) with LIN28 and directly with RNA helicase A (RHA), and is required for hESC self-renewal and cancer cell proliferation. We hypothesize that L1TD1, in complex with RHA-LIN28, regulates OCT4 translation in hESCs and plays an important role in maintaining hESCs pluripotency and self-renewal

F-2063
THE CIRCADIAN CLOCK IN PLURIPOTENT STEM CELLS AND DIFFERENTIATION

Evantal, Naveh, Schyr, Rachel, Meshorer, Eran, Kadener, Sebastian
The Hebrew University of Jerusalem Institute of Life Sciences, Jerusalem, Israel

Circadian clocks keep temporal order and help organisms to anticipate daily changes in the environment. In mammals, most, if not all, differentiated cell types possess a circadian clock, with over 10% of their transcriptome changing throughout the day. This is achieved through a network of activators and repressors that form interconnected feedback loops. Cells likely acquire circadian rhythms only upon differentiation, since the only cell systems in which circadian rhythms have not been found are embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). Thus, pluripotent stem cells either cannot sustain a circadian clock, or the clock they possess neither depends nor causes transcriptional oscillations. The main aim of this work is to decipher the molecular and cellular mechanisms behind the generation of the circadian clock. First, in order to determine whether ESCs express circadian clock components, we profiled their transcriptome before and upon differentiation. Interestingly, all central clock transcriptional components are expressed in ES cells and change upon differentiation. We found that ES cells express the two master regulators of the circadian system, Clock and Bmal1, also at the protein level. Moreover, as in differentiated cells, CLOCK and BMAL1 proteins localize both to the nucleus and cytoplasm in ESCs. These results strongly suggest that although most components are present in ESCs, they are either not enough to support circadian rhythms, or they have other functions in pluripotent cells. In order to determine the transcriptional targets of CLOCK-BMAL in ESCs, we performed ChIP-seq. In order to complement these experiments we have also generated two ESC cell lines in which we have engineered transgenes that can lead to conditional expression (tet-inducible) of wild type or dominant negative variants of CLOCK. Our results strongly suggest a role for CLOCK in ESCs.

F-2065

CANINE YOLK SAC CELLS TRANSDUCED WITH VEGF CAN ENRICH PROGENITOR ENDOTHELIAL CELLS FOR VESSEL FORMATION

Fratini, Paula[1], Alcântara, Dayane[1], de Oliveira e Silva, Fernanda Menezes[1], Carreira, Ana Claudia Oliveira[2], Rodrigues, Marcio Nogueira[1], Miglino, Maria Angelica[1]

[1]Surgery, School of Veterinary Medicine and Animal Science. University of São Paulo, Sao Paulo, Brazil, [2]Institute of Chemistry, University of Sao Paulo, Sao Paulo, Brazil, Sao Paulo, Brazil

The yolk sac (YS) is a source of endothelial precursor cells. However, data about the full differentiation potential of YS cells and their suitability for cell therapies are rare once that therapies where neovascularization is required seems to be of great value. We characterized canine YS cells through expression of vascular endothelial growth factor (VEGF) markers in flow cytometry, immunocytochemistry and real time PCR, and analyzed their potential to enrich progenitor endothelial cells after transduction with VEGF. Immunocytochemistry showed expression for CD105, PCNA, VEGFA and vWF, flow cytometry for CD105, VEGF, PCNA, OCT-4 and qPCR for VEGF, CD31, CD105, PCNA and FLT - 1. After transduction with PLV-VEGF lentivirus, the undifferentiated cells changed their morphology into endothelial-like cells similar to those occurring near developing capillaries. In conclusion, treatment with VEGF resulted as an appropriate method to enrich endothelial cells derived from isolated canine YS cells, suggesting that these cells represents a promising source that could lead to quantitative amounts of progenitor endothelial cells for regenerative cell therapies that require neovascularization.

F-2066

BREAKING DOWN PLURIPOTENCY IN THE PORCINE EMBRYO REVEALS A PREMATURE AND RETICENT STEM CELL STATE IN THE INNER CELL MASS AND UNIQUE EXPRESSION PROFILES OF THE NAÏVE AND PRIMED STEM CELL STATES.

Hall, Vanessa, Hyttel, Poul

Copenhagen University, Fredriksberg C, Denmark

To date, it has been difficult to establish *bona fide* porcine embryonic stem cells (pESC) and stable induced pluripotent stem cells. Reasons for this remain unclear, but may depend on inappropriate culture conditions. Here, we report the most insight to date on genes and proteins expressed in the pluripotent cell populations of the porcine embryo, namely the inner cell mass (ICM), the epiblast (EPI) and the embryonic disc (ED). We performed gene expression analyses using RT-PCR on four embryonic stages (in-vivo produced embryos) including pooled day 5/6 blastocysts containing the ICM, pooled day 7/8 blastocysts containing the late ICM, pooled day 9/10 isolated EPIs and pooled day 11/12 isolated EDs to identify changes in expression of genes identified with pluripotency and regulation of cell renewal. Genes studied include, *OCT4a, NANOG, SOX2, KLF4, cMYC, REX1, NROB1, KLF2, FGFR1, FGFR2, MEK, cFOS, LIFR, GP130, JAK1, STAT3, BMPr1a, SMAD1, SMAD2, SMAD3, SMAD4, SMAD5, SFRP1, GSK3,* and *CRIPTO*. Immunocytochemistry was also performed in the ICM, EPI and ED for LIFR, REX1, NROB1, CRIPTO, pSMAD2, cMYC, KLF4, SSEA3, SSEA4, TRA-1-60 and TRA-1-81. Finally, pluripotent cells isolated from day 5 (containing the ICM) and day 10 (containing the epiblast) porcine in-vivo derived embryos were cultured in embryonic stem cell media in the presence of varying inhibitors blocking either the MEK, JAK/STAT, NODAL, WNT and BMP pathways to identify which pathways are essential for maintaining pESC self renewal. This study revealed that the early porcine ICM represents a premature state of pluripotency due to lack of translation of many key pluripotent proteins, and the late ICM enters a transient, reticent pluripotent state that lacks expression of most genes associated with pluripotency. We describe a unique expression profile of the porcine epiblast, which represents the naïve stem cell state. Here expression of *OCT4, NANOG,* CRIPTO and SSEA1 was observed and weak expression of NrOB1 and REX1. Only a few genes involved in stem cell renewal in other species was detected in the pig epiblast. The porcine ED, reflecting the primed stem cell state, can be characterized by the expression of *OCT4, NANOG, SOX2, KLF4, cMYC,* REX1, CRIPTO, and *KLF2*. The cell culture experiments containing inhibitors revealed the importance of the FGF, JAK/STAT, and BMP pathways for maintaining cell proliferation of pESCs in-vitro. Together this research provides new insight into the regulation of pluripotency, revealing unique stem cell states in the different porcine stem cell populations derived from the early developing embryo.

F-2067

EPIGENETIC PROFILING OF HUMAN EMBRYONIC STEM CELLS AT ENDOGENOUS OXYGEN TENSION

Heravi-Moussavi, Alireza[1], Raghavan, Karthika[2], Bilenky, Misha[1], Carles, Annaick[2], Moore, Richard[1], Mungall, Andy[1], Jones, Steven[1], Marra, Marco A.[1], LaRoque, Nick[3], Fisher, Susan[3], Costello, Joseph F.[4], Hirst, Martin J.[5]

[1]Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada, [2]Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada, [3]University of California San Francisco, San Francisco, CA, USA, [4]Department of Neurosurgery, University of California San Francisco, San Francisco, CA, USA, [5]University of British Columbia and Canada's Michael Smith Genome Sciences Centre, Vancouver, BC, Canada

The majority of molecular and phenotypic descriptions of human embryonic stem cells (hESC) are derived from cells cultured under atmospheric oxygen tension (20%). However, cells of the blastocyst inner cell mass are exposed to a significantly lower oxygen environment. This study investigates the impact of culturing hESC under reduced oxygen tension (8%) as compared with the atmospheric level using RNA-Seq, ChIP-seq and whole genome bisulfite sequencing datasets. The global CpG methylation levels across the samples showed reduced intermediate methylation for cells cultured under 8% oxygen tension. Differentially methylated regions (DMRs) were generally hypomethylated in lower oxygen tension all chromosomes except chromosome X which displayed a more balanced hypo-/hypermethylated profile. Nearest gene analysis of oxygen tension DMRs showed a statistically significant enrichment in pluripotency associated genes suggesting that these regions are contributing to the well-documented increase in pluripotency as measured by established phenotypic markers. Using ChromHMM derived chromatin states, we studied the relationship between histone modification patterns and CpG methylation. A "transcribed flank" state, which showed both H3K4me1 and H3K4me3 enrichments, a "bivalent flank" state with an enrichment for H3K4me1, H3K4me3, and H3K27me3, and "bivalent enhancer" state, enriched for H3K4me1 and H3K27me3, showed lower levels of methylation in the cells cultured under 8% oxygen tension as compared with 20% ($P<0.001$). To identify oxygen tension dependent gene isoform expression changes, we used an alignment-free isoform quantification algorithm, Sailfish , to annotated transcript isoforms, and DESeq to infer isoform differential expression. Isoform analysis showed an enrichment in genes associated with differentiation and system development in the 20% sample suggesting a reduced pluripotent state in the cells cultured under atmospheric oxygen tension. Hypoxia signaling was among the significant canonical

pathways for the 8% sample in concordance with the hypoxic culture conditions. In summary, the results demonstrated that culturing under reduced oxygen tension impacted the epigenetic state of hypoxia and pluripotency associated genes in human embryonic stem cells.

F-2068
IDENTIFICATION OF MEMBRANE PROTEINS THAT ARE ESSENTIAL FOR PLURIPOTENCY MAINTENANCE

Kuo, Hung-Chih, **Hsu, Wei-Ting**
Academia Sinica, Taipei, Taiwan

Membrane proteins play critical roles in many cellular functions, such as cell signaling and cell-cell communication. Additionally, membrane proteins can serve as cell surface markers for stem cell characterization and purification. Quantitative membrane proteomic approaches will provide an in-depth view of the stage- and lineage-specific expression, which potentially can enhance our understanding on the underlying mechanisms of stem cell differentiation. However, the analysis of membrane proteins is experimentally challenging due to their hydrophobic nature and low abundance. Here, we employed gel-assisted digestion-based quantification approaches for quantitative membrane proteomics between human embryonic stem cells (hESCs) and their differentiated derivatives (hESC-dd). Proteomics results were validated by western blot and immunecytochemical staining. The functions of candidate membrane proteins were studied using shRNA-mediated knockdown. Combining membrane proteomic analysis, bioinformatic analysis and experimental validation, we confirmed a panel of membrane proteins whose expression level is significantly higher in hESCs than hESC-dd. Furthermore, the disruption of the RNA expression of the genes encoding the identified hESC-enriched membrane proteins by transfecting hESCs with lentivirus encoding short-hairpin RNA resulted in the lost of pluripotency in hESCs. Together, we have identified hESC-enriched membrane proteins with functional roles in pluripotency maintenance and provide novel insights into the mechanism by which the surface-to-cytoplasm/nucleus signaling regulate the pluripotency maintenance in hESCs.

F-2069
INO80 PROMOTER OCCUPANCY FACILITATES ACTIVATION OF PLURIPOTENCY GENES IN EMBRYONIC STEM CELL SELF-RENEWAL, REPROGRAMMING, AND BLASTOCYST DEVELOPMENT

Hu, Guang, Li, Wang
NIEHS/NIH, RTP, NC, USA

The master transcription factors play integral roles in the pluripotency transcription circuitry of embryonic stem cells (ESCs). How they selectively activate expression of the pluripotency network while simultaneously repressing genes involved in differentiation is not fully understood. Here we define a requirement for the INO80 complex, a SWI/SNF family chromatin remodeler, in ESC self-renewal, somatic cell reprogramming, and blastocyst development. We show that Ino80, the chromatin remodeling ATPase, co-occupies pluripotency gene promoters with the master transcription factors, and its occupancy is dependent on Oct4 and Wdr5. At the pluripotency genes, Ino80 maintains open chromatin architecture and licenses recruitment of Mediator and RNA Polymerase II for gene activation. Our data reveal an essential role for INO80 in the expression of the pluripotency network, and illustrate the coordination among chromatin remodeler, transcription factor, and histone modifying enzyme in the regulation of the pluripotent state.

REGENERATION MECHANISMS

F-2071
NETRIN-1 INDUCES MMP-12-DEPENDENT E-CADHERIN DEGRADATION VIA DISTINCT ACTIVATION OF PKC-ALPHA AND FAK/FYN IN PROMOTING MESENCHYMAL STEM CELL MOTILITY

Lee, Sei-Jung, Jung, Young Hyun, Lee, Hyun Jik, Jeon, Jihoon, Lee, Ki Hoon, **Han, Ho Jae**
Department of Veterinary Physiology, BK21 PLUS Creative Veterinary Research Center, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

Netrin-1 (Ntn-1) is a potent inducer of neuronal cell migration; however their physiological mechanism in guiding the migratory behavior of stems cells has not been characterized. In present study, we investigated the role of Ntn-1 in promoting motility of human umbilical cord blood derived mesenchymal stem cells (UCB-MSCs) and its related signaling pathways. Ntn-1 (50 ng/mL) significantly increased UCB-MSCs motility after a 24 h incubation, which was inhibited by blocking antibodies for deleted in colorectal cancer (DCC) and integrin (IN) α6β4. Ntn-1 in acting DCC stimulated PKCα activation, but not PKCε, PKCθ, and PKCζ, while Ntn-1 in acting INα6β4 induced phosphorylation of focal adhesion kinase (FAK) and Fyn. Notably, Ntn-1 induced phosphorylation of extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), nuclear factor kappa-B (NF-κB), but they were concurrently down-regulated by blocking the activities of PKCα, FAK, and Fyn. Ntn-1 uniquely increased MMP-12 expression of all the matrix metalloproteinase (MMP) isoforms presented in UCB-MSCs, which was significantly blocked by NF-κB inhibitor. Finally, Ntn-1 induced MMP-12-dependent degradation of E-cadherin (E-cad) in promoting UCB-MSCs motility, where Ntn-1 abrogated E-cad and p120-catenin interaction. In addition, Ntn-1 has ability to stimulate cytoskeletal reorganization-related proteins, such as Cdc42, Rac1, profilin-1, cofilin-1, α-actinin-4, and filamentous actin (F-actin) in UCB-MSCs. These results demonstrate that Ntn-1 induced MMP-12-dependent E-cad degradation via distinct activation of PKCα and FAK/Fyn that are necessary for governing the activation of ERK, JNK, and NF-κB in promoting UCB-MSCs motility.

F-2072
BONE MARROW STEM CELL VITALITY AND COUNT DECREASES WITH ELEVATED ALUMINUM LEVELS

Matzner, Michael Paul[1], Shaw, Chris[2], Morrice, Jess[2], Kobinia, George Sebastian[3]
[1]Vienna Medical School, Dep of Orthopaedics, Vienna Stem Cell Group, Vienna, Austria, [2]University of British Columbia, Vancouver, BC, Canada, [3]Vienna Stem Cell Group, Vienna, Austria

Background: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal disease of the motoneurons. Recent studies suggest genetic and environmental mechanisms, but the principal causes still remain undiscovered. Study groups found high aluminum (Al) levels in blood and urine of ALS patients as well as in cerebrospinal fluid (CSF). At this stage it is commonly agreed that Al elevation may lead to neurotoxicity involved in the pathogenesis of ALS. Materials and Methods: We analyzed the CSF of consecutive 72 no-option patients with ALS before autologous stem cell therapy (SCT). Al-levels in CSF samples were analyzed. Stem cells were retrieved using bone marrow aspirate as an autologous source from the iliac crest. Samples were taken immediately after puncture. Results: In all patients we found at least a trance of aluminum (range

from 1 to 87,7 µg/l) with a mean of 13,09±16,4 µg/l. Two groups of patients were compared with a cut-off point in 10 µg/l using t-test. Patients with higher levels of Al were significantly younger (p=0,01) and were found to have a higher level of leucocytes in whole bone marrow aspirate (p= 0,016). There was a tendency seen for less vitality in stem cells after spin dry (p=0,059) than patients with Al-levels under 10 µg/l. These patients also showed lower levels of stem cells in whole bone marrow (p=0,097 linear model). The results support previous hypothesis of metal neurotoxicity might be contributing factor for ALS that is directly related to the outcome. Results suggest that high levels of Al in CSF are directly related to bone marrow and stem cell toxicity interfering with optimal results of the SCT. Conlcusion: Further studies will have to be conducted to unveal more information on metal ion induced toxicity on bone marrow stem cells as well as degenerative diseases such as neurodegenerative ones. According to these results lowering of Al-levels could be beneficial in treating those diseases.

F-2073
IN VIVO IMAGING OF ADULT SKELETAL STEM CELLS EXPRESSING PRX1 IN THE MURINE CALVARIA

Mortensen, Luke J.[1], Runnels, Judith M.[2], Wilk, Katarzyna[3], Lin, Charles[4], Intini, Giuseppe[5]

[1]Harvard School of Dental Medicine and Massachusetts General Hospital, Boston, MA, USA, [2]Massachusetts General Hospital, Boston, MA, USA, [3]Harvard School of Dental Medicine, Boston, MA, USA, [4]Massachusetts General Hospital, Harvard Stem Cell Institute, Boston, MA, USA, [5]Harvard School of Dental Medicine, Harvard Stem Cell Institute, Boston, MA, USA

Skeletal development and regeneration is vibrant and nuanced process important for mechanical support and the maintenance and protection of some of the most important physiological processes. A primary component that is thought to influence the microenvironment and structure of the skeletal system, but whose in vivo identity and behavior has not been fully characterized, is the skeletal stem cell. The transcription factor Prx1 is expressed in high quantities during skeletogenesis and has been suggested to be important in bone and cartilage development. Recent studies have indicated an important role for skeletal cells of a Prx1-lineage in bone regeneration and bone marrow microenvironment maintenance. However, the presence in the intramembranous calvarial bone and the role of an adult cell population actively expressing this transcription factor has not been reported. We hypothesize that Prx1 may be a marker for an adult skeletal stem cell population in the intramembranous bone. To address this hypothesis, we use the Prx1-CreER-EGFP transgenic mouse to label Prx1+ cells with EGFP and cross it with a floxed-tdTomato reporter mouse to label their progeny with tdTomato. To allow in vivo evaluation of cell location and functional bone regeneration response, we have developed a novel 2-photon microscope for intravital bone imaging and femtosecond laser ablation to precisely generate bone defects and dynamically monitor cellular response. We first determined intramembranous cell localization in adult mice, and found that Prx1+ cells are located in the non-fused craniofacial sutures of the mouse. Prx1 expressing cells isolated from the sutures exhibit mRNA expression of stem cell markers and skeletal precursor genes, and have in vitro single cell clonogenic capacity. Young 4 week mice have large quantities of Prx1+ cells, and cell number gradually decreases with age up to 32 weeks. We next investigated the function of these cells and their progeny in the repair of micro-defects created with a femtosecond ablation laser. Prx1+ cells and their progeny are found in the defect after 5-10 days after ablation, with evidence of the progeny providing newly formed bone 30 days later. These results could potentially have implications in understanding the pathogenesis of craniofacial deformations such as craniosynostosis.

F-2074
ARACHIDONIC ACID PROMOTES THE CUTANEOUS WOUND HEALING EFFECT OF HUMAN UMBILICAL CORD BLOOD DERIVED MESENCHYMAL STEM CELLS THROUGH MAMMALIAN TARGET OF RAPAMYCIN COMPLEX 2-DEPENDENT MATRIX METALLOPROTEINASE-16 ACTIVATION

Oh, Sang Yub, Lee, Sei-Jung, Suh, Han Na, Kim, Mi Ok, Kim, Dah Ihm, Song, Eun Ju, Han, Ho Jae

Department of Veterinary Physiology, BK21 PLUS Creative Veterinary Research Center, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

Among microenvironmental factors released from wound sites, arachidonic acid (AA) is major form of polyunsaturated fatty acids (PUFA) and plays important roles in stem cell migration. However, clinical potential as well as functional roles of AA in human umbilical cord blood derived mesenchymal stem cells (hUCB-MSCs) is not fully elucidated. We first tried to characterize the effect of AA-activated hUCB-MSCs in mouse excisional wound splinting model. In contrast to hUCB-MSCs alone, AA-activated hUCB-MSCs enhanced the re-epithelization accompanied by activation of mammalian target of rapamycin complex 2 (mTORC2) and matrix metalloproteinase 16 (MMP-16) at the cutaneous wound site. We further examined the mechanism of AA-induced wound healing hUCB-MSCs. AA (10^{-5}M) promoted hUCB-MSCs migration via mTORC2 phosphorylation which was attenuated by lipoxygenase (LOX) inhibitor. mTORC2 activation triggered by AA induced phosphorylation of Akt Ser^{473} and protein kinase C (PKC). Subsequently AA uniquely stimulated phosphorylation of c-Jun NH(2)-terminal kinase (JNK) and p38 mitogen activated protein kinase (MAPK) but not extracellular signal-regulated kinase (ERK). In addition we found that phosphorylation of MAPK and Akt induced by AA distinctively activated the ubiquitous transcription factor Sp1 that controls gene expression involved in stem cell behavior. Interestingly AA-mediated Sp1 activation resulted in the increased mRNA level of *MMP-16* among *MMP-1, -2, -11, -12, -14, -16, -17* in hUCB-MSCs. Furthermore AA stimulated MMP-16-mediated degradation of fibronectin but not collagens and laminins through Sp1 activation. Finally, inhibition of MMP-16 as well as LOX, mTORC2, PKC, Akt, MAPK and Sp1 abolished migration of hUCB-MSCs. These data suggest that AA-induced mTORC2 activation triggers MMP-16-dependent FN degradation in promoting hUCB-MSCs migration, which is necessary for enhanced wound healing effect of AA.

F-2075
TRANSPLANTATION OF NEURAL STEM CELLS AND THEIR DERIVATIVES IN AN INJECTABLE HYDROGEL TO PROMOTE CELL SURVIVAL AND INTEGRATION FOLLOWING STROKE

Payne, Samantha L.[1], Cooke, Michael[2], Morshead, Cindi M.[3], Shoichet, Molly S.[4]

[1]Chemical Engineering and Applied Chemistry; Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [2]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [3]Institute of Medical Science, University of Toronto, Toronto, ON, Canada, [4]Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON, Canada

Stroke is the leading cause of neurological disability in Canada, with

up to two-thirds of stroke survivors experiencing a loss of function that significantly impacts their quality of life. Currently, the only FDA-approved therapy for ischemic stroke is administration of the thrombolytic agent, tissue plasminogen activator (tPA), which does not regenerate lost cells. Delivery of neuralstem cells, while a promising approach to replace lost neurons and glia, results in poor cell survival and minimal integration into the host tissue. To enhance cell survival, integration, and ultimately functional recovery, we have developed an injectable cell delivery system, comprised of hyaluronic acid and methylcellulose (HAMC). The shear-thinning and inverse thermal gelling properties of HAMC allow it to be injected through a fine gauge needle into the stroke-injured brain. Neural stem/progenitor cells (NSPCs) were transplanted in an endothelin-1 mouse model of stroke and demonstrated increased survival after 7 days when delivered in HAMC vs. in artificial cerebrospinal fluid (aCSF). Furthermore, animals receiving NSPCs in HAMC had significantly greater functional recovery compared to those receiving NSPCs in aCSF. Interestingly, in previous research, we demonstrated that pre-differentiating NSPCs in vitro towards a neuronal phenotype increases cell survival when delivered in a biomaterial composite to the injured spinal cord. Therefore, to explore the influence of cell maturity on transplanted cell survival and integration, we first developed an in vitro method to drive differentiation of rodent NSPCs to neurons. Cells were cultured in a combination of dibutyryl cyclic adenosine monophosphate (dbcAMP) and interferon-γ (IFN-γ) to yield a neuronal population of 70% βIII-tubulin positive cells, as quantified using immunocytochemsitry. Using magnetic activated cell sorting against neural cell adhesion marker (NCAM) we isolated a population of neuronal precursors derived from rodent NSPCs for transplantation into the stroke-injured rat brain. A deeper understanding of the cell-substrate interactions between grafted cells and the host environment and how this can be influenced by biomaterials and cell maturity will be essential for future delivery strategies. Acknowledgments: We are grateful to NSERC and CIHR for partial funding of this research.

F-2076
THE EFFECT OF TOBACCO SMOKING DURING PREGNANCY ON OXIDATIVE STRESS IN BLOOD CELLS OF CORD BLOOD CELLS

Pereira, Thiago de Melo Costa
Pharmaceutical Sciences Graduate Program (UVV), University of Vila Velha (UVV) and Federal Institute of Education, Science and Technology, Vila Velha, Brazil

Background: Studies have shown that some of genotoxic substances or metabolites in cigarette smoke are able to pass through the placenta impairing the health of the newborn. Furthermore, smoking is known as a promoter in the formation of oxidative damage by facilitating the development of various genetic diseases such as cardiovascular and neoplastic diseases, through mechanisms of genotoxicity. The evaluation of these genotoxic damage can be successfully achieved by measuring oxidative stress parameters, such as reactive oxygen species (ROS) , whose main representatives are the superoxide anion ($\bullet O_2^-$) and hydrogen peroxide (H_2O_2) . Recently, our laboratory have been shown that of ROS production by flow cytometry can help the understanding of many diseases " oxidative stress - dependent ". Therefore, the aim of the study was to evaluate the effect of tobacco smoking during pregnancy on oxidative stress in cord blood-derived mononuclear cells (MNC) by flow cytometry analysis. Methods: The MNC were collected after centrifugation with ficoll-paque of cord blood cells. Oxidative stress was assessed by dihydroethidium (DHE) and 2,7-dichlorofluorescein (DCF) by flow cytometer. For apoptosis detection, MNC were resuspended in Binding Buffer and incubated Annexin V-FITC and propidium iodide (PI). A FACSCanto II cytometer was used for the flow cytometric analysis. Data were acquired and analyzed using BD FACSDiva and FCS Express softwares. Data are mean±SEM. Statistical analysis was performed using Student´s t test. Results: In the group of patients analyzed, there was no difference between parameters such as gestational age and mean arterial pressure. In neonates, parameters such as weight and head circumference also showed no difference. Blood samples also showed no difference in hematocrit . However, flow cytometry analysis showed that smoking during pregnancy impairs the quality of blood mononuclear cells from umbilical cord: we observed an increase in the levels of superoxide anion stained with DHE compared to unexposed pregnant women (2118 ± 253 vs 1017 ± 76 MFI , P <0.05 , respectively) and hydrogen peroxide stained with DCF (651 ± 32 vs 488 ± 21 MFI , P <0.05 , respectively). Moreover, there was an impairment in the rate of cell viability (84 ± 2.9 vs 94 ± 1.6 %, p <0,05 , respectively) , but no difference in the levels of apoptosis (31.4 ± 4.8 vs 25.2 ± 4.6). These data suggest that the practice of smoking in pregnancy can trigger an imbalance between reactive oxygen species, increasing oxidative stress and decreasing the cell viability. Conclusion: The present study demonstrated that exposure to cigarette smoke can impair the functionality and viability of mononuclear cells derived from the umbilical cord of women exposed to smoking. These results by flow cytometry provide new directions and alternative approaches of investigations of the impact of smoking in pregnancy.

F-2077
THE ROLE OF TOLL-LIKE RECEPTORS IN CARDIAC REGENERATION MEDIATED BY HUMAN BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS

Rashedi, Iran[1,2], Wang, Xinghua[2], Viswanathan, Sowmya[2], Radisic, Milica[1], Keating, Armand[1,2]
[1]Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada, [2]Princess Margaret Cancer Centre, University Health Network, Toronto, ON, Canada

Mesenchymal Stromal Cells (MSCs) are promising candidates for cell therapy and tissue regeneration due to several properties including immunomodulatory/anti-inflammatory effects. Human MSCs (hMSCs) exhibit various properties in response to microenvironmental cues. Toll-like receptors (TLRs), known for their role in innate immunity, are involved in cardiac tissue pathology. Several TLRs are highly expressed on hMSCs and can affect their differentiation, migration, immunosuppressive and therapeutic properties. The results of studies on the role of TLRs in cardiac tissue regeneration, however, are limited and inconsistent. We previously showed that the capacity of human MSCs to express cardiac-specific proteins was enhanced in cells cultured on collagen scaffolds (3D) versus grown on culture plates (2D). We also showed that the capacity of hMSCs to undergo cardiac lineage reprogramming was not affected by activation of TLR2-4. Here, we investigated the effect of TLR2-4 on the immune properties of hMSC and remodeling of extra cellular matrix (ECM). We grew non-activated and TLR-activated hMSCs in 2D and 3D cultures as well as with or without co-culture with rat neonatal cardiomyocytes (rCM). We showed differential expression of various cytokines including IL6, IL8, IP10, RANTES and IL10 in hMSCs cultured in 2D versus 3D with reduced cytokine production in hMSCs cultured on collagen scaffolds. In co-culture with human peripheral blood mononuclear cells (PBMCs), 3D-cultured hMSCs did not affect the proliferation of PBMCs even after treatment with TLR4 ligand and interferon gamma whereas 2D-cultured hMSCs inhibited the proliferation of PBMC by more than 50%. The viability of PBMCs was significantly decreased in co-culture with 2D-cultured hMSCs whereas in 3D-cultured hMSCs,

only TLR4-activated hMSCs decreased the viability of PBMCs although not as significantly as their 2D counterparts (15.43 ± 1.7% in 2D vs 9.3 ± 2.6% in 3D, n= 3). The level of cytokines produced by hMSCs was not affected by co-culturing with rCM. We also showed a significant difference in the expression of several fibrosis-related genes in TLR-activated hMSCs including Col3α (M= -6.9, 95% CI [-8.19, -1.03], p< 0.05 for TLR2-activated hMSCs), TNFα (M= -4.613, 95% CI [-12.25, -1.54], p< 0.05 for TLR3-activated hMSCs) and αSMA (M= 0.68, 95% CI [0.34, 1.01], p< 0.01 for TLR2-activated hMSCs; M= 0.55, 95% CI [0.21, 0.88], p< 0.01 for TLR3-activated hMSCs; M= 0.75, 95% CI [0.41, 1.09], p< 0.01 for TLR4-activated hMSCs). In addition to showing that activation of TLRs affects the immune phenotype of hMSCs in a culture-dependent manner, we demonstrated that ECM gene expression was also modulated. Our observations have implication for cardiac regeneration mediated by TLR-activated hMSCs and the search for more effective hMSC subsets.

F-2078
DYNAMIC INTERPLAY OF CELLULAR MILIEU LEADING TO ABERRANT REGENERATION IN SKELETAL MUSCLE

Thooyamani, Abinaya Sundari[1], Mukhopadhayay, Asok[2]
[1]Manipal Institute of Regenerative Medicine, Bangalore, India, [2]Stem Cell Biology Labratory, New Delhi, India

Regeneration of skeletal muscle has two main participants the satellite stem cells (SCs) the resident and infiltrating fibroblast cells called myofibroblasts (MFs). The persistence of the fibroblasts post regeneration leads to fibrosis or scar formation. The aim is to understand the interactions between satellite cells and myofibroblasts that may play a role in aberrant regeneration. To study the dynamics of these cells during fibrosis, we established a fibrosis model of mouse using laceration technique. The fibrosis was validated by mason's trichrome stain. The SCs and MFs were identified using immunohistochemistry and analysed for its cell cycling status by FACS. Then the gene expression relating to various time point of regeneration were analysed by real time PCR. The candidate proteins were identified using western blot and ELISA. It was observed that the normal regeneration begun by day 2 with the appearance of activated satellite cells and the immune cells. There was also an increase in the population of fibroblasts and infiltrating cells, suggesting active regeneration. The study was conducted for a period of 120 days, where the pattern of regeneration to fibrogenesis was compared. From day 7 till day 21 of injury, there was a change noticed in the trend of regeneration where the number of satellite cells reduced in the injured area. This variation was further analysed at the gene level to check for probable inhibition of cell propagation due to cell cycle inhibitors such as p21, p27. We further pursued to check genes of different pathways that are known to be involved in myogenesis such as NFkB, TGF-b, p38 etc. Our results suggested that the satellite cells are initially activated but by day 7 post injury are pushed towards differentiation and by day 21, there is reappearance of quiescent satellite cells. The myofibroblasts were observed to inhibit the rate of proliferation of the cells through cytokines as seen in invitro culture.

F-2079
NEW ACTION OF STEM CELLS: A NOVEL ONCOCIDAL MECHANISM - STEM CELL-RELEASED VESICLES

Thu, Mya S.[1], Akers, Johnny[2], Crain, Andrew[1], Winquist, Alicia[1], Li, Jie[2], Adhikari, Bandita[2], Chen, Clark[2], Snyder, Evan Y.[1]
[1]Neuroscience, Aging and Stem Cell Research, Sanford-Burnham Medical Research Institute, La Jolla, CA, USA, [2]Neurosurgery, University of California, San Diego, La Jolla, CA, USA

Over the past 2 decades, a broad variety of stem cells from an array of organs have been employed for a range of therapeutic applications in multiple disease states, including cell replacement, immune modulation, anti-inflammation, promotion of angiogenesis, global provision of metabolic enzymes, targeted delivery of desired exogenous transgenes (e.g., trophic factors, oncocidal molecules). Additionally, there have been attempts to recruit a patient's own resident stem cells through drugs or biologics. Often, in attempting to achieve a given action, a desired outcome was achieved by mechanisms unrelated to the intent of the investigator. For example, in attempting to treat rodent and primate models of Parkinson's disease (PD), impressive behavioral recovery was observed but not because dopaminergic (DA) neurons were replaced - although DA differentiation was attempted - but rather because host nigrostriatal circuitry and host DA neurons were preserved. This stem cell action came to be called the "chaperone" effect and has come to be observed universally in the stem cell field. It was initially attributed solely to the secretion of trophic, protective, anti-inflammatory molecules. Subsequently, passage of yet-to-be-determined molecules through gap junctions following cell-cell contact with stem cells was added to the repertoire of therapeutic actions. Recently, another difficult-to-explain observation came to the attention of stem cell biologists. Unmodified human neural stem cells (hNSC), included simply as negative controls for the therapeutic cargo they would ultimately carry, seemed, nevertheless, to have a consistent anti-tumor effect in and of themselves in vivo and in vitro. Additionally, a Phase IIb clinical trial conducted by Stem Cell Therapeutics (NCT00362414) employing a regimen of ß-hCG+Erythropoietin in patients with acute ischemic stroke, attempting to mobilize their endogenous stem cells, appeared to have failed due to an unexplained high-level response in the placebo group as well as the experiment group. These findings have not only puzzled many researchers but scuttled some hypotheses and clinical trials. To explain these surprising findings we hypothesized that the stem cells must be "launching" vehicles that can, in turn, mediate a more prolonged and sustained effect at the site of pathology. We know that vesicular trafficking is involved in intercellular communication during the normal development of many organs. They have also been implicated in a range of pathologies. We found that endogenous and transplanted stem cells interact with pathologic entities via this unanticipated method of communication: EV secreted by the pathology; EVs secreted by the stem cells themselves, mediating their therapeutic action. We demonstrated this new phenomenon in a prototypical model: the therapeutic use of hNSCs against glioblastoma multiforme (GBM). We found that NSC EVs have consistent intrinsic anti-tumor effect in GBMs (regardless of molecular signature). We were able to confirm that GBM cells uptake NSC EVs in vivo and in vitro and in response, there was delayed disease progression extending survival in tumor-bearing animals. This new paradigm of cell action, we believe, has implications that go beyond the interaction of NSCs with cancer, but rather can elucidate how stem cells (of many types) can communicate with pathology (of many types) through EV, allowing the design of novel, broadly-applicable therapeutic strategies against many classes of disease.

F-2080
SINGLE-CELL PROFILING REVEALS PROMINENT CLASSES WITHIN THE PLANARIAN STEM CELL COMPARTMENT

Wagner, Daniel, van Wolfswinkel, Josien, Reddien, Peter W.
[1]*Whitehead Institute MIT, Cambridge, MA, USA*

Planarians are flatworms well known for their capacity to regenerate any missing body region throughout adult life. A population of proliferative mesenchymal cells, collectively referred to as neoblasts, mediates planarian regeneration and is known to contain pluripotent adult stem cells. Historically, neoblasts have been defined based on morphology, radiation sensitivity, proliferative ability, and shared expression of numerous genes. It is unclear, however, whether neoblasts represent a largely homogeneous population, or exist as a heterogeneous collection of multiple cell types. Here, we used single-cell transcriptional profiling to directly compare gene expression fingerprints from over a thousand individual neoblasts under different conditions. These experiments identified two distinct major classes within the neoblast population that differ in their regenerative properties. Most functional attributes of neoblasts, including the ability to proliferate in response to injury and broad lineage capacity, are properties of one cellular class, the "sigma-Neoblasts". By contrast, the "zeta-Neoblast" class encompasses specified cells that give rise to an abundant post-mitotic lineage, which includes epidermal cells. Identification of these classes presents a new view of planarian neoblasts, in which the population is comprised of distinct and abundant cell compartments (pluripotent stem cells and lineage-committed precursors) that are prominent during regeneration as well as homeostasis.

F-2081
HAIR-FORMING ABILITIES OF SKIN EPITHELIA IN THE WOUND HEALING MODEL - INSIGHT INTO THE REGENERATIVE POTENTIAL AND MORPHOGENETIC COMPETENCE OF EPIDERMIS

Wang, Xiaojie[1], Shatrova, Olga[2], Astrowskaja, Aksana[2], Astrowski, Aliaksandr[2], Plikus, Maksim[1]
[1]*Developmental and Cell Biology, University of California, Irvine, Irvine, CA, USA,* [2]*Department of Medical Biology and General Genetics, Grodno State Medical University, Grodno, Belarus*

Typically, hair follicles form only once in a lifetime during the process of embryonic skin development. However, recent studies show that in the center of large adult wounds many new hairs can form de novo. This phenomenon of wound-induced hair neogenesis (WIHN) implies that epidermal cells in the wound's center acquire enhanced, stem cell-like plasticity. To better understand the plasticity aspect of hair follicle neogenesis, we developed a novel experimental wound model. Large full thickness wounds were generated. Autologous vibrissa-derived dermal papillae were transplanted onto the wound bed. Dermal papillae were used as the source of strong, localized hair-inducing signaling. Simultaneously, autologous interfollicular epidermis was obtained via vacuum suction and transplanted on top of the dermal papillae. Transplanted dermal papillae and epidermis often engaged in morphogenetic interactions. Epidermis often became hyper-proliferative and enveloped the dermal papillae. However, these interactions failed to induce new hair follicles, meaning that transplanted epidermis lacked morphogenetic plasticity, typically displayed by the wound epidermis in the WIHN model. To verify that transplanted dermal papillae maintained their hair-inducing abilities in the presence of hair-fated epithelium, we substituted epidermis for split-thickness skin containing upper segments of hair follicles, rich in follicular stem cells. In this combination, vibrissa-like hair follicles were indeed induced. Our results suggest that by itself the inductive signal, such as in form of transplanted dermal papilla, is not sufficient to initiate de novo regeneration of hair follicles from adult epidermis. We posit that unlike the WIHN model, vacuum-separated epidermis used in our wound model did not go through reepithelialization repair, the state when epidermal cells enter epithelio-mesenchymal-like transition (EMT) state typically associated with extensive epigenetic remodeling. We conclude that EMT-like reepithelialization is an essential prerequisite for epidermal cells to acquire greater stem cell-like plasticity in vivo.

F-2082
RNA CORE AT HOUSTON METHODIST RESEARCH INSTITUTE

Yakubov, Eduard, Diep, Nga, Cooke, John P.
Houston Methodist Research Institute, Houston, TX, USA

Artificially designed RNAs (artRNAs) represent a new class of biomimetic polymers which can be widely used in research. For certain applications, to modulate immunogenicity artRNA may be synthesized with modified nucleotides. Despite intense interest in artRNA, the challenges of generating RNA have hindered its efficient adoption. There are technical issues in this laborious, multi-stage process that typically result in sub-optimal product. To address these hurdles, the RNAcore @HMRI has developed and optimized a plasmid-based, high-throughput process for generating high-quality artRNAs. This approach takes advantage of the effective proof-reading mechanisms of in vivo plasmid expansion. Consequently, our plasmid-based approach allows for the generation of longer error-free RNAs (up to 15 kb). By contrast, PCR-based approaches may introduce errors in the amplified dsDNA template due to suboptimal exonuclease proofreading activity of thermostable DNA polymerases and/or DNA thermal damage. We have validated our methods by synthesizing and testing numerous artRNAs encoding factors related to reprogramming, transdifferentiation and gene therapy applications. Our mission is to efficiently generate high quality mRNA (or mmRNA) of any gene of interest, microRNA cassettes and noncoding RNA for advanced research applications.

F-2083
EFFECT OF REACTIVE OXYGEN SPECIES OVERPRODUCTION ON OSTEOGENESIS OF POROUS TITANIUM SCAFFOLD IN THE PRESENT OF DIABETES MELLITUS

Zhang, Zhiyong
Shanghai 9th People's Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

Clinical evidence indicates diabetes mellitus as a risk factor for metal implant treatment with higher failure rates in diabetic patients, but the underlying mechanism involved remain elusive. It is well known that reactive oxygen species (ROS) is extremely produced in diabetic patients and plays a role in the pathogenesis of diabetic bone disorder. This study was designed to test the hypothesis that sustained ROS may contributed to the impaired osteogenesis of porous titanium implants under diabetic conditions. In vitro study was performed on primary rat osteoblasts subjected to normal serum (NS), diabetic serum (DS), DS + NAC (a potent ROS inhibitor) and NS + H2O2. The porous titanium was implanted in bone defects that generated bilaterally in the femoral condyle of alloxan-induced diabetic rabbits, and then the rabbits were randomized to receive vehicle and NAC treatment. Compared with NS group, DS or NS + H2O2 group exerted significantly increased ROS generation both in the supernatant and intracellular, together with suppressed osteoblast functions such as depressed cell attachment

and morphology, decreased proliferation and alkaline phosphatase activity, as well as more severe cell apoptotic injury. In comparison, scavenging ROS with NAC significantly attenuated the DS-induced osteoblast dysfunction and apoptosis. Micro-CT and histological study showed much less new bone formation within the porous titanium scaffolds under diabetic conditions compared with the control group at 12 weeks after implantation, while treatment with NAC markedly improved the osteogenesis of porous titanium in diabetic rabbits. Our findings demonstrate that ROS overproduction under diabetic condition induces depressed osteoblasts behaviors and abundant cell apoptosis, resulting in impaired bone ingrowth within the porous titanium. Anti-oxidative treatment may become a valuable adjunctive strategy in accelerating osteogenesis of porous titanium scaffolds, with great clinical potentials for bone defect treatment in diabetic patients.

TISSUE ENGINEERING

F-2086
NOVEL DETERGENT FOR ORGAN DECELLULARIZATION CAN PRESERVE CELL SURFACE STRUCTURES

Kirita, Yuhei[1], Kami, Daisuke[2], Kawasaki, Takanori[3], Ishida, Ryo[1], Adachi, Takaomi[1], Kitani, Tomoya[2], Gojo, Satoshi[4]

[1]Nephrology, Kyoto Prefectural University of Medicine, Kyoto, Japan, [2]Kyoto Prefectural University of Medicine, Kyoto, Japan, [3]Cardiovascular medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan, [4]Regenerative Medicine, Kyoto Prefectural University of Medicine, Kyoto, Japan

Background: Organ transplantation is a safeguard against nonreversible and life-threading organ failure, and demonstrates outstanding outcomes. Therefore, the demand for organ transplantation is growing so much, but it cannot be met by the shortage of donor organs. Allogeneic organ transplantation still has drawbacks, which mainly consist of chronic rejection caused by immunological reaction even under immunosuppression, and the adverse effects of immunosuppressive drugs, such as carcinogenesis. Whole organ tissue engineering holds the potential to solve these drawbacks through the implementation of decellularization-recellularization technology. Recently, some groups reported the decellularization of an entire heart and kidney through perfusion with sodium dodecyl sulfate (SDS) with preserving original architecture and original microvascular network and allowing for a feasibility of recellularization. However, SDS induces strong biological reactions that may denature and remove out proteins and glycans on both extracellular matrix and cellular surface, which are involved in cell to cell interactions. The preservation of these structures might acquire better engraftment of donor cells at the recellularization. In this study, we investigated the potential of a novel detergent in search of better biocompatibility. Methods: We decellularized cadaveric rat hearts using coronary artery perfusion and kidneys using renal artery perfusion with a new detergent (Detergent X) and 1% SDS. To evaluate cellular removal, Hematoxylin-Eosin and DAPI stain, and quantification of residual DNA were performed. To evaluate retaining glycoproteins in decellularized organs, Periodic acid Schiff stain, Alcian blue stain and quantification of glycosaminoglycans were performed. To evaluate retaining extracellular matrix (ECM) content, Masson's trichrome stain, immunohistochemistry (Collagen I, IV, Laminin, Fibronectin) and quantification of collagen were performed. Although hemocompatibility includes a lot of biological reactions, the platelet adhesion and complement activation is the first step of reactions to foreign materials. In this study, platelet adhesion was assessed in vitro using platelet rich plasma (PRP). The presence of residual DNA, glycosaminoglycans and collagens within the decellularized organs was measured with spectrophotometric quantification. Decellularized hearts were transplanted into syngeneic mice, and examined the coagulopathy, including clotting formation and coagulation pathway activation. Results: Both detergents completely removed cellular component (<50 ng DNA/mg dry tissue) and morphologically retained 3-dimentional structure. Detergent X significantly retained more glycoproteins and tended to retain ECM contents and total collagen. In vitro platelet adhesion assay demonstrated that decellularized organs treated with Detergent X had fewer platelets than those treated with SDS. The grafts treated with Detergent X attenuated the coagulopathy, which was experienced in the hearts decellularized with SDS. Conclusion: Our data showed that Detergent X preserved the cell surface structures and lead better hemocompatibility. It suggests that Detergent X is more suitable for whole organ decellularization and recellularization towards the ultimate goal of making solid organs.

F-2087
IMMORTALIZATION OF MOUSE PITUITARY CELLS AND OPTIMIZATION OF THEIR CULTURE CONDITIONS

Kokubu, Yuko[1], Kurisaki, Akira[2]

[1]Tsukuba University, Tsukuba, Japan, [2]National Institute of Advanced Industrial Science and Technology (AIST)/Tsukuba University, Tsukuba, Japan

Pituitary gland is the center of the endocrine system that controls homeostasis of whole body via the secretion of many hormones. The anterior pituitary is composed of 5 different endocrine cells expressing each specific hormones. However, it is not well known about their regulatory mechanisms of the differentiation into each cell types, and even their appropriate culture conditions. Moreover, there is no specific protocol for the differentiation into each mature type of pituitary cells from their progenitors or ES/iPS cells. Here, we attempted to immortalize mouse pituitary cells by infecting TERT, E6 and E7 genes using pseudo-lentivirus system. These established cell lines express TSH beta (TSHb), which is the specific hormone of thyrotropes in the anterior pituitary. Currently, we are analyzing the characteristics of these established cell lines. We will use these cell lines to optimize the culture conditions for mouse pituitary endocrine cells.

F-2088
RAFT TISSUE EQUIVALENTS FOR CO-CULTIVATION OF HUMAN CORNEAL LIMBAL EPITHELIAL AND STROMAL STEM CELLS FOR TRANSPLANTATION

Kureshi, Alvena[1], Funderburgh, James L.[2], Daniels, Julie T.[3]

[1]Ocular Biology and Therapeutics, Institute of Ophthalmology, University College London, London, United Kingdom, [2]Department of Ophthalmology, University of Pittsburgh, Pittsburgh, PA, USA, [3]University College London, London, United Kingdom

We investigated whether we could use RAFT (Real Architecture For 3D Tissue) tissue equivalents as a substrate for co-culturing human corneal limbal epithelial stem cells (LESCs) and corneal stromal stem cells (CSSCs) for transplantation of a cell therapy. Since these cell types are observed in close proximity in vivo in the corneal limbal stem cell niche, we wanted to re-create this element of their native environment in vitro, in an attempt to optimize culture conditions and investigate epithelial-stromal cell interaction. Methods: We used RAFT tissue equivalents as a three-dimensional substrate to co-culture a mixed population of LESCs and CSSCs. Cell morphology, stem cell marker expression, epithelial cell confluence and epithelial and stromal cell physical interaction were assessed with light microscopy, immunohistochemistry, fluorescein-diacetate

staining and transmission electron microscopy analysis respectively. A monolayer of epithelial cells that maintained positive expression of p63α, indicative of the LESC phenotype, formed on the surface of RAFT within 13 days post-seeding of cells. CSSCs remained in close proximity to LESCs and maintained positive expression of mesenchymal stem cell markers. The onset of epithelial cell layering and differentiation was also observed. We have demonstrated a simple 3D, co-culture method to support the cultivation of LESCs on RAFT tissue equivalents suitable for transplantation. This method of co-culturing with another niche cell type has advantages including the elimination of animal fibroblasts as feeder cells, a short preparation time and the capacity to support multilayering of epithelial cells. This system could be used to investigate other cell-cell interactions or form the basis of a corneal epithelial-stromal equivalent for bioengineering applications in the future.

F-2089

BUILDING BRAINS IN SPIDER WEBS: STEM CELL-DERIVED AMPA-RESPONSIVE NEURONS GROWN IN 3D MATRICES OF RECOMBINANT SPIDER SILK PROTEIN

Lewicka, Michalina[1], Rebellato, Paola[1], **Lewicki, Jakub**[1], Uhlén, Per[1], Rising, Anna[1], Hermanson, Ola[2]
[1]Karolinska Institutet, Stockholm, Sweden, [2]Karolinska Institute Neuroscience, Stockholm, Sweden

A major problem in stem cell research is the lack of reproducible and easy-to-use three dimensional (3D) culture systems, in combination with limited access of synthetic matrices providing optimal conditions for culturing stem cells. Here we demonstrate that 3D structures generated from recombinant spider silk protein (4RepCT) provided excellent matrices for the generation of functional excitatory neuronal circuits from cortical neural stem cells (NSCs). NSCs isolated from the cerebral cortices of rat embryos were cultured on either 4RepCT matrices shaped as foam structures, or on conventional polystyrene plates coated with poly-L-ornithine and fibronectin. Upon treatment with the growth factor BMP4 or a combination of BMP4 and the signaling factor Wnt3a, the cortical NSCs grown on 4RepCT 3D structures differentiated efficiently into neurons that responded to glutamate receptor agonists, such as AMPA, to at least the same extent as or even to a greater degree than control cultures. Current experiments are aiming at testing the 4RepCT matrices for culturing iPS-derived NSCs as well as neuroblastoma cells in 3D. Matrices derived from recombinant spider silk proteins thus provide a functional 3D microenvironment for neural stem cells and can be employed in the development of new strategies in stem cell research and regenerative medicine.

F-2090

DIFFERENTIATION OF NESTIN-EXPRESSING STEM CELLS IN THE SCIATIC NERVE IN GELFOAM® HISTOCULTURE AND IN VIVO

Li, Lingna[1], Cao, Wenluo[1], Liu, Fang[2], Tran, Benjamin[1], Mii, Sumiyuki[3], Amoh, Yasuyuki[3], Katsuoka, Kensei[3], Hoffman, Robert M.[4]
[1]AntiCancer, Inc, San Diego, CA, USA, [2]Department of Anatomy, Second Military Medical University, Shanghai, China, [3]Department of Dermatology, Kitasato University School of Medicine, Kanagawa, Japan, [4]Department of Surgery, University of California San Diego, San Diego, CA, USA

In our previous studies, we have demonstrated that nestin-expressing stem cells exist in both the hair follicle and sciatic nerve. We have also shown that nestin-expressing cells from the hair follicle can differentiate into Schwann cells, neurons and other cell types in vitro and when implanted into injured nerves or spinal cord. We have termed these cells HAP (hair accessible pluripotent cells). In the present study, to further understand the role of nestin-expressing cells in peripheral nerve injury and regeneration, we characterized nestin-expressing cells in the sciatic nerve from nestin-driven GFP transgenic mice (ND-GFP mice) by immunofluorescence (IF) staining and confocal fluorescence microscopy. In the uninjured sciatic nerve in vivo, nestin-GFP expressing cells co-expressed p75NTR (a neural crest stem cell marker), but neither S100 (a mature Schwann cells marker) nor βIII-tubulin, (a mature neuron marker). In the injured sciatic nerve in vivo, nestin-GFP expressing cells preferentially proliferated, with the majority of these cells located in the distal injured area compared to the proximal area. The proliferating nestin-GFP cells in the injured sciatic nerve co-expressed p75NTR but not βIII-tubulin, a mature neuron marker. A fraction of the nestin-GFP expressing cells co-expressed S100 one week after injury. When the severed sciatic nerve was cultured on Gelfoam® in nerve growth medium, preferential proliferation of nestin-GFP cells occurred after 2-3 weeks in culture. The nestin-GFP expressing cells proliferating in the severed nerve on Gelfoam® co-expressed S100, as well as βIII-tubulin, as we have previously reported. In the βIII-tubulin positive cells in the sciatic nerve of Gelfoam® culture, nestin-GFP was expressed mainly in the nucleus. These results suggest that nestin-expressing stem cells in the peripheral nerve play a critical role in nerve injury and regeneration. Gelfoam® culture of nestin-expressing cells from hair follicles or peripheral nerves provides an effective scaffold for nerve injury repair and regeneration with high potential of neuron differentiation.

F-2092

RECONSTRUCTION AND REGENERATION OF THE RAT UTERUS THROUGH NOVEL DECELLULARIZATION AND RECELLULARIZATION TECHNIQUES

Miyazaki, Kaoru, Maruyama, Tetsuo, Masuda, Hirotaka, Hida, Naoko, Uchida, Hiroshi, Yoshimura, Yasunori
Department of Obstetrics and Gynecology, Keio University School of Medicine, Tokyo, Japan

Patients with entire or partial uterine structural defects need uterine transplantation or uterine tissue engineering. Several bioengineered organs including heart and liver have recently made through recellularization of decellularized scaffolds (DS) prepared from the corresponding organs. The aim of this study was to develop a technique for decellularization of the rat uterus and to elucidate the potential of the resulting uterine DS (uDS) for uterine regeneration. Rat uteri were excised along with large vessels and perfused with detergent to prepare uDS. uDS was then injected and incubated with rat uterine cells and rat mesenchymal stem cells in vitro for recellularization. For in vivo regeneration, uDS was cut into small rectangles and then subjected to transplantation into partially excised uteri in rats (excision and replacement group, ER). As a control group (CO), the uterine horns were left intact without excision after abdominal incision. As an excision only group (EO), the uterine horns were left open after partial uterine excision as done in ER. Histological and electron microscopic analysis revealed that uDS contained no intact cells but retained extracellular matrix and microvasculature. Recellularization of uDS resulted in regeneration of endometrium comprised of stroma and epithelium, as determined by immunohistochemistry. Uterine tissue consisting of endometrium and myometrium was also regenerated at the transplantation site of uDS in vivo 28 days after surgery. Recipient rats were mated with male rats 28 days after surgery. Statistical analysis revealed that the number of fetuses per uterine horn was significantly larger in CO (4.9 ± 1.0, mean ± SD) than ER (1.3 ± 1.3) and EO (0.6 ± 1.8) ($P<0.05$). The pregnancy rate and average weight of fetuses

were comparable in CO (100% and 1.78 ± 0.58 g) and ER (75% and 1.65 ± 0.62 g), and significantly lower in EO (12.5% and 0.77 ± 0.04 g) (P<0.05). We have developed a decellularization method to prepare rat uDS and found that uDS have potentials as a three-dimensional scaffold to regenerate uterine tissues in vivo and in vitro through recellularization. These results implicate uDS as a possible ideal material for uterine reconstruction for patients with uterine factor infertility. Furthermore, these techniques may enable us to develop a research tool to investigate the contribution of the specific cell group such as stem cells to each uterine component.

F-2093
ENGINEERING BIO-ARTIFICIAL HEART BY HUMAN STEM CELLS USING NATURE'S THREE DIMENSIONAL PLATFORM OF RAT HEART

Okuyama, Michihiro[1], Ousaka, Daiki[1], Ishigami, Shuta[1], Kobayashi, Junko[1], Sano, Shunji[1], Oh, Hidemasa[2]
[1]Cardiovascular Surgery, Okayama University Hospital, Okayama City, Japan, [2]Regenerative Medicine, Center for Innovative Clinical Medicine, Okayama University Hospital, Okayama City, Japan

Background: While many studies have suggested the efficacy of direct stem cell infusion or cardiac patches implantation in patients with cardiac dysfunction, heart failure is the leading cause of mortality globally. Heart transplantation remains the definitive treatment for end-stage heart failure, but the supply of donor organs is very limited. Once a heart is transplanted, recipients need lifelong immunosuppression, and often face transplanted heart failure caused by hypertension, diabetes and renal failure. However, enhancement of the geometrical cell engraftment to improve the global cardiac function remains as significant challenges. Here, we used non-immunogenic extracellular matrix as a scaffold to seed the human stem cells. We reseeded cardiac progenitor cells (hCPCs) and human umbilical vein endothelial cells (HUVEC) or vascular endothelial cells derived from human induced pluripotent stem (hiPS) cells on the platform with acellular architecture. Methods: We generated a modified Langendorff apparatus which had two motor pumps, and located the apparatus in the clean benches. The apparatus was applied on an 8-week-old male Sprague-Dawley rat's heart to decellularize the entire heart-derived cells. The heart was decellularized with 0.02% trypsin, 1% SDS, 3% Triton X-100, and 4% deoxycholic acid followed by serial perfusion. The acellular constructs contain architecture of original tissues, including the vessels, components of valves, and intact chamber geometry. We perfused 0.1% gelatin solution to fortify these structures at first. To generate whole heart grafts, we perfused hCPCs and HUVEC with 5% CO_2 incubation followed by perfusion reseeding system. As a next step, hiPS cells-derived vascular endothelial cells were employed to enhance the cell engraftment efficiency. Two weeks after the recellularization, we evaluate the heart histologically by hematoxylin and eosin stain and Masson's trichrome stain. Cell retention and engraftment were also monitored by lentiviral-GFP infected hCPCs. Results: Decellularized rat heart constructs contain architecture of original tissues, including the vessels, components of valves, and intact chamber geometry. A subtype of collagens, laminin, and fibronectin were confirmed by immunofluorescence. Indocyanine green perfusion through the aorta demonstrated the preserved coronary arteries and capillary vasculatures. Fluorescent images of lentiviral-GFP infected hCPCs grafted extracellular matrix also showed the cell retention and engraftment particularly on the left ventricular portion. We found that the three dimensional scaffolds reseeded under these conditions for two weeks could achieve significant hCPCs engraftment and distribution along with coronary perfusion area. HUVEC or vascular endothelial cells derived from hiPS cells could promote to substantial recellularization with enhanced micro-circulation. The recellularized cardiac constructs kept growing as thickening tissues with constant and physiological loading conditions. Conclusions: Our initial results suggest that hCPCs can be reseeded onto rat decellularized scaffolds to develop three-dimensional cardiac grafts. HUVEC or hiPS cells-derived vascular endothelial cells could reinforce the vascular endothelium to enhance the cell engraftment and survival. Further challenge could apply by cardiac and smooth muscle cells directly differentiated from hiPS cells to provide mechanically contractile heart organ.

F-2094
CANONICAL WNT-10B SIGNALING EXERTS AN ABILITY OF MAINTAINING MOUSE DERMAL PAPILLA CELLS

Ouji, Yukiteru, Fukumi, Nakamura-Uchiyama, Yoshikawa, Masahide
Department of Pathogen, Infection and Immunity, Nara Medical University, Kashihara, Japan

Dermal papilla (DP) cells, the specialized fibroblasts in the hair follicle, and they are associated with the development and cycle regulation of hair follicles. It is known that DP cells cultured in the ordinary fibroblast culture conditions lose their hair induction-ability. Although the conditioned medium obtained from skin epithelial cells are reported to maintain the hair induction-ability of DP cells, Wnt proteins are also candidates with few works on it. In hair follicles in postnatal skin, the expressions of Wnts (e.g. Wnt-3a, 5a, 10b and 11) have been reported. In the present study, we investigated the effects of Wnt-3a, Wnt-5a, Wnt11 and Wnt-10b on DP cells that were primarily cultured or passaged. DP cells were dissected from vibrissae follicles of 4-week-old C3H/HeN mice by microdissection method, and the single DP cells were cultured in the medium with or without bioactive Wnts (Wnt-3a, 5a, 10b, and 11). Only Wnt-10b exerted an evident promotion on the proliferation of the cultured DP cells and most of the proliferated cells remained to be positive for alkaline phosphatase (ALP). Further, the DP cells cultured with Wnt-10b could form the hair follicle and induce hair growth after co-transplantation with epithelial cells, while a limited and insufficient maintenance by Wnt-3a was observed and no effects by Wnt-5a or Wnt-11. Our results suggest that canonical Wnts, specifically Wnt-10b, play an important role in the maintenance of DPs and trichogenesis.

F-2095
HUMAN TISSUE-ENGINEERED ADIPOSE TISSUE PRODUCED FROM ADIPOSE-DERIVED STEM CELLS : IN VIVO ANALYSIS OF TISSUE VASCULARIZATION AFTER GRAFTING

Proulx, Maryse[1], Mayrand, Dominique[1], Aubin, Kim[1], Vincent, Caroline[1], Boisvert, Annie[1], Lagueux, Jean[2], Fortin, Marc-André[3], Fradette, Julie[1]
[1]Centre LOEX de l'Université Laval, Centre de recherche du CHU de Québec - Axe Médecine régénératrice, Département de chirurgie, Faculté de médecine, Université Laval, Québec, QC, Canada, [2]Centre de recherche du CHU de Québec - Axe Médecine régénératrice, Québec, QC, Canada, [3]Centre de recherche du CHU de Québec - Axe Médecine régénératrice, Département de génie des mines, de la métallurgie et des matériaux and Centre de recherche sur les matériaux avancés (CERMA), Université Laval, Québec, QC, Canada

Reconstructed human adipose tissues (rhATs) produced by tissue engineering represent a promising alternative to autologous fat grafts for reconstructive surgeries. We took advantage of the properties of adipose-derived stem/stromal cells (ASCs), in combination with the self-assembly approach of tissue engineering, to produce rhATs rich in human extracellular matrix and adipocytes but devoid of exogenous biomaterials. rhATs containing differentiated adipocytes as well as an

in vitro preformed capillary network were also obtained upon addition of human microvascular endothelial cells (ECs). rhATs enriched or not in ECs were grafted subcutaneously onto nude mice (n=6 per group per time-point, 3 independent experiments). At day 3, 7 and 14, grafted tissues were excised and the presence of adipocytes as well as vascularization were analyzed. Histology revealed that rhATs without ECs were vascularized from day 7 after grafting and that large quantities of capillaries were present in EC-enriched tissues at all time-points examined. Adipocytes persistence after grafting was also confirmed by histology. Immunolabelings for the EC marker CD31 revealed a stable human capillary network with a gradual connection with the host vascular network (inosculation visible as early as day 3) and murine red blood cells that perfused the grafts. Additionally, we produced thicker rhATs (± 1 mm) enriched or not in ECs and grafted them onto nude mice (2 independent experiments). We confirmed that non-invasive magnetic resonance imaging (MRI) was an excellent method to visualize grafted rhATs and follow their volume maintenance after grafting. Moreover, vascularization (indicative of network functionality) was analyzed by dynamic contrast-enhanced MRI (DCE-MRI) using the Gadomer 17 contrast agent. Volume retention analysis revealed no differences between both groups of rhATs, with an average retention of 54.3 ± 2.9% 21 days after grafting. DCE-MRI confirmed that both rhAT groups were perfused by day 9 (signal enhancement factor of 1.20, n=4 per group) but no differences in the perfusion extent were observed between rhATs enriched in ECs or not. In conclusion, reconstructed human adipose tissues rich in matrix and human cells produced without exogenous components by the self-assembly approach were rapidly vascularized as needed to ensure graft survival and represent promising substitutes for soft tissue regeneration.

F-2096
ADDITIVE FABRICATION TECHNOLOGIES FOR CELL DELIVERY AND 3D SCAFFOLDS

Quigley, Anita Frances[1], Chung, Johnson HY[2], Kita, Magdalena[1], Foroughi, Javad[2], Cornock, Rhys[1], Crook, Jeremy[2], Wallace, Gordon G.[2], Kapsa, Robert MI[1]

[1]Neurosciences and Intelligent Polymer Research Institute, St Vincent's Hospital and ARC Centre of Excellence for Materials Science, University of Wollongong, Melbourne, Australia, [2]Intelligent Polymer Research Institute, ARC Centre of Excellence for Materials Science, University of Wollongong, Wollongong, Australia

Additive fabrication refers to a class of manufacturing processes where structures are fabricated by cumulatively adding layers of material. Our laboratories are developing additive fabrication technologies for tissue engineering and for the integration of devices with biological tissues. We have been investigating the use of bio-printing and fiber spinning methodologies to produce 3D scaffolds suitable for both cell delivery (progenitors and stem cells), drug delivery and guidance of tissue regeneration. These technologies allow increased spatial control over 3D structures and the integration of biological complexity at a level that has previously been unachievable. In particular, we have been investigating the use of alginate and alginate-gelatin hydrogel blends for the creation of wet-spun cell laden hydrogel fibers and printed 3D structures. To date we have produced hydrogel fibers with encapsulated human neural stem cells, Schwann cells and muscle progenitor cell (myoblasts) and have developed hydrogel based coaxial fibers to facilitate spatial control over the distribution of different cell types within single fibers. Progenitor and stem cells encapsulated within fibers and printed 3D structures show high cell viability and can be sustained in vitro for at least three weeks. Over this period encapsulated cells can proliferate within the majority of cells remaining viable. In addition we have used muscle progenitor cells (myoblasts) encapsulated in wet-spun hydrogel fibers to successfully deliver wild type muscle progenitor cells to muscle in a mouse model of muscular dystrophy (mdx mouse), achieving large areas of muscle regeneration. We are currently investigating the use of these fibers for cell delivery to the peripheral nerve as well as for guidance of axonal growth in biodegradable nerve conduits. These experiments demonstrate the versatility of additive fabrication based approaches in tissue engineering, particularly in the fields of nerve and muscle regeneration.

F-2097
MECHANICAL STRESS CONDITIONING PROMOTES FUNCTIONAL MATURATION OF IPS-DERIVED HUMAN CARDIAC TISSUES

Ruan, Jia-Ling[1], Tulloch, Nathaniel L.[2], Razumova, Maria[1], Saiget, Mark[1], Muskheli, Veronica[3], Korte, Steven[1], Pabon, Lil[4], Reinecke, Hans[4], Regnier, Micheal[1], Murry, Charles[5]

[1]Bioengineering, University of Washington, Seattle, WA, USA, [2]University of Washington, Seattle, WA, USA, [3]Pathology, University of Washington, Seattle, WA, USA, [4]University of Washington, Seattle, WA, USA, [5]University of Washington - Center for Cardiovascular Biology, Seattle, WA, USA

Generation of engineered myocardiums makes possible the study of tissue level mechanics of stem cell-derived cardiomyocytes. Here we generated bioengineered human myocardiums via collagen-based scaffolding techniques and human induced pluripotent stem (iPS) cells. These tissues demonstrate Frank-Starling curve-type force-length relationship, an essential feature of native myocardium. With static stress conditioning, the engineered myocardium shows a 2.5-fold increase in contractility and a 2-fold increase in the upstroke velocity of the calcium transient. Furthermore, stress conditioning increases the stiffness of engineered myocardium 2-fold, yielding passive mechanics more similar to native myocardium, and decreases the negative slope of force frequency relation that generally happens in immature cardiac tissues. The contractility of engineered myocardium is responsive to extracellular calcium and L-type calcium channel agonist and blockers but not sensitive to sarcoplasmic reticulum (SR)-related drugs. Immunohistological staining showed more expression of SR calcium regulated proteins, ryanodine receptors and SERCA2 under static stress conditioning. The study demonstrates stem cell-derived human myocardium is functionally contractile and demonstrates characteristic tissue-level responses such as the Frank-Starling mechanism. Moreover, the engineered tissue is highly responsive to static stress conditioning, showing substantial maturation of active force production, calcium transient dynamics, and tissue compliance.

F-2098
GROWING MULTIPLE VASCULARIZED ORGAN BUDS FROM DISSOCIATED MIXED PROGENITORS THROUGH SELF-DRIVEN DYNAMIC AGGREGATION

Takebe, Takanori, Enomura, Masahiro, Yoshizawa, Emi, Kimura, Masaki, Koike, Hiroyuki, Takahashi, Yoshinobu, Koike, Naoto, Sekine, Keisuke, Taniguchi, Hideki

Yokohama City University, Yokohama, Japan

Over the last decade, numerous publications have described protocols that can induce the cellular differentiation by sequential addition of combinatory factors such as hepatocytes, insulin-producing cells or dopaminergic neurons. However, conventional uni-directional cell induction approach ignores 3-D and chronological multicellular communications, which occur along with physiological cell differentiation during organogenesis. In order to derive therapeutically

effective functional cells from stem cells, it is essential to develop a 3-D culture platform, allowing the cells underwent developmental cellular interactions to follow the organogenesis including biophysical stimuli, biochemical and molecular signals and the spatial organization. In this context, we have recently showed that specified hepatic cells self-organized into three-dimensional organ buds in a dish by co-cultivated with stromal cell populations; human endothelial and mesenchymal progenitors, which are required for the initiation of hepatogenesis, the budding of the rudimentary liver (liver bud) in the foregut. By transplanting in vitro grown organ bud, we have demonstrated the generation of functional hepatocytes in an immunodeficient animal. Here, we examined the translatability of this principle to the other organ systems to grow organ buds from human and mouse embryonic tissue derived mixed progenitors. Surprisingly, recapitulation of endothelial-mesenchymal interaction in culture was shown to provide a widely conserved morphogenetic force against multiple tissue-derived progenitors, resulting in the formation of 3D and self-organized tissues in vitro. Generated pre-vascularized tissues were intravitally proved to be re-perfused quickly within 2-3days in vivo, and successfully reconstituted a mature islet-like tissue with functional human vascular networks. Furthermore, transplantation of in vitro derived pancreatic organoids showed a therapeutic potential against type 1 diabetic animal model. Considering a critical shortage of donor organs for treating end-stage organ failure, manipulation of autologous iPSCs holds great promise for regenerative medicine. However, classic clinical trials of cell transplantation, currently an important target of the stem-cell-based approach, have presented unsatisfactory results. Our proposed concept, i.e. organ-bud transplantation, offers an alternative approach to the generation of a three-dimensional, vascularized organ. This will highlight the enormous therapeutic potential using in vitro-grown organ-bud transplantation for treating organ failure.

F-2099
PRESERVING THE REGENERATIVE POTENTIAL OF WHOLE RENAL TISSUE SCAFFOLD FOLLOWING LONG TERM FREEZING

Talwar, Baldeep Chani
Center for Stem Cell and Tissue Engineering, Panjab University Chandigarh, Chandigarh, India

The multicellular nature of the renal tissue makes it most difficult organ for regeneration purpose. The cellular therapy including direct stem cell injection for tissue repair therefore remains difficult proposition. Therefore, still today whole organ transplantations remain the definitive treatment to the patients having end stage renal disease (ESRD). The ever increasing demand for the transplantable organ albeit remains the concern as well as an unsolved problem. Currently, combination of cellular therapy with tissue engineering for generating functional tissue is now gaining ground for the ready availability of functionally compatible organ. In this regard generation of whole organ scaffold through decellularization followed by regeneration of the whole organ by recellularization is now being viewed as novel way of generating whole organs, including the renal tissue. Long term freezing help to retain regeneration potential of decellularized renal bioscaffold. Whole kidneys from Sprague Dawley rats were isolated and immediately cryopreserved at -80°C in deep frezer. Three months following cryopreservation, the organ was thawed and perfused employing SDS solution for a period of 3 days to achieve decellularization. Fresh kidneys isolated from rats were used for the comparison. Following decellularization, the native architecture of both the cryopreserved and the freshly isolated organs was analyzed through staining with Haematoxylin and Eosin, Masson Trichrome Immunohistochemistry and Scanning electron microscopy (SEM). mesenchymal Stem Cells were employed to regenerate the decellularized kidney bioscaffold. Based on these observations, it is suggested that cryopreserved kidneys retained the architecture suitable for recellularization following decellularization to similar extent as was seen for the freshly isolated kidneys. This work presents methodologies for the process development in generation of non-destructive decellularized tissue following long term preservation as an alternative for regenerating functional organ in the cure of ESRD.

F-2100
POROUS BONE CEMENT SCAFFOLDS FOR ENGINEERING BONE GRAFTS WITH HUMAN INDUCED PLURIPOTENT STEM CELLS

Sladkova, Martina[1], Engqvist, Håkan[2], **de Peppo, Giuseppe Maria[1]**
[1]The New York Stem Cell Foundation Research Institute, New York, NY, USA, [2]Division of Applied Material Sciences, Uppsala University, Uppsala, Sweden

Introduction: Treatment of large bone defects remains a clinical challenge worldwide. Approaches of bone engineering using human induced pluripotent stem cells and compliant biomaterial scaffolds open the opportunities to grow therapeutically relevant grafts for personalized applications. Among others, calcium phosphate cements create a stable bond with the host bone, are biocompatible, bioresorbable and osteotransductive (replaced by new bone tissue), thus representing suitable biomaterials for the construction of porous scaffolds for optimal skeletal reconstructions. The objective of this study was to engineer bone cement scaffolds of adequate porosity and mechanical properties for the construction of functional bone grafts using osteocompetent progenitor cells derived from human iPSC lines. Materials And Methods: Macroporous synthetic cement scaffolds (8 mm x 3 mm height) were fabricated using a dissolving phase approach by mixing monocalcium phosphate monohydrate and β-tricalcium phosphate with poly(ethylene glycol) particles of different size (100-600 μm) in distilled water. Fabricated scaffolds were then characterized for chemical composition, porosity and mechanical properties. Human iPSC lines were derived from dermal fibroblasts and bone marrow stromal cells using non-integrating reprogramming vectors, expanded and induced into the mesenchymal lineage for 7 days. Mesenchymal progenitors were then expanded and characterized prior to culture on bone cement scaffolds for 5 weeks. Constructs of cells seeded onto decellularized bone scaffolds were used as control for all experiments. Cell viability, expression of bone-specific markers, bone tissue development and mineralization and mechanical properties of engineered bone tissues were investigated along the experimental period. Results: Our results demonstrate that functional and highly proliferative osteocompetent cells can be derived from iPSC lines, and their culture on bone cement scaffolds result in the increased expression of a large set of markers associated with osteogenesis and bone development, and the formation of mature bone-like tissue. Conclusion: The use of ostecompetent cells derived from iPSC lines in combination with innovative biomaterials open the possibility to engineer next-generation bone grafts that provide a faster and cheaper healing in critical size skeletal defects.

TECHNOLOGIES FOR STEM CELL RESEARCH

F-2101
UNIQUE MICRO-RNA AND PROTEOMIC PROFILES OF UMBILICAL CORD BLOOD- DERIVED STEM CELLS WITH EMBRYONIC-LIKE CHARACTERISTICS

Zuba-Surma, Ewa[1], Karnas, Elzbieta[1], Bobis-Wozowicz, Sylwia[1], Kedracka-Krok, Sylwia[2], Jankowska, Urszula[2], Kmiotek, Katarzyna[1], Ratajczak, Mariusz Z.[3], Boruczkowski, Dariusz[4], Madeja, Zbigniew[1]

[1]Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland, [2]Department of Physical Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland, [3]Stem Cell Biology Institute, James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA, [4]Polish Stem Cell Bank, Warsaw, Poland

Human umbilical cord blood (CB) represents a vast source of several stem cell (SC) populations including not only hematopoietic SCs (HSCs) but also non-hematopoietic mesenchymal SCs (MSCs), unrestricted somatic SCs (USSC) and progenitor neural SCs (HUCB-NSC) as well as recently discovered population of small SC with embryonic-like features (VSELs). These cells exhibit: 1) very small size (smaller than erythrocytes), 2) CD45-/Lin-/CD133+ phenotype, 3) several features of pluripotency on untrastructural and genetic levels and 4) share several markers with not only ESCs (e.g. Oct-4, Nanog, PLAP), but also with more mature HSCs (e.g. CD34, CXCR4). Although, the presence of non-hematopoietic SC with embryonic-like features has been reported in human CB, neither global proteomic profile nor extended multiantigenic phenotype of such stem cells has been investigated. Similarly, microRNA transcriptome of VSELs has never been compared to other more tissue committed CB- derived SC fractions. By employing multiparameter flow cytometry (LSR II; BD Bioscience) and modern imaging cytometry (ImageStream; Amnis Corp.), we examined the presence app. 200 surface antigens present on CB-derived CD45-/Lin-/CD133+ cells (Lyoplate array, BD Bioscience). We established that these cells represent heterogeneous fraction greatly co-expressing CD34 antigen (54.2±5.3%) and only modestly CXCR4 receptor (0.025 ± 0.004%). Importantly, we found small Oct-4+ cells among CD45-/Lin-/CD133+ cells co-expressing CD47, CD50 or CD81 antigens. By using SC- focused microRNA panel (quantifying 85 distinct SC- related miRNAs; Exiqon), we established the presence of 23 different miRNAs enriched in VSELs when compared to HSCs including miR-520 and miR-302 families that have been widely described in both ESCs and iPS cells (pluripotent SC-miRs). Interestingly, by employing mass spectroscopy (Shutgun array; micrOTOF-Q, Bruker), we found that FACS sorted CD45-/Lin-/CD133+ CB- derived SC posses higher level of proteins engaged in tissue development, proteins responsible for answer to stress and external stimuli when compared to purified CD45+/Lin-/CD133+ HSCs. Interestingly, CD45-/Lin-/CD133+ VSELs expressed more adhesion and membrane proteins involved in cell-cell junction and communication when compared to HSCs, which corresponds to antigenic profile identified by flow cytometry. Importantly, we found low expression of proteins involved in metabolic and biosynthesis processes, RNA processing, gene expression, replication and translation in purified CB- derived embryonic-like SC when compared to HSCs that goes along with quiescent status of these cells. In summary, we confirmed the presence of primitive CD45-/Lin-/CD133+ SC exhibiting several features of embryonic-like cells in human umbilical CB. Such CD45-/Lin-/CD133+ cells represent a rare population of quiescent SC with unique proteomic, antigenic and miRNA profiles that are distinct from more mature HSCs and greatly correspond to ESCs features. Thus, umbilical CB may be a source of embryonic- like cells which functional properties need to be further investigated. Acknowledgements: Supported by the European Union structural funds, Innovative Economy Operational Programme, grant No. POIG 01.02-00- 109/09 "Innovative methods of stem cells applications in medicine".

F-2102
THE EFFECT OF SCAFFOLD MODULUS ON THE MORPHOLOGY AND REMODELING OF FETAL MESENCHYMAL STEM CELLS

Abdul Jalil, Rufaihah[1], Mohd Mazlan, Muhd Danial[1], Han, Tianyang[1], Lim, Kenrich[1], Ong, Wen Fang[1], Cheyyatraivendran, Suganya[2], Chong, Mark[3], Plotkin, Marian[2], Mattar, Citra[4], Chan, Jerry[5], Seliktar, Dror[6], Kofidis, Theo[7]

[1]Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, [2]Nanoscience and Nanotechnology Initiative, National University of Singapore, Singapore, [3]School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore, [4]Obstretics and Gynecology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, [5]Department of Obstretics and Gynecology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, [6]Faculty of Biomedical Engineering, Technion-Israel Institute of Technology, Haifa, Israel, [7]Department of Cardiac, Thoracic and Vascular Surgery, National University Heart Centre Singapore, National University Health System, Singapore

Hydrogel biomaterials are becoming more prevalent as scaffolds in cell therapy and tissue engineering. Advantages of hydrogel include their tissue-like water content and controllable mechanical properties. The scaffold's mechanical properties are known to critically influence anchorage-dependent cells' behavior. Recent advances in hydrogel design have introduced semi-synthetic biomaterials that are capable of activating cell signaling and biodegradation via cell-mediated proteolysis. In this study, we evaluated the influence of our hydrogel properties on fetal mesenchymal stem cell (fMSC) proliferation and morphology with a specific focus on scaffold modulus. Our semi-synthetic hydrogel consists of fibrinogen peptide, a biologically active component for cells that is conjugated with polyethylene-glycol (PEG), an inert polymer in order to create a protein-polymer mosaic (PEG-fibrinogen). PEG precisely interacts with fibrinogen according to its relative size and quantity of the conjugation sites to alter both physical and biochemical properties of the cell culture environment. Five different scaffold formulations were prepared with shear modulus ranging from 30 to 2400 Pa. The scaffolds moduli were measured using a strain rate controlled rheometer. fMSCs were seeded on top of the PEG-fibrinogen hydrogel for 2D culture or encapsulated within it for 3D culture either singly or in aggregates. They were cultured in 2D and 3D for 1 and 2 weeks respectively. Cellular morphogenesis in the PEG-fibrinogen hydrogels was documented using confocal laser scanning microscopy (CLSM). The hydrogels stiffness modulus was correlated with specific patterns of fMSC morphogenesis observed by CLSM. Cell proliferation and viability assessment were performed using alamarBlue and LiveDead assay respectively. Phalloidin staining was done to assess the cell morphology and actin organization. Cell survival was excellent in both environments throughout the period studied. In 2D environment, higher cell proliferation rate was observed when cultured in PEG-fibrinogen hydrogel with lower modulus (≈30Pa) but degree of cell elongation was lowest in this hydrogel. Singly-seeded fMSCs migrated towards each other, forming clusters before elongating on the hydrogel surface and invading the hydrogel beneath them. At lower modulus, the cells first showed flattened morphology before remodeling themselves into tube-like networks.

At higher modulus, they formed flattened, well-spread morphology throughout the 2D culture. When seeded in aggregates, they elongated and formed extensive multicellular tube-like networks in all hydrogel moduli. On the other hand, fMSCs proliferated better in stiffer hydrogel (≈800 to 1200Pa) when cultured in 3D. fMSCs also migrated towards each other, forming clusters before elongating themselves within the hydrogels. Changes in 3D cellular morphology was dynamic with alternating flattened to tube-like network in hydrogel moduli of 30 to 800Pa and only wide spread out flattened morphology in hydrogel moduli of 1200 to 2400Pa. In both environments, optimal cell density was crucial before any remodeling was observed. Extensive formation of lamellipodia and filopodia were clearly observed in the later time period of the experiments.The data obtained from this study can be applied towards rational scaffold design for different medical applications and also contribute in the optimization of cell culture in cell responsive hydrogel scaffolds.

F-2103
E-CADERIN-FC CHIMERIC PROTEIN-COATED CELL-COOKING PLATE IS A SPECIAL DEFINED MATRIX THAT GENERATES SINGLE-CELL PHENOTYPE OF STEM CELL CULTURE AS WELL AS ENHANCED DIFFERENTIATION TO AND IN-SITU PURIFICATION OF TARGET LINEAGE FROM STEM CELLS

Akaike, Toshihiro
Tokyo Institute of Technology, Yokohama, Japan

Culturing stem cells on defined matrices is critical for application of relevant products in regenerative medicine and tissue engineering protocols. E-Cadherin is a marker for pluripotent stem cells that mediate tight interaction of neighboring cells, which has been known critical for survival of stem cells. We have exploited this classical phenomenon, and by fusing the N terminal extracellular domain of E-Cadherin (E-Cad) with the Fc domain of IgG have generated a highly efficient selective cell-recognizable biomaterial E-Cad-Fc. E-Cad-Fc-coated plate, we termed as cell-cooking plate, was sufficient to maintain murine ESC and iPSC without any need of feeder layer or other matrices. Remarkably, unlike normal tight-colony phenotype, the cultured ESC and iPSC showed single-cell phenotype on E-Cad-Fc cooking-plate. We further found that such single colony phenotype also can be obtained for human ESC and iPSC on E-Cad-Fc cooking-plate by controlling ROCK-Rho pathway. Obtaining of such single-cell phenotype is very special compared with any other known defined or non-defined stem cell culture system including recently reported laminin-511 and -521. Such single-cell phenotype stem cell culture showed superior transfection efficiency and proliferation property. Normal tight-colony phenotype is associated with heterogeneous environment and resulted mixed cell populations for any targeted differentiation protocols. However, our E-Cad-Fc cooking-plate generated much higher purity of target cells as observed for hepatocyte and neural differentiation protocols. Additionally, we have recently, for the first time, characterized the cell-division properties of murine stem cells at single-cell level on E-Cad-Fc cooking-plate. Directed differentiation of stem cells need understanding of cellular signaling circuitry, and to the best of our knowledge E-Cad-Fc is the only defined matrix that generates single-cell phenotype of stem cells therefore it provides unique scope to analyze the precise signaling cascade in stem cell at a single-cell level. Collectively, E-cad-Fc is a superior cell-recognizable biomaterial for coating of cell culture plates for establishing xenogeneic-agent free monolayer stem cell culture and their maintenance, enhanced directed differentiation to specific lineages, and non-enzymatic on-site one-step purification of target cells.

F-2104
REDUCED OR NO FEEDING PROCEDURE FOR HESCS ON LN521 MATRIX

Alatar, Shemim, Kallur, Therese, Ericsson, Jesper, Sun, Yi
BioLamina, Stockholm, Sweden

The conventional methods for culturing human embryonic stem cells (hESCs) have required a daily-based medium change. Apart from being tedious and private life disturbing because of weekend feeding, it is also very costly due to the large amount of medium consumed during the whole culture period. Moreover, frequent medium changing can result in unnecessary environmental stressing to the cultured cells. Recently Rodin and colleagues have shown that LN-521™, a human recombinant laminin protein and naturally expressed in the inner cell mass of the early embryo, allows robust long-term self-renewal of human pluripotent stem cells. LN-521™ provides a biorelevant niche for hPSCs and we are now able to show that hESCs can be cultured more efficiently and economically even without the need of daily feeding. In this study, three different feeding patterns, daily feeding, one feeding and no feeding, were applied to maintain hESC line HS181 on LN-521™ for 10 consecutive passages. Data show no significant differences observed between the three feeding patterns based on the microscopic observation of cell morphology and proliferation. After only 4 days the single-cell plated cultures were ≥80% confluent and cell viability was at all times ≥90% for all three groups. The pluripotency of cells after 10 passages was evaluated with immunocytochemistry (ICC) and the results show >90% Nanog expression, and >80% Oct-4 expression. RT-PCR showed consistent result with ICC staining in terms of transcription levels of Nanog and Oct-4. Moreover, no differentiation marker (Pax6, Brachyury, and sox17) was detected. Chromosome analysis after 10 passages indicated normal female karyotype for all groups. Our preliminary conclusion is that by culturing hESCs on the biologically relevant niche-protein LN-521™ reduced feeding frequency or no feeding at all is possible in an easier and more predictable manner than previously shown. Ultimately this benefit most researchers in terms of both labor and material cost savings and also allows for more standardized culture protocols.

F-2105
LIPOFECTAMINE® 3000: A NEW TRANSFECTION REAGENT FOR IPSC GENERATION AND STEM CELL GENOMIC ENGINEERING

Andronikou, Nektaria, Geng, Yue, Roark, Natasha, Yu, Xin, Sridharan, Mahalakshmi, Lakshmipathy, Uma, de Mollerat du Jeu, Xavier
Life Technologies, Thermo Fisher Scientific, Carlsbad, CA, USA

Stem cells, specifically induced pluripotent stem cells (iPSCs), hold immense promise for the future of regenerative medicine and personalized therapeutic treatments for a myriad of diseases and conditions. However, the lack of advanced technologies has been hindering the current pace of research and discovery. One of the greatest challenges lies in the manipulation of stem cells. Lipofectamine® 3000, a new transfection reagent, has been developed for nucleic acid delivery to enable the use of new technologies for stem cell applications. In the present study, we first demonstrated that Lipofectamine® 3000 can be used to efficiently deliver the EPi5™ episomal reprogramming vectors to BJ skin fibroblasts for the generation of iPSCs. This method allows researchers to perform efficient in-situ reprogramming at lower cost, providing a great alternative over the standard electroporation techniques typically used for iPSC generation. Furthermore, it was discovered that Lipofectamine® 3000 can achieve optimal transfection efficiency of various sizes of plasmid DNA and low toxicity in both

embryonic stem cells (ESCs) and iPSCs which have been traditionally proven to be hard to transfect. More importantly, manipulation of stem cells can be achieved utilizing TALENs and CRISPRs for genome engineering purposes. Transcriptional activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPRs) allow for editing and engineering of genomic DNA at specific loci. However, the effectiveness of these tools depends on the intrinsic properties of the locus of interest and efficient delivery. Lipofectamine® 3000 demonstrated efficient delivery of TALENs and CRISPRs into various iPSC clones and H9 human embryonic stem cells for targeted genome engineering. Taken together these advancements in delivery greatly improve downstream workflows, enable easier stem cell manipulation, and enhance site-specific insertion or deletion of transgenes in the cellular genome for the generation of knock-in or knock-out cell models and transgenic small animal models.

F-2106
ADVANCING HUMAN EMBRYONIC STEM CELL BANKING PROCESSES USING 3-D SUSPENSION CULTURE SYSTEMS

Bahia, Harsharon K., Young, Lesley, Stacey, Glyn
UK Stem Cell Bank, National Institute for Biological Standards and Control, Potters Bar, United Kingdom

The increasing use of stem cells in understanding cellular development, modelling disease, toxicological studies and as the basis of novel regenerative therapies is resulting in an increased demand. Traditional methods of producing banks of human embryonic stem cells (hESCs) need to be advanced to support the requirement for reproducible large-scale production and maintenance of undifferentiated hESCs. The UK Stem Cell Bank (UKSCB) produces banks of undifferentiated hESCs for worldwide distribution and continually strives to optimise methodologies for production of hESC banks whilst reducing production costs and minimising labour requirements. Traditionally, hESCs have been grown as 2-D adherent cultures on various matrices such as layers of fibroblast feeder cells or on culture vessels coated with extracellular matrices. These methods are labour intensive and have limited ability to meet the increasing demand for scaled-up production of undifferentiated hESCs. The UKSCB has been evaluating the abilities of commercially available 3-D culture systems to support increased yield of undifferentiated hESCs in a cost and time effective manner. We have compared the morphology and viability of hESCs grown in 2-D adherent cultures with cells grown in 3-D suspension cultures. For adherent cultures cells were grown in 6-well plates on gelatin, laminin, extracellular matrix and on layers of mouse embryonic fibroblasts (MEFs). For suspension cultures, cells were grown in 50 ml vessels with and without beads coated with the same matrices used for 2-D cultures, with the exception of MEFs. With the microcarrier bead system hESCs attached covalently to the matrix coating of the beads and were able to proliferate across the surface of the bead. Several hESC lines were maintained for five serial passages by both adherent and suspension culture systems. Suspension cultures produced a greater yield of undifferentiated hESCs with 20-25% increase being observed with the microcarrier bead system in comparison to 2-D cultures of hESCs on MEFs. In addition, suspension cultures utilised less medium than adherent cultures, thus the 3-D systems produced a greater cell yield, in less time with reduced volumes of media. Cells that had been cultured in suspension systems were seeded onto MEFs and post attachment they displayed normal undifferentiated stem cell morphology. Viability of undifferentiated hESC cultures was assessed at each sub-culture point and no loss of viability was detected with the 3-D culture system whereas 1-2% loss of viability was observed in adherent culture systems. Furthermore, the microcarrier bead system was deemed suitable for cryopreservation with >80% post-thaw viability being observed. Work is currently being undertaken to assess the maintenance of the undifferentiated state of hESCs and their differentiation potential when cultured in 3-D suspension systems. Initial findings indicate that adopting 3-D suspension culture systems into the banking process will support scaled-up production of cell banks for distribution in a more cost and time effective manner than is currently being achieved with adherent culture techniques.

F-2107
NEW MEDIUM SUPPORTING THE ACTIVITY AND FUNCTION OF HUMAN IPSC-DERIVED NEURONS IN VITRO

Bardy, Cedric[1], Van den Hurk, Mark[1], Marchand, Cynthia[1], Eames, Temaji[1], Kellogg, Mariko[1], Boyer, Leah[2], Simon, Suzie[1], Gage, Fred H.[2]
[1]Sanford Consortium for Regenerative Medicine, Salk Institute, La Jolla, CA, USA, [2]Salk Institute, La Jolla, CA, USA

Induced pluripotent stem cell (iPSC) technologies offer access to live human neurons derived from patients and a new alternative to model neurological and psychiatric disorders in vitro. Effective models of neuronal circuits require physiological conditions that sustain neuronal functions. Therefore, we specifically examined the neuronal activity in different media currently used to culture neurons. Neurons from rodents and humans are routinely grown in vitro in basal media with a variety of supplements. We found that classic media based with Neurobasal and DMEM strongly impair fundamental neuronal functions, including action potential firing and synaptic communication. To overcome these limitations and better reproduce in vitro the physiological conditions of the human brain, we designed a new medium (BrainPhys). This new medium supports optimal electrophysiological activity like physiological cerebrospinal fluids in vivo, but in addition it also sustains optimal long-term survival in-vitro. BrainPhys was essentially designed to culture healthy and functionally mature human neurons derived from patient iPS cells. Importantly it provides more realistic in vitro conditions to model human neurological diseases. We believe this in turn will likely contribute to the discovery of new relevant treatments and improve translational success.

F-2108
TETR-REGULATED ALL-IN-ONE LENTIVIRAL VECTORS FOR CONDITIONAL EXPRESSION IN HUMAN EMBRYONIC STEM CELLS.

Benabdellah, Karim[1], Muñoz, Pilar[1], Garcia-Perez, Angelica[2], Gutierrez-Rodriguez, Alejandra[1], Cobo, Marién[1], **Martin, Francisco[1]**
[1]Genomic variability, GENYO. Centre for Genomics and Oncological Research, Fundación Pública Andaluza Progreso y Salud, Spain, [2]Mobile DNA, Max-Delbrück-Center for Molecular Medicine, Berlin, Germany

Objective: Human embryonic stem cells (hESCs) are pluripotent cells derived from the inner cell mass of human embryos. These cells have the unique potential to differentiate to any cell type of fetal and adults' tissues. These proprieties confer hESCs a great potential for basic research and for future use in regenerative medicine. However, the generation of doxycycline-responsive hESCs lines expressing different transgenes has been very difficult and time-consuming to achieve due to the low efficiency of existing delivery methods, the strong silencing of the transgenes, and the toxicity of the regulators. Here, we described an efficient and non toxic method, based on lentiviral-TetR vectors for the conditional gene expression in hESCs and their differentiated derivatives. Methods: We optimized our previously published CEST All-In-One Lentiviral vectors: 1. We modify the TetR protein including two different nuclear localization signals (TetRnls1 and TeRnls2). 2. We use the EF1alpha promoter to express TetR instead of the SFFV

promoter. 3. We combined several insulator sequences to favour stability of expression of the different components. Our final constructs, named CEETnls2 and CEETnls2IS2 were able to obtain conditional expression in hESCs after one single transduction. Results: CEETnls2 and CEETnls2IS2 were able to obtain conditional expression in hESCs after one single transduction and in the absence of any selection. This conditional expression was maintained in undifferentiated hESCs under different passages as well as in hESC-derived cells. We have demonstrated a doxycycline-dependant expression of eGFP on hESCs derived hemangioblasts (CD31+CD45-), hematopoietic progenitors (CD34+CD45+) and mature hematopoietic cells (CD34-CD45+). Conclusions: This is to our knowledge the first All-In-One Dox-regulated vector system that allow the generation of Dox-regulated hESCs lines in the absence of drug selection.

F-2109
DEVELOPMENT OF AN AUTOMATED IMAGING-BASED SCREENING ASSAY FOR PLURIPOTENT STEM CELL RESPONSES TO COMMERCIALLY AVAILABLE SUBSTRATES

Blit, Patrick[1], **Khattak, Shahryar[1],** Norman, Andreea A.[1], Titus, Emily[1], Alvarez, Manuel[1], Fromstein, Joanna D.[1], Zandstra, Peter W.[2], Timmins, Nicholas E.[1]

[1]Centre for Commercialization of Regenerative Medicine (CCRM), Toronto, ON, Canada, [2]University of Toronto Institute of Biomaterials and Biomedical Engineering, Toronto, ON, Canada

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) have broad applicability for cell therapy, regenerative medicine and personalized medicine applications. Originally derived and cultivated on feeder cells, a variety of substrates are now available that enable feeder-free pluripotent stem cell (PSC) culture. These feeder-free approaches eliminate the risks associated with contamination from feeder cells and reduce process variability. As the performance of any given PSC line may vary on different substrates, the objective of this study was to develop a rapid screening method in order to inform substrate choice and the development of next generation stem cell maintenance substrates. Eight commercially available substrates and Matrigel™, which served as the control, were prepared as per manufacturer's instructions in a 48-well plate format. A single-cell adapted hES2 cell line was seeded onto the substrates at a density of 20,000 cells/cm2 and grown using mTesR™ medium. Both a fluorometric DNA based assay (Cyquant®, Life Technologies) and an imaging based assay (BioStation CT, Nikon) were used to determine cell responses to the substrates. Percent cell adhesion was assessed 24 hours post-seeding and proliferation was monitored over a 3 or 4 day period. Cell adhesion results were normalized against results obtained on Matrigel™ substrates, and cell expansion was calculated as the increase in cell number, or cell area at a given time-point divided by the same measure at 24 hours. After 4 days of culture, cell seeded substrates were immunostained to determine Oct4 expression and imaged on the BioStation CT. No significant differences in cell adhesion were observed at 24 hours post-seeding using the fluorometric Cyquant® assay or the BioStation CT. However, after 3 days of culture, phase contrast images of the substrates demonstrated clear differences in cell morphology and cell number on the various substrates. Cell expansion data were indicative of substrate-specific differences in proliferation, with the Cyquant® assay demonstrating four substrates performing comparably to Matrigel™. The BioStation CT method showed enhanced discrimination between substrates, indicating only three substrates with comparable proliferation rates as observed on Matrigel™. Immunostaining revealed differential Oct4 expression for the various substrates, with three commercial substrates demonstrating similar expression levels as on Matrigel™. In conclusion, this study demonstrated the feasibility of using the BioStation CT to develop a rapid and effective screening assay for PSC substrates able to detect cell adhesion, proliferation, and Oct4 expression at levels comparable to Matrigel™ in two of the eight commercially available substrates. This non-destructive screening tool was demonstrated to allow for automated acquisition of data related to cell morphology, adhesion, proliferation and pluripotency all in one analytical device, while maintaining temperature, humidity and carbon dioxide levels.

F-2110
ASSESSING THE MODULATION OF CELLS BY MODIFIED MRNAS IN VITRO AND IN VIVO

Wild, Stefan[1], Beclin, Christophe[2], Jurk, Marion[1], Diener, Yvonne[1], Bissels, Ute[3], Cremer, Harold[2], Knoebel, Sebastian[4], **Bosio, Andreas**[1]

[1]R and D, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany, [2]CNRS-IBDM, Marseille, France, [3]Miltenyi Biotech GmbH, Bergisch Gladbach, Germany, [4]Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

The transfer of genetic information to cells is in the center of interest as it allows deciphering the function of genes and proteins and programming of cells. A wealth of methods have been described for transient gain or loss of function experiments and an impressive toolbox is available for modification of of genes including Cre and FLP recombinase, Zinc finger nucleases, TALEN, CRISPR/CAS. Due to the elaborated defense mechanisms of cells, all these methods depend on the efficacy of delivering genetic material and are hampered by potential genotoxicity. In our study we aimed at exploring the versatility of mRNA to i) extend DNA free gain of function experiments to cells which have been difficult to address *in vitro* and *in vivo* and ii) to increase the efficacy and precision of recombination events *in vitro* and *in vivo*. To this end we have set up a standardized, animal component free, sterile workflow to generate mRNAs with optimized 5´- and 3´-UTRs, human codon optimized sequences and optionally Ψ-UTP and 5-mCTP modified nucleotides. We have validated the performance of our mRNA in a number of cell lines, primary cells, and pluripotent stem cells and derivatives thereof. When transfecting eGFP mRNA into human fibroblasts we gained a transfection efficiency of up to 90% (n=12) with an average survival of 90-99%. Electroporating CD133(+) hematopoietic stem cells with eGFP mRNA (n=10) yielded 97% (+/-2.9%) GFP(+) cells after 24h with a survival of 45% (+/-17.8%). eGFP expression was detectable for more than 7 days. *In vivo* we observed that postnatal brain electroporation of eGFP mRNA showed considerably higher transfection efficiencies in neural stem cells than DNA based approaches. Moreover, a wider spectrum of cells, including fully differentiated ependymocytes, that are refractory to DNA electroporation, could be efficiently targeted *in vivo*. We next assessed mRNAs in gain of function experiments by transferring transcription factors to primary cells. Notably, we could successfully reprogram newborn fibroblasts to pluripotency with 10-12 consecutive transfections using different sets of transcription factors. Finally, we explored the recombination efficiency of iCRE mRNA when transfecting or electroporating mES cells carrying a GAGG-Stop(flox)-eGFP cassette. We observed a very high recombination efficiency in transfected cells resulting in up to 98% GFP(+) cells. The rate of GFP(+) cells correlated with the amount of iCRE mRNA indicating the possibility to control the degree of recombination rate. Ongoing experiments are addressing *in vivo*-recombination and fate switching of cells as well as direct transfection of mature neurons in the brain. Altogether we demonstrate that mRNAs are a versatile, broadly applicable, fast acting agent with low toxicity *in vitro* and *in vivo* showing an extraordinary high efficiency for gain of function and DNA recombination experiments.

F-2111

UNDERSTANDING TRANSLATIONAL REGULATION OF MUSASHI1 (MSI1) RNA BINDING PROTEIN USING RNA-SEQ OF RIBOSOME PROTECTED MRNA FRAGMENTS

Brady, Lee[1], Kuersten, Scott[1], De Araujo, Patricia[2], Vo, Dat[2], Burs, Suzanne[2], Qiao, Mei[2], Radek, Agnes[1], Bahrami, Emad[3], Uren, Phil[3], Smith, Andrew[3], Penalva, Luiz[2]
[1]Illumina, Madison, WI, USA, [2]University of Texas Health Science Center, San Antonio, TX, USA, [3]University of Southern California, Los Angeles, CA, USA

Musashi1 (Msi1) is an evolutionarily conserved RNA-binding protein that has been implicated in a variety of cellular processes such as stem cell maintenance, nervous system development, and tumorigenesis. Msi1 is highly expressed in many cancers including glioblastoma, and is emerging as a potential therapeutic target in both regenerative medicine and cancer. Our goal is to better understand the regulatory role of Msi1 by identifying the RNAs targeted by Msi1 at the level of translation. Our approach is to use RNA-Seq to compare total mRNA to the ribosome protected mRNA fragments (ribosome profiling or ribosome footprinting) in order to obtain quantitative analysis of translationally active versus repressed pools of transcripts present at a particular time or condition. In the course of our work, we developed a simplified method for preparing RNA-Seq libraries from Ribosome Protected fragments sequencing. Existing ribosome-profiling protocols require sucrose-gradient ultracentrifugation, many purification steps and can take 5-7 days to perform. The resulting RNA-seq libraries are frequently highly contaminated with unwanted rRNA sequences. In contrast, we have streamlined these protocols to replace ultracentrifugation with a simple and rapid column-based procedure and have eliminated many of the purification steps. In addition, we incorporate an rRNA removal technology that greatly reduces the rRNA contamination in the library. Our results show that using this new method of producing RNA-seq libraries from ribosome protected fragments offers a viable approach to understanding the translational regulation role of Msi1.

F-2112

A NOVEL SYSTEM TO DISSECT PROTEIN FUNCTION IN MAMMALIAN STEM CELLS

Brosh, Ran, Hrynyk, Iryna, Shen, Jessalyn, Lemischka, Ihor
Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

We have developed a novel strategy to study gene function, specifically adapted for use in mammalian stem cells. This strategy features shRNA-based silencing of an endogenous gene-of-interest, complemented by expression of a degradable protein-of-interest. Our system imports key components of the plant hormone-induced degradation pathway into mammalian cells. Plant-specific hormone receptors are expressed, which, upon binding their cognate media-supplemented hormones, recruit the SCF ubiquitin ligase complex to target proteins that are marked by a specific degron domains, thereby promoting their degradation. The system is designed as a single lentiviral vector and allows efficient, rapid, titratable, and reversible control of protein degradation. Importantly, by utilizing different sets of plant hormones, hormone-receptors and degrons, our system offers combinatorial studies of protein function that are otherwise unfeasible.

F-2113

BARCODING PROTEINS FOR SELECTION VIA CDNA DISPLAY

Bruckner, Annalise Michelle
Bioengineering, University of California, San Diego, San Diego, CA, USA

We are currently developing a proximity ligation based technology to study protein-protein interactions. Our goal is to develop a cDNA display, which is a stable improvement upon mRNA display, an in vitro display technology used to screen for proteins of interest via puromycin linker. Here we use the protein-cDNA fusion to select for proteins with desired properties and enrichment of proteins that bind to a target of interest, while tagging the protein with its cDNA as a barcode. Here, we describe the use of cDNA display as a powerful selection tool to screen the proteome of embryonic stem cells (ESCs) using the self-contained transcriptome, specifically mRNA isolated from E14 mouse ESCs. Following double stranded cDNA synthesis, we attach directional linkers and cassettes on both ends of the cDNA. The 5' cassette contains the T7 promoter sequence, which allows for the directional RNA synthesis, and a Kozak sequence to facilitate the protein translation. After size selection, the cDNA was amplified using PCR. Subsequently, we perform in vitro transcription, add a linker containing puromycin at the 3' end to enter the ribosomal A site and bind to the nascent amino acid chain, and conduct in vitro translation. Following the in vitro translation, we will use standard cDNA synthesis to produce double stranded cDNA attached to the protein. This technology has a broad range of applications and similar technology has been utilized to understand enzyme-substrate interactions and identify drug-binding targets.

F-2114

A FLUORESCENCE-BASED IMAGE CYTOMETRY METHOD FOR ENUMERATION AND VIABILITY MEASUREMENT OF CANINE STROMAL VASCULAR FRACTION CELLS

Chan, Leo Li-Ying[1], Cohen, Donald A.[2], Kuksin, Dmitry[1], Paradis, Benjamin D.[1], Qiu, Jean[1]
[1]Technology R and D, Nexcelom Bioscience, Lawrence, MA, USA, [2]Microbiology, Immunology, and Molecular Genetics, University of Kentucky, Lexington, KY, USA

In recent years, the lipoaspirate collected from adipose tissue has been seen as a valuable source of adipose-derived mesenchymal stem cells for autologous cellular therapy. For multiple applications, adipose-derived mesenchymal stem cells are isolated from the stromal vascular fraction (SVF) of adipose tissue. Because the fresh stromal vascular fraction typically contains a heterogeneous mixture of cells, determining cell concentration and viability is a crucial step in preparing fraction samples for downstream processing. Due to a large amount of cellular debris contained in the SVF sample, as well as counting irregularities standard manual counting can lead to inconsistent results. Advancements in imaging and optics technologies have significantly improved the image-based cytometric analysis method. In this work, we validated the use of fluorescence-based image cytometry for SVF concentration and viability measurement, by comparing to standard flow cytometry and manual hemocytometer. The concentration and viability of freshly collected canine SVF samples are analyzed, and the results are highly correlated all three methods, which validated the image cytometry method for canine SVF analysis, and potentially for SVF from other species.

F-2115

A NUCLEOLUS-PREDOMINANT PIGGYBAC TRANSPOSASE, NP-MPB, MEDIATES ELEVATED TRANSPOSITION EFFICIENCY IN HUMAN AND MOUSE EMBRYONIC STEM CELLS

Chen, You-Tzung[1], Hong, Jin-Bon[1], Chou, Fu-Ju[1], Ku, Amy T.[1], Fan, Hsiang-Hsuan[1], Yang, Tsung-Lin[2], Yu, I-Shing[3], Lin, Shu-Wha[4], Chien, Chung-Liang[5], Ho, Hong-Nerng[6]

[1]Institute of Medical Genomics and Proteomics, National Taiwan University College of Medicine, Taipei, Taiwan, [2]Department of Otolaryngology, National Taiwan University College of Medicine, Taipei, Taiwan, [3]Laboratory Animal Center, National Taiwan University College of Medicine, Taipei, Taiwan, [4]Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University College of Medicine, Taipei, Taiwan, [5]Institute of Anatomy and Cell Biology, National Taiwan University College of Medicine, Taipei, Taiwan, [6]Department of Obstetrics and Gynecology, National Taiwan University College of Medicine, Taipei, Taiwan

PiggyBac is a popular transposon system in embryonic stem cells and cell lines. The important features of piggyBac transposon are the relatively low insertion site preference and the ability of seamless removal from genome, which allow its potential uses in functional genomics and regenerative medicine. The function of piggyBac transposase (PBase) has been demonstrated to remain intact when PBase is fused with different proteins, such as with the Gal4 or zinc-finger DBD, to create a targeted transposition system. In addition, the PBase has been revised in several ways by engineering the corresponding transposase to improve its transposition efficiency. Through screening for mutant variants, hyperactive PBase was generated. In this study, we developed a nucleolus predominant PBase, NP-mPB, by adding a nucleolus predominant (NP) signal peptide from HIV-1 TAT protein to a mammalian codon optimized PBase. We demonstrated that NP-mPB was restricted to the nucleus and associated with fibrillarin and cyclin T1. Unexpectedly, the transposon insertion sites were relatively random, except for a tendency to be in the gene region, in the study of mouse embryonic stem cells. There was no obvious preference for the nucleolar organizer regions. Instead a 3-fold increase in piggyBac transposition efficiency was reproducibly observed in human and mouse embryonic stem cells.

F-2116

GENERATION OF A CRISPR/CAS9-MEDIATED GENE KNOCKOUT LIBRARY USING A HUMAN PLURIPOTENT STEM CELL CRISPR GUIDE RNA KNOCK-IN LINE

Chen, Shuai[1], Mercy, Guillaume[2], Veres, Adrian[3], Hendriks, William[4], Cowan, Chad[4]

[1]Faculty of Biomedical Science, University of Amsterdam, Amsterdam, Netherlands, [2]Systems and Synthetic Biology, University of Evry Val d'Essonne, Evry, France, [3]Faculty of Arts and Sciences, Harvard Medical School, Boston, MA, USA, [4]Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA

The recently developed Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) RNA-guided Cas9 nuclease-mediated genome engineering technology has rapidly become one of the most important tools for stem cell biology, enabling researchers to edit almost any DNA sequence in the genome of (human) pluripotent stem cells (hPSCs). The RNA-guided Cas9 is targeted to a genomic site by complexing with a synthetic guide RNA that hybridizes a 20-nucleotide DNA sequence beginning with G and immediately preceding an NGG motif recognized by Cas9. Introducing a guide RNA with Cas9 into hPSCs creates a double-strand break (DSB) in the targeted gene that is either repaired by error-prone non-homologous end joining (NHEJ) or homology-directed repair (HDR). The power of CRISPR/Cas9-mediated gene editing for interrogating gene function genome wide has recently been shown by two independent labs that used a CRISPR-RNA library to knockout thousands of genes in mammalian cell lines. The work presented here tries to build on those studies and describes the generation of hPSC gene knockout lines using Transcription Activation-Like Effector Nuclease (TALEN)-mediated targeting of a DNA donor vector containing an antibiotic selection and CRISPR guide RNA expresssion cassette to the AAVS1 safe harbour locus in an hPSC line. Subsequent transient overexpression of Cas9 in successful targeted cell lines results in a DSB at the target locus that is repaired by NHEJ. This will cause a frameshift mutation and essentially knock out the targeted gene. Currently, we are building DNA donor vectors with CRISPR guide RNA sequences targeting 10 different loci in hPSCs using this approach. Successful completion of this pilot study will enable us to adapt a more high-throughput approach in order to build a genome wide library of hPSC-knockout lines. Such a hPSC knockout library would be an extremely valuable resource for the stem cell research community.

F-2117

SELECTIVE PHOTOTOXICITY OF PLURIPOTENT STEM CELLS LOWERS TERATOMA RISK

Cho, Seung-Ju[1], Kim, So-Yeon[2], Moon, Sung-Hwan[3], Cha, Hyuk-Jin[1]

[1]College of Natural Sciences, Deptartment of Life Sciences, Sogang University, Seoul, Republic of Korea , [2]Sogang University, Seoul, Republic of Korea, [3]Department of Biomedical Science, College of Life Science, CHA University, Pochon, Republic of Korea

Risk of teratoma formation from the residual undifferentiated pluripotent stem cells (PSCs) has been considered to be one of the most critical technical barriers in PSCs based cell therapy. Although, a number of approaches have been developed to resolve this, among them, utilization of stem-toxic small molecules, which can deplete undifferentiated PSCs by promoting selective cell death, cannot be free from the critic of unexpected toxicity or side effect on the differentiated cells despite its relatively high efficiency. Herein, we took advantage of light-sensitive suicide gene to amply the phototoxicity that can be induced by light through production of reactive oxygen species (ROS) at the mitochondria. Thereby, PSCs of which suicide gene expression was designed to precisely restricted, while pluripotency is maintained, undergo selective phototoxicity but not differentiated cells, after light exposure. Due to the high selectivity of phototoxicity in PSCs expressing suicide gene, teratoma formation after light exposure can be inhibited. In special, endothelial cells (ECs) derived from suicide gene expressing PSCs remain functional *in vitro* and *in vivo*, suggesting that genetic approach to express suicide gene would be a viable strategy to inhibit teratoma formation by selectively inducing phototoxicity of the residual undifferentiated PSCs for future safe PSCs based therapy.

F-2118

LARGE SCALE PRODUCTION OF HIGH PURITY HUMAN CYTOKINES FROM HUMAN CELLS TO ENABLE CLINICAL MANUFACTURING OF CELL THERAPY PRODUCTS.

Doctor, Sean

Humanzyme, Chicago, IL, USA

Cytokines play an essential role in expansion and differentiation of human stem cells, which are often the single largest component of the cost for clinical manufacturing. Clinical production of cell lines for cell therapy requires large amounts of high purity cytokines and growth factors. HumanZyme is a world leader in providing highly authentic serum free/xeno-free (SF/XF) cytokines that conventional non-human

cell expression systems either cannot produce at all or cannot produce economically. We are currently offering over 50 high purity human cytokines and growth factors. All our proteins are expressed in human cells to ensure authentic glycosylation and correct post translational processing. HumanZyme plans to bolster its leadership position in enabling clinical production of human cell therapy products by scaling up production of major cytokines essential for human stem cell production including Activin A, TGF β-1, and thermo-stable FGFbasic. HumanZyme products are made under strict quality control to meet our customer needs. In addition, we are committed to provide cytokines in compliance with cGMP guidelines in foreseeable future.

F-2119
EFFICIENT CRYOPRESERVATION OF HPSCS IN A DEFINED SERUM XENO AND DMSO FREE FREEZING MEDIUM PRODUCED ACCORDING TO CGMP

Ericsson, Jesper[1], Hagbard, Louise[1], Sun, Yi[1], Karlsson, Camilla[2], Kallur, Therese[1]
[1]BioLamina, Stockholm, Sweden, [2]Vitrolife, Gothenburg, Sweden

Human pluripotent stem cells (hPSCs) show great promise in regenerative medicine, drug discovery, as well as importance for basic research. It is therefore a priority to establish efficient, user-friendly, and robust methods for bulk hPSCS cryopreservation. So far, obtaining good survival and high quality cultures after thawing have been problematic when using current slow-freezing protocols since most cryopreservation media contain dimethyl sulfoxide (DMSO) which have been reported to have toxic effects and promote cellular differentiation. We have developed a novel, chemically defined, xeno- and dimethyl sulfoxide (DMSO)-free cryopreservation medium denoted FREEZEstem. FREEZEstem is produced according to current good manufacturing practice (cGMP) and ISO13485 and is optimized for single-cell slow freezing for cryostorage and subsequent banking of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), ideal for a defined, xeno-free hPSC culture system supporting single-cell passage such as the LN-521™. Human pluripotent stem cells (hPSCs) frozen in FREEZEstem were compared to a commonly used cryomedium containing 10% DMSO. We found that the number of hPSC colonies growing 7 days post-seeding were similar however, by leaving the cells 4h post-thaw in room temperature and thereby testing acute toxicity, we counted more colonies emerging 7 days post-seeding when frozen in FREEZEstem. No differences were detected in the hPSC expression of stem cell markers (Oct-4 and Tra-1-60) or lack of expression of differentiation marker (SSEA1) early after thawing. Cells from both cryomedia tested formed embryoid bodies with components of the three germ layers present. FREEZEstem not only has the advantage of being cGMP manufactured and DMSO-free, it is also a user-friendly, robust freezing medium with very low toxicity enabling total control over hPSC cultures, thus offering an excellent, simple option for quality banking hPSCs.

F-2120
POSITIONAL INFLUENCE ON THE TRANSCRIPTIONAL ACTIVITY OF ENGINEERED TALE AND CAS9 ACTIVATORS

Hu, Jiabiao, **Feng, Bo**
School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China

The newly developed engineered transcription factors TALE-TF and CRISPR/Cas9-TF offered a powerful and precise approach for modulating gene expression. In this study, we found that these engineered transcription factors (TF) displayed a positional preference that has not been reported before. Targeting the silenced master pluripotency gene Oct4 in mouse and human somatic cells, TALE-VP64 binding to −120 ~ −80 bp region or sgRNA/dCas9-VP64 targeting −147 ~ −89 bp region upstream of transcription starting sites exhibited the highest activity to activate the transcription of Oct4 promoter. Similarly, highly effective TALE-VP64 also bind to a similar region in human NANOG, KLF4, c-MYC and CDH1 promoters. This finding could serve as a simple guideline for designing effective engineered TF. In addition, we observed synergistic effect among multiple combinations of TALE-VP64 and sgRNA, which resulted in up-regulation of the endogenous Oct4 transcription up to 30-fold in mouse NIH3T3 cells and 20-fold in human HEK293T cells. More importantly, this enhancement of OCT4 transcription ultimately generated mature OCT4 proteins. Furthermore, histone acetyltransferase p300 enhanced both TALE-VP64 and sgRNA/dCas9-VP64 induced transcription of Oct4. Taken together, our study suggested that the engineered TALE-TF and dCas9-TF are useful tools for modulating gene expression in mammalian cells.

F-2121
SCALABLE XENO-FREE CULTURE SYSTEMS FOR HUMAN INDUCED PLURIPOTENT STEM CELL EXPANSION

Badenes, Sara M.[1], **Fernandes, Tiago G.**[1], Boucher, Shayne E.[2], Kuninger, David[2], Vemuri, Mohan C.[2], Diogo, Maria Margarida[1], Cabral, Joaquim M.S.[1]
[1]Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, Lisboa, Portugal, [2]Thermo Fisher, Biosciences, Cell Biology, Life Sciences Solutions, Frederick, MD, USA

In order to fully realize the potential of human induced pluripotent stem (hiPS) cells for clinical applications in cellular therapy, the development of standardized and scalable processes to produce clinically relevant cell numbers, while maintaining critical biological functionality and safety, are of huge importance. Moreover, envisaging further bioprocess translation to Good Manufacturing Practice (GMP) standards, a great effort has also been made towards the translation of scalable culture systems to chemically-defined and xeno-free conditions. hiPS cell culture using E8 xeno-free medium was successfully implemented, under adherent and suspension conditions. For adherent culture, defined xeno-free matrices Synthemax II and vitronectin were chosen for long-term hiPS cell proliferation. These matrices were able to support the expansion of hiPS cells either in coated-plates or on polystyrene coated-microcarriers, and cells maintained their functionality and pluripotency features. The long-term expansion of hiPS cells as undifferentiated aggregates using E8 medium was also implemented. Xeno-free stirred suspension culture systems, either using adherent microcarriers or cell aggregates, therefore provided a potent approach for the scale-up of hiPS cell expansion under clinical-compliant conditions.

F-2122
EVALUATION OF HUMAN PLATELET LYSATE AS A CELL CULTURE MEDIA SUPPLEMENT IN CONJUNCTION WITH A CRYOPRECIPITATE-COATED BIOREACTOR FOR THE EXPANSION OF HUMAN MESENCHYMAL STEM CELLS IN THE QUANTUM SYSTEM

Frank, Nathan D., Nguyen, Kim, Peters, Rebecca, Hill, Domicinda M., Vang, Boah, Jones, Mark E., Killian, Rachel, Startz, Thomas
TerumoBCT, Lakewood, CO, USA

The number of institutions pursuing the development of cellular therapies based on the ex vivo culture of adherent human stem cells continues to grow. As such, the necessity for a scalable process that will yield a clinically relevant number of expanded cells in

a closed fashion is increasing significantly. The Quantum Cell Expansion System is a functionally closed and automated continuous perfusion hollow fiber bioreactor system that is designed to expand both adherent and suspension cells in a reproducible manner. Expansion of adherent cell types in the bioreactor of the Quantum system requires coating the membrane surface of the bioreactor's hollow fibers with an adhesion promoting compound to facilitate cell attachment and proliferation. Human fibronectin (FN) has been used as the standard adhesion promoter in the Quantum system. However, as FN can be a cost-prohibitive reagent, other, less costly alternatives have been explored. Pooled cryoprecipitate (CPPT) derived from human plasma is one such product that has already been demonstrated to be an effective adhesion promoter for human mesenchymal stem cells (hMSCs) in the Quantum system. Previous work using CPPT as an adhesion promoter in the Quantum bioreactor has been conducted using cell culture media supplemented with fetal bovine serum (FBS). However, as human cellular therapies move closer to wider release in consumer markets, concerns regarding potential contamination with zoonotic pathogens or altered cellular function due to infiltration of non-human elements into expanded cellular products must be addressed. Human platelet lysate (hPL) has been demonstrated to be an effective and reliable replacement for FBS as a source of proteins and cytokines in cell culture media. This study examines the efficacy of hPL as a suitable substitute for FBS as a culture media supplement when culturing hMSCs in a CPPT-coated Quantum bioreactor. hMSC from 4 donors were cultured in the Quantum system using the following combinations of adherence promoter and media supplements: FN and FBS or CPPT and hPL. Bioreactors were seeded with P1 hMSC from bone marrow aspirate that had been culled from a mixed population in the P0 stage using the Quantum system. 1.05 E^{+7} hMSC were seeded at an approximate density of 500 cells/cm^2 into a Quantum bioreactor that had been coated overnight with either 5mg single-donor-equivalent human CPPT or 5mg human FN. MSC were cultured for between 4.9 and 6.9 days in the Quantum system. Harvest products were evaluated by assessing total cell yield, cell morphology, biomarker expression, and potential for tri-lineage differentiation. The mean cell yield from Quantum harvests incorporating CPPT as an adherence promoter and cultured using hPL as a media supplement was 3.31E^{+8} cells (n=9); mean doubling time for this group was 30.47hrs. Quantum harvests using FN as an adhesion promoter and FBS as a media supplement had a mean cell yield of 2.52 E^{+8} cells (n=3); mean doubling time for this group was 31.00hrs. Cell products from all harvests displayed plastic adherence capability as well as fibroblastic morphology after 1-3 days in culture. Expression of CD73, CD90, and CD105 were all >95% while expression of CD14, CD19, CD34, CD45, and HLA-DR were <2% for all harvest products regardless of culture conditions. Tri-lineage differentiation assays demonstrated that cells from both the CPPT+hPL and FN+FBS harvests were capable of being induced to differentiate into adipocytes, osteocytes, and chondrocytes.

F-2123
SIMULATED MICROGRAVITY MODIFIES CDDP SENSITIVITY OF CANCER CELLS POSSIBLY THROUGH THE P53 PATHWAY

Fukazawa, Takahiro[1], Tanimoto, Keiji[2], Furukawa, Takuma[3], Ookura, Yunosuke[3], Otsuru, Naofumi[3], Kawahara, Yumi[1], Yuge, Louis[3]
[1]*Space Bio-Laboratories Co. Ltd., Hiroshima, Japan,* [2]*Department of Radiation Medicine, Hiroshima University, Hiroshima, Japan,* [3]*Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan*

We have interestingly demonstrated that simulated microgravity modifies CDDP-sensitivity of cancer cells, possibly through the p53-pathway. Although all of our biological system is governed by gravity on the earth, molecular mechanisms of its regulation are mostly unknown. Previously, we demonstrated that simulated microgravity using a 3D-clinostat allowed mouse embryonic stem (ES) cells proliferation in conditions of LIF-, serum-, and feeder cell-free culture, suggesting a novel method to maintain stemness. We thus tried to clarify the molecular mechanisms of acquired drug resistance relating to cancer stemness under simulated microgravity. In our experiments, conditions of simulated microgravity were created using an originally developed multi-directional gravity device, the Gravite (KITAGAWA IRON WORKS Co., Ltd., Japan), controlled by rotation of two axes, resulting 10^{-3} G average over time. A hepatocellular carcinoma, HepG2, and a breast cancer cell, MDA-MB-231, were firstly treated with CDDP, and found to be effectively suppressed their growth with 500 ng/mL of CDDP until 72 hours. Therefore, both cells were treated with 500 ng/mL of CDDP under normal 1G-environment (group 1G) or microgravity environment (group 0G). As a result, they surprisingly showed opposite responses: growth inhibition of HepG2 cells was significantly higher in group 1G, but that of MDA-MB-231 was higher in group 0G. Immunoblot analyses importantly demonstrated that CDDP-induced p53 protein expression in HepG2 cells decreased in group 0G, but did not in MDA-MB-231. Furthermore, activated caspase-3 in HepG2 cells detected in group 1G but did not in group 0G. These results interestingly suggested that microgravity decreased CDDP-induced p53 protein expression leading to anti-apoptotic effects.

F-2124
HIGH-THROUGHPUT DEVELOPMENTAL TOXICOLOGY SCREENING ASSAYS USING INDUCED PLURIPOTENT STEM CELLS

Elcheva, Irina A., Frazee, Scott A., **Garcia, Bradley H.**
Primorigen Biosciences Inc., Madison, WI, USA

Human induced pluripotent stem cells (hiPSC) and their capacity for differentiating into virtually any cell types present an attractive model for use in disease, drug discovery, and toxicology screening applications. Consistent and efficient differentiation of hiPSCs into specific lineages is paramount for evaluating the impact of drugs or other chemical compounds on human cells during different stages of development and maturation. The ultimate goal of this project was to establish feasibility of novel toxicity assay systems suitable for screening the effects of various compounds on a) hematopoietic progenitor cells (HPCs) and cardiomyocytes derived from hiPSCs and b) hiPSC differentiation into these lineages. The assay system was designed to feature i) robust and consistent miniaturized differentiation of hiPSCs to $CD45^+/CD43^+$, $CD34^+$ HPCs and beating cardiomyocytes (Troponin T+, α-Actinin+); ii) functionality across different hiPSC lines; and iii) screening formats suitable for high-throughput applications. As a critical first step in achieving these goals, we developed a revised formulation of our existing MesoTotal™ HPC differentiation medium and identified a highly efficient cardiomyocyte protocol. We also identified the optimal hiPSC seeding density and substrate for each differentiation. Multiple hiPSC lines differentiated in high-throughput formats using the HPC and Cardiomyocyte differentiation systems displayed robust development of $CD45^+/CD43^+$, $CD34^+$ HPCs with multi-lineage colony forming potential (CFU-GEMM), and spontaneously beating cardiomyocytes with > 80% of cells expressing Troponin T. The systems then were used to screen a modest library of well studied control compounds to determine whether toxic effects during and after hematopoietic and cardiac differentiation could be identified using simple cell characterization and viability assays. Preliminary results and future plans will be presented.

F-2125

CRISPR BASED GENOME EDITING AND ITS APPLICATION IN LARGE SCALE GENOME AND STEM CELL ENGINEERING

Garza, Johan[1], Ravinder, Namritha[2], Roark, Natasha[2]
[1]California State University San Marcos, San Marcos, CA, USA, [2]Thermo Fisher Scientific, Carlsbad, CA, USA

Precision genome engineering has improved in recent years by the incorporation of highly specific DNA cleavage endonucleases. These targetable endonucleases introduce a double-strand break (DSB) at specific genomic locus, initiating the cell's error prone repair system by nonhomologous end joining (NHEJ) that creates targeted mutations. By co-transfecting modified donor DNA that is flanked with target specific homology arms and leveraging Homology Directed Repair (HDR) one can introduce the foreign DNA at user defined genomic locus. These mechanisms can be harnessed to repair a disease specific mutation or create engineered stem cell model systems for understanding disease manifestation and drug screening studies. Clustered regularly interspaced short palindromic repeat (CRISPR), a bacterial adaptive immune system from *Streptococcus pyogenes* has been recently engineered and demonstrated to function as a genome editing tool. A small non-coding RNA called the CRISPR RNA (crRNA) drives the specificity and guides Cas9, a DNA endonuclease to the target genomic DNA sequence via Watson and crick base pairing. Since target specificity in CRISPR system is defined by a small 19-20 bp non coding RNA sequence within crRNA, it is easy to design this system to target any locus of interest there by making it an attractive genome editing tool. Discussed here are different CRISPR based editing tools and its applications in multiplex genome engineering across wide range of cell types including stem cells. Also described here is a complete CRISPR based editing workflow including target specific CRISPR designing, construction and screening of edited clones using a PCR based Genomic cleavage detection assay and next generation PGM sequencing. These experiments were executed both pre and post enrichment of transfected population to test % enrichment in edited population. Tools and workflows described through this work include a combination of CRISPR based editing tools that not only reduce the hands on time needed for generating target specific CRISPR systems but also allows the user to edit multiple loci or alleles simultaneously. The simple and easy-to-design tools described here holds great promise in large scale genome and stem cell engineering applications.

F-2126

HIGH-THROUGHPUT CHEMICAL SCREENING REVEALS NOVEL REGULATORY MECHANISMS OF HUMAN PLURIPOTENT STEM CELL PLURIPOTENCY AND NEAR-HOMOGENEITY MESODERM AND ENDODERM DIFFERENTIATION CONDITIONS

Geng, Yijie
University of Illinois at Urbana-Champaign, Urbana, IL, USA

Understanding and controlling the fate decisions of human pluripotent stem cells (hPSCs) is central to human developmental studies and their potential applications in disease modeling, drug discovery and cell-based therapies. Here we developed and applied a simple and reliable high-throughput screening (HTS) platform of hPSC pluripotency to large-scale chemical screening of >170,000 compounds, leading to the identification of 122 lead chemicals that potently disrupt cellular pluripotency. By exploiting target identification, biochemical, molecular and cellular analyses, we discover a novel small-molecule inhibitor of heat shock 70-kDa protein 8 (HSPA8), identify HSPA8 as a critical regulator of pluripotency, and reveal a mechanism by which HSPA8 promotes auto- and mutual regulation of the core transcription circuitry of pluripotency. In addition, through characterizing the lead chemicals we identify a second small molecule that, when used in conjunction with extrinsic factors, leads to robust, close-to-homogeneity mesoderm and endoderm differentiation (≥90%) of human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). Together, we provide novel mechanistic insights into pluripotency and establish new tools and technologies for monitoring and manipulating hPSC fate, which should greatly facilitate the biological and therapeutic applications of hPSCs. Our findings can potentially improve the therapeutic strategies for cancer and other diseases involving the function of heat shock proteins.

F-2127

A FLUORESCENT SWITCH FOR SPECIFIC HIGHLIGHTING OF YOUR CELLS OF INTEREST

Georges, Adriana, Moal, Yohann
Vectalys, Toulouse, France

The characterization of various lineages populations is crucial because (i) one needs to evaluate how efficient a differentiation protocol is and (ii) how the presence of cells of an undesirable phenotype may pose problems for further use. Current identification methods are heavy, time consuming and often not suitable for any applications. For this purpose Vectalys has developed accurate, easy to use, innovative tools to specifically monitor the occurrence of different cell types thanks to promoters that drive bright fluorescent reporters' expression. The key to success resides also in the use of highly concentrated and purified lentiviral vectors which are then the best tool to transduce without damaging any sensitive cells (embryonic or adult stem cells, induced pluripotent stem cells (iPS) and all differentiated populations issued from them). Data concerning lineages of differentiation of the liver and the brain are presented here. Stem cells that can be isolated, expanded and induced to differentiate into fully functional hepatocytes *in vitro* are an ideal source of cells for the treatment of chronic liver diseases or for drug discovery. In delicate cells such as ESCs (Embryonic Stem Cells), classical transfection protocols are not only inefficient but they also induce cytotoxic effects and/or proliferative arrests. Gene transfer using lentiviral vectors is safer as it minimizes any deleterious effects upon transduction of target cells. Concentrated and purified lentiviral suspension expressing a fluorescent reporter driven by an APOA-II promoter has then been developed as a cell marker specific to hepatic progenitors. The APOA-II driven reporter expression allows FACS sorting of hepatic progenitors specifically expressing APOA-II, yielding a population highly enriched (>99%). This purification approach makes possible the subsequent differentiation of the sorted hepatoblasts into more mature hepatocytes. The ability to visualize and genetically manipulate specific cell populations in the Central Nervous System (CNS) is fundamental to a better understanding of brain functions at the cellular and the molecular levels. Tools to selectively target cells in the CNS can be valuable for studying and treating neurodegenerative diseases. Our data illustrate preferential targeting of neurons, astrocytes and oligodendrocytes *in vitro* on rat cortical neurons culture and *in vivo* by stereotactic injections into adult mice. We also show efficient targeting of neural stem cells (NSCs) by transduction of mouse neurospheres in culture and by *in vivo* injections of highly purified lentiviral vectors into the subventricular zone or the dentate gyrus of mice brains. These data of *in vitro* and *in vivo* lentivector-based gene transfer highlight the relevance of such tool to follow cell differentiation and its interest in regenerative medicine. But it emphasizes the need of cell preservation by using pure lentiviral suspensions. Based on these convincing results, Vectalys is currently developing a bank of lentiviral

vectors expressing specific promoters for any tissues and for various differentiation stages, starting from the embryonic layers up to the most specialized cells of a given organ. This approach represents a technological advance in many areas of research because it can be adapted for the purification of any stem cell derived populations for therapeutic purposes and also for *in vitro* applications such as drug screening.

F-2128
ONE-STEP HIGH-THROUGHPUT REPROGRAMMING AND DIFFERENTIATION ON A CHIP

Luni, Camilla, **Giulitti, Stefano**, Serena, Elena, Zambon, Alessandro, Onelia, Gagliano, Ferrari, Luca, Michielin, Federica, Elvassore, Nicola
University of Padova, Padova, Italy

An emerging need in biological and clinical studies is to increase the number of patients or patient-derived samples, in view of the profound impact of human population heterogeneity, in terms of genetic profiles and ethnicity, on biological and therapeutical responses. Conventional strategies of drug discovery for development of ad hoc patient-specific therapies need to be substantially improved to offer cost-effective and feasible new solutions for both common and rare diseases. Human induced pluripotent stem cells (hiPSC) opened a breakthrough new perspective on research and clinical applications, but have not been included in translational procedures applicable to study inherited pathologies and human heterogeneity yet. The down-scale of the reprogramming process could provide a unique opportunity to derive cost-effective hiPSCs and valuable human in vitro tissues. The full process of tissue-specific cell derivation involves multiple steps, from cell reprogramming and expansion for several passages to differentiation towards a final phenotype. A recent method for the expression of reprogramming factors is based on the use of modified mRNA (mmRNA). This delivery system is non-integrating, transient, and after multiple transfections of the reprogramming factors is able to produce vector-free hiPSCs in short time with high efficiency, to the point that the production of clinical-grade hiPSC is now feasible, although new technological advancements are required. We developed a comprehensive micro-scale platform with a translational potential for a population-based cell reprogramming and programming without any intermediate stage of expansion. mmRNA technology and chemically defined long-term culture environments were adopted to safely and efficiently derive clinical-grade hiPSCs with unmatched minimum requirements of reagents (few microliters per day). Microfluidic-derived hiPSC emerged as soon as 7 days from the initial transfection and all clones tested exhibited a pluripotent phenotype. The reprogramming of ~100 cells per sample in feeder-free conditions and the direct and stable expansion of hiPSC with chemically-defined media were effective. Due to the transient nature of mmRNAs, we verified that the expansion passages after colony formation are not required to fully exploit hiPSC differentiation potential in tissue engineering applications. Microarray analyses revealed no significant differences between fresh and expanded hiPSC obtained without the use of integrating or long-lasting viral vectors. In the perspective of obtaining population-based tissue libraries, we further optimized the micro-scaled reprogramming system obtaining an efficiency of up to 16% with the exclusive use of mmRNAs. A fully automated microfluidic chip was developed to simultaneously derive hiPSC from 192 independent samples without need of human intervention for the entire process. Pluripotent cells were then directly programmed into functional cell types valuable for in vitro human assays. Without any expansion procedure, we obtained advanced cardiac and hepatic phenotypes in less than one month, starting from human fibroblasts. Our technology paves the way to high-throughput screening studies on hiPSC-derived functional tissues, after robust and systematic tissue production. This work represents an important step towards the effective use of hiPSC-derived tissue libraries for studies of large cohorts of patients for personalized medicine.

F-2129
USING 100 STRAINS OF MICE TO FIND ASSOCIATIONS BETWEEN DNA METHYLATION AND CLINICAL PHENOTYPES

Go, James L.[1], Orozco, Luz D.[1], Malone, Cindy S.[2], Pellegrini, Matteo[1]
[1]*University of California, Los Angeles, Los Angeles, CA, USA,* [2]*California State University, Northridge, Northridge, CA, USA*

The Hybrid Mouse Diversity Panel is an ongoing study of 100 inbred strains of mice. Sixty clinical phenotypes, such as cholesterol levels and bone mineral density, were measured in the mice. To study how the clinical phenotypes are associated with DNA methylation, epigenome-wide association studies were performed on the individual phenotypes using variable CG methylation sites . To further analyze the relationship between methylation and phenotype, principal component analysis was used on the clinical phenotype data to capture the most variation within the set and form a meta-trait from the sixty individual traits. Then epigenome-wide association tests were performed on the rotated phenotype data to find methylation sites with strong associations. We identified new sites of association with the principal components that were not found in the previous epigenome-wide associations that looked at individual traits. The first component captured variation between glucose to insulin ratio and fat related traits while the second component captured variation between blood and fat related traits. Associations to the second component of clinical traits yielded the most interesting results, including a strong association to the gene mTOR, which plays a fundamental role in mouse metabolism. These results can help identify gene targets that will be used for further study of the phenotypes. In addition, we wanted to determine how quantitative methylation data could be used to predict clinical phenotypes. The top 1000 methylation sites associated with each phenotype were used to build a linear model for prediction. Many of the phenotypes were very accurately predicted, such as hemoglobin concentration and free fatty acid levels. Our results show that CG methylation can be used as a quantitative predictor for several phenotypes, which may have clinical applications as diagnostics.

F-2130
SCALABLE ENZYME-FREE PASSAGING OF HUMAN PLURIPOTENT STEM CELLS CULTURED IN MTESR™1 OR TESR™-E8™ WITHOUT SCRAPING

Hadley, Erik, Norberg, Jessica, Liu, Rachel, Eaves, Allen C., Thomas, Terry E., Louis, Sharon A.
STEMCELL Technologies Inc., Vancouver, BC, Canada

Recent progress in human pluripotent stem cell (hPSC) research has been facilitated by the availability of defined culture media such as mTeSR™1 and TeSR™-E8™ (STEMCELL) that permit the maintenance of the pluripotent state in the absence of feeder cells; both of these media were developed based on publications from James Thomson's laboratory [Ludwig et al., 2006; Chen et al., 2011], and may be used with enzyme-free passaging reagents such as Gentle Cell Dissociation Reagent (GCDR, STEMCELL). However, a key limitation of this and other passaging reagents has been the need to mechanically scrape the culture surface to generate cell aggregates. Repeated generation and re-seeding of appropriately sized hPSC aggregates is critical, since repeated passaging as single cells can lead to the accumulation

of chromosomal abnormalities [Mitalipova et al., 2005]. We have developed an improved enzyme-free passaging reagent, ReLeSR™, for the passaging of hPSC aggregates without the need for scraping or complicated manipulation to obtain the desired aggregate size. ReLeSR™ enables the use of culture flasks and other closed vessels where the use of a cell scraper is not practical, thus facilitating culture scale-up and automation. We evaluated ReLeSR™ by assessing its performance using two human embryonic stem cell (hESC; H1, H9) lines and one human induced pluripotent stem cell (hiPSC; WLS-4D1) line cultured in either TeSR™-E8™ or mTeSR™1 medium on Corning® Matrigel®. Cells were passaged every 5 - 7 days using a standard GCDR protocol (control) or a protocol developed for ReLeSR™; each of these passaging protocols are outlined below. When using GCDR, the culture medium was aspirated and replaced with 1 mL of GCDR, followed by incubation at room temperature for 6-8 minutes. GCDR-treated aggregates were scraped and collected into fresh medium, dissociated to the desired size by repeated pipetting, and then re-seeded onto wells pre-coated with Matrigel® containing TeSR™-E8™ or mTeSR™1 for subsequent culture. Alternately, in the passaging protocol using ReLeSR™, the culture medium was first aspirated, and then 1 mL of ReLeSR™ was added to each well and immediately removed (i.e. colonies were exposed to a thin film of liquid). After 8 minutes incubation at room temperature, 2 mL of TeSR™-E8™ or mTeSR™1 was added. The plate was firmly tapped against the palm of the hand for a total of approximately 45 seconds to dislodge the cell aggregates. The suspension of cell aggregates was then transferred to a 15 mL tube and re-seeded as above without further manipulation of aggregate size by pipetting. The performance of ReLeSR™ was compared to a standard GCDR protocol by observing cell morphology, expression of hPSC markers, plating efficiency (# aggregates seeded/# aggregates attached at 48 h) and cell expansion (# aggregates harvested/# aggregates seeded). Cells passaged using GCDR and ReLeSR™ were found to have indistinguishable morphology, and in each culture >90% of cells maintained expression of undifferentiated cell markers (TRA-1-60 and OCT4). High fold-cell expansion per passage was achieved using either ReLeSR™ (H9/mTeSR™1: 65 ± 9; 8 passages, H1/TeSR™-E8™: 86 ± 12; 10p, WLS-4D1/TeSR™-E8™: 48 ± 5; 10p) or GCDR (H9: 55 ± 5; 8p, H1: 75 ± 12; 10p, WLS-4D1: 45 ± 8; 10p). In conclusion, ReLeSR™ is a convenient passaging reagent for hPSCs that can be used to maintain high quality cultures in mTeSR™1 or TeSR™-E8™ without the need for scraping, thus making scale-up and automation easier.

F-2131
A ONE STEP SYSTEM FOR CONVENIENT AND FLEXIBLE TALEN ASSEMBLY

He, Lixiazi
Shanghai Institute of Biochemistry and Cell Biology, Shanghai, China

Transcription activator-like effector nuclease (TALEN) is a robust tool for genome editing. TALENs can recognize and bind to specific genome sequence and make a double strand break, which will lead to frame-shift or specific replacement of sequence and cause gene knock-out, knock-in, and other modification on the target site. Methods for TALEN assembly are keeping upgrading, but the current methods are too difficult to operate in common molecular laboratories, either due to tedious assembly steps or a large size of module libraries, impeding widespread application of TALEN technology. We hereby report a one-step ligation system for TALEN assembly system based on a 172 TALE module library. This one-tube system is not only convenient, enabling researchers to construct TALEN vectors and verify them to ready-to-use within a short period of time, but also flexible, allowing TALEN with repeats of consecutive number from 11.5 to 18.5 to be assembled with high efficiencies ranging from 33.3% to 100%. We have tested our system on HEK293T cell lines by targeting four sites on three human genes, after a step of TALEN pair selection for each site, high targeting efficiency were obtained at all four sites.

F-2132
TALEN- AND CRISPR-MEDIATED GENOME EDITING OF MECP2 IN HUMAN PLURIPOTENT STEM CELLS

Hendriks, William[1], Zuckerman, Eric[2], Chen, Shuai[3], Daheron, Laurence M.[4], Cowan, Chad[5]
[1]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA, [2]Harvard College, Harvard University, Cambridge, MA, USA, [3]Faculty of Biomedical Science, University of Amsterdam, Amsterdam, Netherlands, [4]Harvard University, Cambridge, MA, USA, [5]HSCRB, Harvard University, Cambridge, MA, USA

Using custom-engineered nuclease-mediated genome editing, such as Transcription Activator-Like Effector Nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) RNA-guided Cas9 nucleases, we can create human pluripotent stem cell (hPSC) lines with knockout or mutant alleles that can be used for differentiation into various cell types. This strategy of genome engineering in hPSCs will prove invaluable for studying human biology and disease. TALENs are arrays of 34-amino-acid DNA binding domains fused to FokI-nuclease domains. These arrays can be easily engineered to bind almost any DNA sequence. The RNA-guided Cas9 is targeted to a genomic site by complexing with a synthetic guide RNA that hybridizes a 20-nucleotide DNA sequence beginning with G and immediately preceding an NGG motif recognized by Cas9. Introduction of a pair of TALENs or a guide RNA with Cas9 into hPSCs creates a double-strand break (DSB) in the targeted gene that is either repaired by error-prone non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ typically results in frameshift mutations in the targeted locus, generating a knockout hPSC line when both alleles are targeted. Simultaneous delivery of a TALEN pair or a CRISPR/Cas9 nuclease and a DNA donor template can result in HDR and can be used to introduce specific mutations, repair endogenous mutations in disease-specific hPSC lines, or create hPSC reporter lines. Using both TALEN and CRISPR/Cas9 technology, we targeted *MECP2* (methyl CpG binding protein 2) with a single stranded oligodeoxynucleotide (ssODN) in order to introduce a R306C mutation, implicated in Rett Syndrome. The targeting efficiencies for *MECP2* with TALENs generating insertions and/or deletions (indels) were between 1.25% and 4.2%. Using CRISPRs, we observed a targeting efficiency for *MECP2* of 8.3% (indels), which is higher than with TALENs like described previously. However, we did not observe successful targeting of the R306C mutation using TALENs or CRISPRs. Currently, we are functionally validating the generated *MECP2* knockout lines. In addition, we are investigating CRISPRs to introduce mutations and specific deletions through "double-nicking" and the use of guide RNA pairs. This study reveals that both TALEN and CRISPR/Cas9 technology can be used for successful genome engineering in hPSCs and that the CRISPR/Cas9 nuclease is more efficient in generating indels. CRISPR- and TALEN-mediated genome engineering in hPSCs is available as a fee-for-service offered through the Harvard Stem Cell Institute iPS Core Facility.

REPROGRAMMING

F-2133
DIRECT REPROGRAMMING OF CARDIAC FIBROBLAST INTO CARDIOMYOCYTES USING SMALL MOLECULES FOR REGULATION OF MUSCLE SPECIFIC-MICRORNAS

Lee, Se-Yeon[1], Ham, Onju[1], Lee, Chang Youn[2], Park, Jun-Hee[2], Lee, Jiyun[1], Seo, Hyang-Hee[1], Seung, Minji[1], Yun, INa[1], Han, Sun M.[1], **Choi, Eunhyun**[1], Hwang, Ki Chul[3]

[1]Yonsei University College of Medicine, Brain Korea 21 Plus Project for Medical Science, Seoul, Republic of Korea, [2]Yonsei University, Department of Integrated Omics for Biomedical Sciences, Seoul, Republic of Korea, [3]Yonsei University College of Medicine, Severance Biomedical Science Institute, Seoul, Republic of Korea

Efficient induction of fully reprogrammed functional cardiomyocytes using specific transcription factors and microRNAs (MiRNAs) has showed enhanced functional recovery of infarcted heart after reprogramming treatment *in vivo* and *in vitro*. Notably, cardiomyocytes which generated via direct reprogramming *in vivo* are known to be more mature than those from direct reprogramming *in vitro* and indicated functional enhancements similar to natural ventricular cardiomyocytes. Here, we hypothesized that small molecules regulate the muscle specific-miRNAs, miRNA-1, -133a, -208a, and -499, to induce *in vivo* and *in vitro* direct reprogramming of fibroblasts into cardiomyocytes and advance the heart regeneration in rat myocardial infarction (MI) models. The conversion of endogenous fibroblasts directly into cardiomyocytes was induced by regulating HDAC4, which represses MEF2, and repressing SRF expression after treatment of small molecules. Sequential expression of different genes related to mesoderm formation (DKK1), cardiogenic mesoderm (MESP1), cardiac-specific progenitors (NKX2.5, GATA4, TBX5, HAND1/2), and cardiomyocytes maturation (MYH6) were increased in the fibroblasts reprogrammed into cardiomyocyte by treatment with small molecules. Cardiac functions such as cardiac output and ejection fraction were enhanced while cardiac fibrosis was reduced in small molecules-treated MI models. Small molecules, which are able to increase the 4 miRNAs, regenerated new muscle in situ, improved cardiac function, and reduced cardiac fibrosis. Interestingly, these small molecules also induced cardiac differentiation of fibroblasts as well as mesenchymal stem cells (MSCs). Taken together, this study suggested that direct cardiac reprogramming *in vivo* and *in vitro* by regulating miRNAs using small molecules may represent a powerful strategy and a promising therapy for regenerative medicine that could enhance cardiac function and reduced fibrosis in ischemic heart disease.

F-2134
OPTIMIZING IPSC GENERATION WITH A NON-INTEGRATIVE AND FDA APPROVED SYSTEM

Fernandez Munoz, Beatriz[1], Lomas-Romero, Isabel[1], Martin, Maria[1], Ordonez-Luque, Angel[1], Arribas, Blanca[1], Montiel, Miguel Angel[1], Carmona, Gloria[2], González-Muñoz, Elena[1], **Laricchia-Robbio, Leopoldo**[2], Cibelli, Jose B.[3]

[1]Andalusian Laboratory of Cellular Reprogramming, Sevilla, Spain, [2]Andalusian Initiative for Advanced Therapies, Sevilla, Spain, [3]Michigan State University, East Lansing, MI, USA

iPSCs serve as a potentially ideal platform to produce patient-specific cells to be used in individualized cell therapies. Nevertheless, cell reprogramming is a relatively new technology that has been poorly translated to a clinical use. Our aim is to develop a protocol for iPSC generation more amenable for a clinical use. EMA and FDA prefer non-integrative vectors for clinical approval of a reprogrammed cell to be used in patients. We use therefore fibroblasts transfected with three episomal vectors containing the usual reprogramming factors. First we compare the efficacy of fibroblasts isolation from different areas of the body using both mechanical and enzymatic protocols. Two skin biopsies from different donor areas were processed by either the collagenase technique or "explants technique", comparing their efficiency in isolating fibroblasts in terms of time and number of fibroblasts obtained. The quality of the isolated fibroblasts was also assessed by measuring fibroblasts proliferation capability, viability, identity, morphology and genetic stability. We found that the "explant technique" is a more efficient approach to obtain fibroblasts under our experimental conditions. Once the primary fibroblast culture is established there are no significant differences between the techniques although fibroblasts isolated by the collagenase approach acquire an earlier senescent-like morphology. The back area, near the scapula seems to be an appropriate area to isolate dermal fibroblasts. The next step has been the optimization of the reprogramming method by an electroporation system approved by the FDA (MaxCyte® GT™ system). We electroporated fibroblasts from 5 different donors with several electroporation programs. We then selected the best program with these data and we used this program to generate iPSC lines from 2 adult donors. We obtained an average of 20 colonies for donor (efficiency of 0,001%). iPSC lines generated from each donor were evaluated for pluripotency by different tests: expression of pluripotency markers (Tra-1-60, Tra-1-81, SEAA4, CD13, DNMT3B, TERT, GDF3, Rex1, DPPA4), karyotyping, lost of episomal vectors and in vitro differentiation test. We found that the lines were positives for the pluripotency markers. Here we demonstrate that generation of iPSC with this FDA approved and closed electroporation system is possible and has a similar efficiency to other protocols using episomal vectors.

F-2135
CYTOTUNE®-IPS 2.0 SENDAI REPROGRAMMING KIT FOR HIGH EFFICIENCY REPROGRAMMING OF FIBROBLASTS AND BLOOD-DERIVED CELLS

MacArthur, Chad C., Quintanilla, Rene H., Lakshmipathy, Uma

Thermo Fisher Scientific, Carlsbad, CA, USA

Induced pluripotent stem cells (iPSCs) are valuable research tools for many different applications, including drug discovery, disease modeling, and regenerative medicine. Given the importance and potential of iPSC, it is vital that researchers have access to reprogramming methods that can safely and efficiently generate iPSCs from a wide variety of patient-derived cell types. The CytoTune®-iPS Sendai Reprogramming kit has emerged as an efficient method for generation of footprint-free iPSC from a wide variety of cell types, including blood cells. However, issues that were observed in some donor cells included toxicity, low reprogramming efficiency, and persistence of the virus beyond passage 10. To address the above challenges, novel configurations of the virus were identified and used for the development of the CytoTune®-iPS 2.0 Sendai Reprogramming Kit. The resulting new configuration consists of the Oct4, Sox2, and Klf4 genes in a single polycistronic vector in a new backbone, with c-Myc remaining on a separate vector, and an additional separate Klf4 vector used to enhance efficiency. The polycistronic vector was designed for greater efficiency of reprogramming in a new backbone that allows for lower cytotoxicity and faster viral clearance. The additional Klf4 vector further aids in achieving maximum efficiency with minimal viral load. Consistent with this, CytoTune® 2.0 was found to have 2-5 fold greater efficiency of reprogramming in fibroblast and blood reprogramming. In addition, the level of toxicity in primary cells was significantly reduced with CytoTune® 2.0, relative to the previous version. Clones derived with

CytoTune® 2.0 had positive pluripotent marker expression, tri-lineage differentiation potential, and normal karyotype. In addition, the viral backbone was diluted from the majority of clones as early as passage 3. Thus, the CytoTune® 2.0-iPS Sendai Reprogramming kit provides a robust system for higher efficiency, low toxicity, and faster viral clearance from resulting iPSC clones.

F-2136
SCORING CELL IDENTITY FROM TRANSCRIPTION PROFILES

Mah, Nancy, Andrade-Navarro, Miguel
Computational Biology and Data Mining, Max Delbrueck Center for Molecular Medicine, Berlin, Germany

Transitions in cell identity, whether induced by experimental means or as a part of a developmental or maintenance process, are complex yet coordinated events. The framework of common regulatory networks in these processes are often subject to modifications, which facilitate cell identity changes that drive induced reprogramming, differentiation and cancer. For example, genes involved in epithelial to mesenchymal transition (EMT) and its reverse process (MET) are active in cancer stem cells and the generation of induced pluripotent cells from somatic fibroblasts, respectively. Although the mechanisms behind the processes can be regulated at many different levels, we start to dissect the cell code by first identifying marker genes from RNA transcription profiles. We then use these genes to evaluate the success of a reprogrammed cell type in relation to its desired target cell type, by generating a cell identity score. Moderate scores indicate that a cell type is only partially reprogrammed. By pinpointing which genes are deficient in partially reprogrammed cells, we hope to identify points of intervention to overcome these reprogramming roadblocks, thereby improving the reprogramming efficiency of experimental protocols.

F-2137
RAPID DERIVATION OF NEURAL PRECURSORS BY DIRECT CONVERSION OF HUMAN CD34+ CELLS USING STANDARD REPROGRAMMING TOOLS

Mallon, Barbara[1], Shi, Yi-Jun[1], Park, Kyeyoon[1], Sabatino, Marianna[2], Civini, Sara[2], Stroncek, David[2], Robey, Pamela G.[3]
[1]National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA, [2]Department Transfusion Medicine, CC, National Institutes of Health, Bethesda, MD, USA, [3]National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, USA

The rapid generation of large numbers of neural cells for drug screening or disease modeling is of great interest to the neuroscience community. In the course of investigating methods for generating clinically relevant induced pluripotent stem cells (iPSCs), we found a high propensity for human CD34+ cells to generate cells of a neural phenotype in addition to the iPSCs. These ubiquitous neural cells expressed nestin, could be propagated using standard neural cell culture protocols and differentiated to express TuJ1 and MAP-2. This direct conversion phenomenon was initially observed when using a non-standard substrate for iPSC derivation with a standard PSC culture medium supplemented with small molecules used to enhance reprogramming. Previously, we had found this standard medium to not only support PSC growth but also to induce limited neuronal differentiation, albeit with continued proliferation, of neural precursors. Direct conversion to neural precursors was not observed when reprogramming fibroblasts under similar conditions and we hypothesize that CD34+ cells, being of a stem cell character, may be more easily converted to other stem cell types. We originally used a Sendai virus kit including all 4 Yamanaka reprogramming factors which distinguishes this approach from the original direct conversion studies of fibroblasts to neurons using neuronal transcription factors. We are currently investigating other methods of reprogramming, such as episomal plasmids and miRNA/mRNA, under the same culture conditions. We also plan to test different combinations of the viruses as well as various substrates. In addition, we will optimize methods to efficiently isolate the neural cells from the iPSCs, examine their multilineage potential and characterize the neuronal subtypes generated. If this approach is found to generate reproducible neuronal profiles, such efficient and rapid direct reprogramming would be of interest to those studying neuronal disorders wishing to derive neurons from large patient cohorts as well as for drug screening.

F-2138
PRECISELY ACTIVATION OF EC-REGULATORY-NETWORKS UNDERPINNING DIRECT REPROGRAMMING OF VASCULAR ENDOTHELIAL CELLS FOR USE IN REGENERATIVE MEDICINE

Margariti, Andriana
Queen's University Belfast, Belfast, United Kingdom

Vascular disease is the leading cause of death worldwide and is characterised by endothelial cell (EC) dysfunction. Replacing damaged ECs could be a potential therapeutic option but the availability of appropriate cell types has been a major limitation. Recently, a new paradigm of direct reprogramming strategy has been devised. We reasoned that, at earlier time points during reprogramming, we could direct the epigenetically activated cells which are induced by the iPS cell factors into lineage specific cell types such as ECs under defined conditions without traversing pluripotency. To this end, we adapted a method to generate partially reprogrammed cells, which did not form tumours in vivo and clearly displayed the potential to differentiate into ECs. However, the efficiency of direct reprogramming is very low and the underlying mechanisms remain unclear. In this study we have identified that the four reprogramming factors precisely activate EC-Regulatory-Networks in early stages of reprogramming. Interestingly, our data has revealed that up to 5 EC-Regulatory-Networks are induced in the early stages of reprogramming. These unique EC-Regulatory-Networks respond to specific stimuli such as Vascular Endothelial Growth Factor and Fibroblasts Growth Factor and enable EC reprogramming. These transcriptional networks which are activated during reprogramming revealing that the cells have reached an intermediate stage thereby "priming" critical signalling networks which could respond to specific stimuli and induce EC reprogramming. It is noteworthy to highlight that the expression levels of these networks are significantly decreased in later stages of reprogramming. These EC-Regulatory-Networks are consisted of a secreted protein which is mainly expressed in the ECs; a potential new marker of ECs, which is implicated in EC activation; a tyrosine kinase receptor which is critical for EC-smooth muscle cell communication in venous morphogenesis; a novel protein which encodes an element of the machinery for trafficking in ECs; a key regulatory protein of ERK, Akt, and eNOS activation pathways, which is implicated in cytoskeletal reorganization; and a secreted binding glycoprotein which regulates activin and BMP2 signalling in differentiation of hematopoietic progenitor cells. Additional experiments have revealed a powerful role of these EC-Regulatory-Networks to induce EC reprogramming in a robust and highly efficient manner. This knowledge has allowed us to establish homogeneous populations of reprogrammed ECs. Importantly, this work demonstrates the potential of reprogrammed ECs to enhance angiogenesis and neovascularisation in vitro and in vivo. Together, these findings may establish the therapeutic potential of

reprogrammed ECs which would have transforming consequences for regenerative and personalised medicine.

F-2139
PHYSIOLOGICAL SUB-LETHAL STRESS VIA CONTROLLED AUTOPHAGY AND METABOLIC TRANSITION INDUCES LINEAGE-RESTRICTED CELLULAR REPROGRAMMING IN AGED HUMAN CELLS

Mason, Rebecca[1], Bray, Elen[1], Pryor, Paul[1], James, Sally[1], McKeegan, Paul[2], Sturmey, Roger[2], Genever, Paul[1]
[1]Department of Biology, University of York, York, United Kingdom, [2]Centre for Cardiovascular and Metabolic Research, University of Hull, Hull, United Kingdom

Recent work has shown that exposure to non-physiological sub-lethal stress (acidic pH) can initiate dedifferentiation of somatic cells to a pluripotent state (so-called "STAP" cells); however the causal mechanisms were not identified. Cellular reprogramming not only requires resetting of the transcriptional network, but metabolic conversion and organelle/cytoplasmic remodelling to restructure somatic cell complexity: ESCs and iPSCs rely on a glycolytic ATP-generating pathway rather than mitochondrial oxidative phosphorylation; cytoplasmic clearance must be achieved to convert organelle-rich, phenotypically mature cell types to organelle-poor primitive cells. Autophagy is a physiological response to cell stress that removes dysfunctional organelles, including mitochondria, and protein aggregates to promote survival. We therefore hypothesised that controlled, sub-lethal autophagy, provides a biological mechanism to induce reprogramming, without requiring exogenous, non-physiological stimuli. To test this hypothesis, we established 3D in vitro spheroid models, using human bone marrow stromal cells (MSCs) from aged donors (median = 66.5 years), which decrease in size over time and so can be used to scale nutrient deprivation and cell stress by varying initiating cell numbers and culture time. We demonstrated that optimal sub-lethal stress conditions were achieved when 60,000 MSCs were cultured for 5 days. 3D culture induced significant increases in expression of TFEB (up 5-fold vs 2D MSCs), the master regulator of autophagy and lysosomal biogenesis, however we also identified a putative TFEB-binding motif in the Sox2 promoter. Levels of the lysosome marker LAMP1 were increased in 3D MSCs, along with loss of cytoplasmic LC3 I, indicative of enhanced autophagy. 3D MSCs contained numerous cytoplasmic vacuolar structures, typical of increased autophagy. We also observed regression to rounded, immature mitochondria, similar to those observed in embryonic blastomeres and ESCs. This was accompanied by a shift from oxidative to 'anaerobic-type' metabolism, evidenced by decreased basal oxygen consumption and elevated/maintained lactate excess; a metabolic phenotype that has been confirmed in pluripotent cells. 3D MSCs expressed maximal levels of Oct4, Nanog and Sox2 transcripts at 5 days of culture (up to 35-fold higher than 2D MSCs, $p<0.05$), but did not form teratomas in vivo, suggesting a post-pluripotent state. Rather, 3D MSCs generated mesodermal tissues with organised histology following subcutaneous implantation in nude mice, and expressed significantly increased levels of early mesoderm markers (Brachyury, GSC, KDR, Mixl1 and CXCR4, up-regulated 3.5-45 fold vs 2D MSCs, $p<0.05$). Following disaggregation, 3D MSCs expanded as colonies in ESC-semi-solid medium and maintained their dedifferentiated state. Remarkably, 3D culture rejuvenated in vitro-aged, post-mitotic MSCs; they reversed senescence-associated hypertrophy, regained a fibroblastoid non-senescent morphology and re-entered active cell cycling. We have identified a novel mechanism by which somatic cells can be developmentally reprogrammed to a teratoma-free, primitive state, simply by stimulation of a physiological survival response through controlled cell stress. Our evidence that intrinsically controlled autophagy flux is alone sufficient to induce dedifferentiation suggests a fundamental role in human cellular reprogramming and tissue regeneration.

F-2140
HYPOXIA INDUCIBLE FACTORS HAVE DISTINCT AND STAGE-SPECIFIC ROLES DURING REPROGRAMMING OF HUMAN CELLS TO PLURIPOTENCY

Mathieu, Julie[1], Zhou, Wenyu[1], Xing, Yalan[1], Sperber, Henrik[1], Ferreccio, Amy[1], Agoston, Zsuzsa[1], Kuppusamy, Kavitha[1], Moon, Randall T.[1], Ruohola-Baker, Hannele[2]
Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, WA, USA,

Pluripotent stem cells have distinct metabolic requirements, and reprogramming cells to pluripotency requires a shift from oxidative to glycolytic metabolism. Here, we show that this shift occurs early during reprogramming of human cells and requires Hypoxia Inducible Factors in a stage-specific manner. HIF1α and HIF2α are both necessary to initiate this metabolic switch and for acquisition of pluripotency, and stabilization of either protein during early phases of reprogramming is sufficient to induce the switch to glycolytic metabolism. In contrast, stabilization of HIF2α during later stages represses reprogramming, due at least in part to up-regulation of TNF-related apoptosis-inducing ligand (TRAIL). TRAIL inhibits iPSC generation by repressing apoptotic caspase 3 activity specifically in cells undergoing reprogramming, but not hESCs, and inhibiting TRAIL activity enhances hiPSC generation. These results shed light on the mechanisms underlying the metabolic shifts associated with acquisition of a pluripotent identity during reprogramming.

F-2141
POU3F2 REGULATES ENDOTHELIAL CELL DIFFERENTIATION AND VASCULAR DEVELOPMENT

Matrone, Gianfranco, Tian, Xiao YU, Wong, Wing T., Cooke, John P.
Department of Cardiovascular Sciences, Houston Methodist Research Institute, Houston, TX, USA

Background: Discovery of molecular factors involved in endothelial development and differentiation may lead to new therapeutic avenues for vascular diseases and regeneration. We have developed the use of heterokaryons as a model system for discovery of novel factors required for endothelial lineage. Our approach combines RNA sequencing with bi-species heterokaryons [generated by fusion of mouse embryonic stem cells (mESC) and human endothelial cells (hEC)]. Our preliminary data suggests that the determinants of endothelial phenotype in the hEC (eg. transcription factors, miRNA, epigenetic modifiers) act on the mESC to recapitulate ontogeny. In addition, the RNAseq data has implicated novel transcription factors in endothelial specification, such as POU3F2, also called BRN2 or N-Oct3. This factor has not previously been implicated in endothelial specification. My project aims to assess the role of POU3F2 in the endothelial cell differentiation and in the zebrafish vascular development. Methods: Mouse embryonic stem cells (mESC) and human induced pluripotent stem cells (hiPSC) were used to study differentiation towards endothelial lineage. Endothelial cell (EC) differentiation was induced by culture of hiPSC with growth factors (VEGF, bFGF and BMP4). POU3F2 loss-of-function was induced by lentiviral shRNA in both mESCs and hiPSCs. Tg(Fli1:EGFP) zebrafish embryos were used to analyse the vascular development following morpholino knockdown of POU3F2. Real Time PCR and Western blotting were used to analyse gene and protein expression respectively.

Results: Pou3f2 knockdown in mESCs reduced Flk1+CD144+ cell population in endothelial differentiation of mESCs. Pou3f2 KD also reduced endothelial cell markers in mESC derived endothelial cells. In normal hiPSCs, our endothelial differentiation protocol generates an endothelial cell yield of 32% which was significantly reduced in hiPSC exposed to POU3F2-specific shRNA. In zebrafish embryos, micro-injection of morpholinos targeting POU3F2 significantly reduced POU3F2 protein at 24 and 48 hpf. This was associated with an embryo phenotype characterized by severe vascular aberrations. Conclusion: Our heterokaryon studies implicated the transcription factor POU3F2 in endothelial cell development. We validated the role of POU3F2 in the reprogramming of pluripotent stem cell to EC lineage. In addition, we provide data that POU3F2 is required for normal vascular development in the zebrafish.

F-2142
GENERATION OF COMMITTED NEURAL PROGENITORS FROM HUMAN FIBROBLASTS BY DEFINED FACTORS

Miura, Takumi, Sugawara, Tohru, Fukuda, Atsushi, Tamoto, Ryo, Umezawa, Akihiro, Akutsu, Hidenori
National Research Institute for Child Health and Development, Tokyo, Japan

Some researchers recently reported that multipotent neural stem/progenitor cells (NSCs) can be directly induced from fibroblasts using neural progenitor-specific transcription factors or the Yamanaka factors as reprogramming agents. Here, we also present a novel method for the generation of self-renewable, multipotent, neural lineage-restricted, and nontumorigenic LIF-dependent induced primitive NSCs (LD-iNSCs) from human fibroblasts. These LD-iNSCs could be directly generated from human fibroblasts by means of a doxycycline-inducible lentivirus encoding the genes of reprogramming factors: OCT4, SOX2, KLF4, L-MYC, and NANOG. After infection of human fibroblasts with the lentivirus, culturing in a chemically defined medium (containing LIF, MEK1/MEK2 inhibitor PD0325901, glycogen synthase kinase [GSK]-3β inhibitor CHIR99021, and doxycycline) resulted in generation of neuroepithelial colonies as early as day 14 but not iPSCs up to day 30. LD-iNSCs exhibit characteristic morphology, gene expression patterns, growth rate, as well as predictable in vitro differentiation potentials. Importantly, these stable expandable LD-iNSC clones show the plasticity of NSCs and can differentiate into functional neurons (motor neurons and dopaminergic neurons), astrocytes, oligodendrocytes, and Schwann cells. Furthermore, even after long-term expansion and repeated passaging in the presence of LIF, CHIR99021, and PD0325901, LD-iNSCs retain high neurogenic potential. On the other hand, the LD-iNSCs lost the capability for differentiation to both mesoderm and endoderm. Interestingly, changes in the growth factor environment for hiPSCs/ESCs could induce the conversion of LD-iNSCs into a stable pluripotent state. This finding indicates that LD-iNSCs are derived from intermediate partially reprogrammed cells. Taken together, our results demonstrate that functional, expandable human primitive NSCs can be directly generated from somatic cells using the same factors known to reprogram cells for pluripotency under special conditions. Therefore, this method may facilitate the generation of patient-specific human neurons for studies and treatments of neurodegenerative diseases.

F-2143
IPSC COLONIES ARISE FROM MULTIPLE FOUNDER CELLS

Moore, Jennifer C.[1], Swerdel, Mavis R.[2], Toro-Ramos, Alana[2], Sahota, Amrik[1], Sheldon, Michael H.[1], Tischfield, Jay[1], Hart, Ronald[2]
[1]*Department of Genetics, Rutgers University, Piscataway, NJ, USA,* [2]*Department of Cell Biology and Neuroscience, Rutgers University, Piscataway, NJ, USA*

Induced pluripotent stem cells have the power to transform medicine by generating human cells for use in drug discovery assays, serving as models of tissue development and disease and providing a source for cell transplantation. In the past iPSC were most often generated through the use of lenti- or retroviral vectors, both of which integrated into the genome. Because integration could affect the pluripotency, differentiation and genomic stability of the resulting iPSC lines, it was important to select, expand and characterize multiple, independent colonies. Today, most iPSC lines are created in the absence of genomic integration and it is unclear if it is still necessary to isolate multiple, independent colonies. If a colony arises from a single reprogramming event (founder cell), the isolation and expansion of multiple colonies is still important, as the completeness of reprogramming may be different in the individual founder cells. However, if multiple founder cells contribute to a colony, the need to isolate individual colonies may be lessened as a single colony likely represents the mixed reprogrammed states of multiple founder cells. Using $CD4^+$ T cells reprogrammed with Sendai viral vectors, we have shown that newly generated iPSC colonies usually arise from multiple founder cells that migrate and expand to form a colony. We further show that the reprogrammed state of the individual cells from the colony shows little or no dependence on the founder cells. Since all T cells undergo V(D)J receptor recombination as they mature, we used a T cell receptor recombination (TCR) assay to determine if colonies arose from single or multiple founder cells. The TCR assay used a mixture of PCR primers that targeted conserved regions within the variable (V) and the joining (J) regions of the T cell receptors which allowed us to distinguish colonies that arose from a single founder cell from colonies that arose from multiple founder cells. Single colonies were isolated immediately after formation and before the first passage and the TCR results showed that only 5 out of 21 colonies arose from a single founder cell. In order to quantitate the fraction of each colony that arose from a single founder cell, an equal mixture of male and female $CD4^+$ T cells was reprogrammed using Sendai viral vectors. These colonies were analyzed by PCR using primers that targeted the sex determining region Y gene (SRY), present only on the Y chromosome. In combination with a standard curve, we were able to determine that the 24 colonies analyzed contained between 0.8% and 65.8% male cells. Although the colonies arose from multiple founder cells, single cell PCR showed that hierarchical clustering of the cells based on the expression of pluripotency genes and germ layer specific genes did not result in segregation based on the gender of the cell analyzed. Even though our results show that colonies arose from multiple founder cells, we continued to follow this apparent diversity through multiple passages. These longitudinal studies revealed that after 3 passages only one gender remained in all colonies, suggesting that some founder cells had a growth advantage and overtook the colony. Therefore our data suggest that it is still important to select multiple individual iPSC colonies when establishing iPSC lines in order to obtain a cell line that is completely reprogrammed.

F-2144

SEARCH OF MACHINERY FOR EFFICIENT REVERSION FROM EPISC TO ESC-LIKE CELL

Murayama, Hideyuki[1], Nakauchi, Hiromitsu[2]
[1]*The Institute of Medical Science the University of Tokyo, Tokyo, Japan,* [2]*University of Tokyo, Tokyo, Japan*

Pluripotent stem cells (PSCs) can be classed as either naïve or primed. Mouse embryonic stem cells (ESCs) are naïve PSCs derived from inner cell mass (ICM) of pre-implantation blastocysts. Their naïve state is maintained in an appropriate culture medium containing leukemia inhibitory factor (LIF). Epiblast stem cells (EpiSCs) are primed PSCs derived from post-implantation epiblasts; their self-renewal ability is maintained by activin A and basic fibroblast growth factor (bFGF) signaling. Naïve and primed PSCs are distinguished from one another by differences in signaling pathways that maintain pluripotency. In contrast to mouse ESCs, however, mouse EpiSCs are barely able to contribute to chimeras when injected into blastocysts, suggesting that a definitive difference between naïve and primed PSCs exists with respect to ability to contribute to chimeras. Transition of mouse EpiSCs to ES-like cells (rESCs) rarely occurs even after stimulation with LIF-STAT3 signaling. This finding suggest that derivation conditions affect pluripotent features of mouse EpiSCs. However, the cellular mechanisms that limit reversion efficiency remain unclear. Pluripotency in non-rodent PSCs is more like that in rodent primed-PSCs, so that chimeric animals derived from PSCs are reported only in work with rodents. Non-rodent PSCs thus are expected not to contribute to chimeras (one reason why knockout or transgenic studies have not been done using non-rodent mammals). Therefore, we investigated the conditions for efficient reversion of primed PSCs to naïve-like PSCs as part of generation of non-rodent naïve PSCs. Here we demonstrated dramatic improvement of reversion efficiency from primed to naïve-like PSCs through up-regulation of E-cadherin in the presence of the cytokine LIF. Analysis revealed that attenuation of Wnt / β-catenin signaling with small-molecule inhibitors significantly enhances reversion efficiency of mouse EpiSCs. We showed reverted EpiSCs contributed extensively to development and succeeded in germline transmission. Although activation of Wnt / β-catenin signals has been thought desirable for maintenance of naïve PSCs, this study provides the first evidence that inhibition of Wnt / β-catenin signaling enhances reversion of mouse EpiSCs to naïve-like PSCs (rESCs). Our investigations thus provide insight into the significance of E-cadherin and Wnt / β-catenin signaling as well as into approaches for increasing efficiency of reversion of primed PSCs to naïve-like PSCs.

F-2145

ROBUST LINEAGE BARRIERS ARE MAINTAINED THROUGH EPIGENETIC REGULATION OF A CORE SET OF GATEKEEPER GENES IN EARLY-EMBRYO DERIVED STEM CELLS

Murray, Alexander John, Cambuli, Francesco, Dudzinska, Dominika A., Hemberger, Myriam
Epigenetics, The Babraham Institute, Cambridge, United Kingdom

The earliest differentiation event during mammalian development results in the segregation of the embryonic and extraembryonic lineages. Embryonic stem (ES) cells, derived from the inner cell mass of the blastocyst, are pluripotent with the ability to differentiate into all cell types of the adult, but are excluded from the placental trophoblast lineage. Conversely, the trophectoderm layer of the mouse blastocyst gives rise to trophoblast stem (TS) cells that are multipotent with the potential to contribute only to trophoblast cell types of the placenta. The stable commitment of ES and TS cells to their respective lineages is ensured by epigenetic modifications, notably DNA methylation, regulating key 'gatekeeper' genes such as the transcription factor *Elf5*. Although global hypomethylation or forced expression of *Elf5* permits ES cells to overcome the lineage barrier and to transdifferentiate into trophoblast cell types, the TS cell state cannot be maintained. ES-to-TS cell reprogramming has also been reported in ES cell models with manipulation of lineage-determining transcription factors (*Oct4* and *Cdx2*) and, remarkably, at the level of signaling (Erk1/2 activation). Here, we have examined the progression of reprogramming in these models. Despite the acquisition of some trophoblast-like characteristics, we find that lineage conversion remains incomplete in all of these reprogramming models, with some key genes retaining an epigenetic memory of their ES cell origin. These genes include additional trophoblast-specific transcription factors as well as some signaling proteins that may function, like *Elf5*, as 'gatekeeper' genes preventing crossover between embryonic and extraembryonic lineages. Simultaneous overexpression of these non-reprogrammed genes improved the efficiency of ES-to-TS cell reprogramming, yet still failed to confer a stable TS cell phenotype. This study highlights the importance of 'gatekeeper' genes in cell lineage specification and stem cell plasticity as they ensure the robustness of the very first cell fate decision in development.

F-2146

A RAPID AND EFFECTIVE METHOD TO GENERATE SUBTYPE SPECIFIC INDUCED NEURONAL CELLS

Ng, Yi Han, Wernig, Marius
Stanford University, Stanford, CA, USA

Reprogramming of pluripotent stem cells by specific transcription factors has proven to be a fast and effective method of obtaining neuronal cells that resembles bona fide neurons. In particular, the overexpression of the proneural bHLH transcription factor Ngn2 can effectively give rise to a highly enriched population of mature excitatory neurons1. Among the Ngn2 ES cells derived induced neuronal cells (ES-iN), there exists rare cells exhibiting subtype-specific markers. This raises the interesting possibility of rapidly inducing specific subtypes using defined transcription factors. Our study identifies a minimal set of neural transcription factors that induces a population positive for tyrosine hydroxylase. Further refinement of the experimental conditions increased the efficiency of TH+ cells induction. By employing electrophysiology studies and HPLC analysis of the released neurotransmitter, we aim to characterize the population obtained. This method of rapidly inducing neuronal subtypes can prove advantageous over existing methods that are expensive, cumbersome and highly variable.

F-2147

ROLE OF THE EPIGENETIC LANDSCAPE IN STEM CELL IDENTITY: DERIVATION OF MOUSE TROPHOBLAST STEM CELLS THROUGH MICRORNA-MEDIATED REPROGRAMMING

Nosi, Ursula, Cox, Brian
Physiology, University of Toronto, Toronto, ON, Canada

Introduction: Pre-implantation development is dependent on the ability of a totipotent cell to specify two distinct and equally indispensable lineages, the inner cell mass (ICM) and outer trophectoderm. Emergence of transcriptional programs in concert with epigenetic modifications, ensures specification of lineage characteristics and maintenance of their respective stem cell niche. Understanding this fundamental process will provide insight to the emergence of trophoblast stem cells (TSCs) and elucidate their biology. TSCs are progenitors of the placenta, the

keystone organ of mammalian reproduction. Dysfunctional TSCs lead to aberrant development of the placenta and consequently, numerous pathological conditions. Derivation of TSCs will provide a model by which we can study trophoblast lineage development and their deviant behavior in disease. Transcription factor (TF) Cdx2 has been shown to successfully reprogram mouse embryonic stem cells (ESCs) to TSCs, yet this method faces limitations, suggesting alternate gene regulatory mechanisms may be required. microRNA regulation of gene expression remains relatively unexplored in the trophoblast lineage. miRNAs exert their effect on multiple gene networks by binding and degrading target mRNA. Evidently, they are required for specification and maintenance of stem cell niches, thereby serving as a powerful new tool for cell reprogramming. We have conducted an extensive data-mining analysis of 300 miRNAs, to construct an interactive network, highlighting 3 TSC-specific miRNAs, predicted to target 56 ESC mRNAs. We hypothesize that introduction of TSC-specific miRNAs in ESCs will lead to successful reprogramming, and TSC derivation, through miRNA-mediated repression of ESC fate. Methods: To test our hypothesis, mESCs were transfected with doxycycline-inducible piggyback constructs containing one or all three microRNAs of interest. Transgenic lines underwent a 12-day time course induction with and without the addition of valproic acid (VPA), an HDAC inhibitor. microRNA expression levels were assessed using miRNA Taqman qRT-PCR. Gene expression analysis was performed using qRT-PCR, and FACS screens were performed using ESC and TSC-specific antibodies. Results: In the presence of an HDAC inhibitor, miRNAs down-regulate pluripotency genes as early as 6 hours post induction. As predicted, induction of these miRNAs leads to a slight up-regulation of TSC markers Cdx2 and Elf5 by the 4th day of induction, although not at levels similar to embryo-derived TSCs. This trend is apparent in single miRNA transgenic lines, though becomes increasingly evident in the triple transgenic lines. Interestingly, without the addition of VPA, miRNA targets return to similar expression levels as the parent ESC after 48 hours of induction in single transgenic lines. Conclusions: These results confirm that forced expression of TSC-specific miRNAs in ESCs partially reprogram ESCs to a TSC-like state, and that multiple miRNAs have a stronger affect. Our results also suggest a role for HDAC inhibition in potentiating or stabilizing this process. We are similarly deriving stable lines containing a lineage tracer in preparation for blastocyst injection, to observe the contribution of these cells to the trophectoderm in vivo. Future experiments involve the role of the microenvironment on TSC generation efficiency through the utilization of co-culture systems. Furthermore, we are investigating the role of epigenetics in more detail.

F-2148
LACTIC ACID BACTERIA CONVERT HUMAN FIBROBLASTS TO MULTIPOTENTIAL CELLS

Ohta, Kunimasa, Kawano, Rie, Ito, Naofumi
Kumamoto University, Kumamoto, Japan

Humans are in contact with components of the microflora from birth. Thus, a delicate balance exists in the symbiotic relationship between microorganisms and the human host during metabolic activities. Lactic acid bacteria (LAB) form a group of related, low-GC-content, gram-positive bacteria that are considered to offer a number of probiotic benefits to general health. While the role of LAB in gastrointestinal microecology has been the subject of extensive study, little is known about how commensal prokaryotic organisms directly influence eukaryotic cells. Here, we demonstrate the generation of multipotential cells from adult human dermal fibroblast (HDF) cells by incorporating LAB. LAB-incorporated cell clusters are similar to embryoid bodies derived from embryonic stem cells and can differentiate into endodermal, mesodermal, and ectodermal cells in vivo and in vitro. LAB-incorporated cell clusters express a set of genes associated with multipotency, and microarray analysis indicates a remarkable increase of NANOG, a multipotency marker, and a notable decrease in HOX gene expression in LAB-incorporated cells. During the cell culture, the LAB-incorporated cell clusters stop cell division and start to express early senescence markers without cell death. Next, we roughly purified the LAB-derived materials from the homogenized LAB through ion-changed and gel-filtration chromatography by observing their cell clusters forming activity. When we applied the LAB-derived materials to our culture assay system, they could form the cell cluster for not only HDF cells but also cancer cells. Thus, the LAB-derived materials have potentially wide-ranging implications for cell generation, reprogramming, and cell-based therapy.

F-2149
IDENTIFICATION OF SMALL MOLECULES THAT INCREASE DIRECT NEURAL CONVERSION OF HUMAN FIBROBLASTS

Pfisterer, Ulrich[1], Ek, Fredrik[2], Olsson, Roger[2], Parmar, Malin[1]
[1]Lund University, Lund, Sweden, [2]Chemical Biology and Therapeutics, Lund University, Lund, Sweden

We have previously shown that overexpression of three transcription factors Ascl1, Brn2 and Myt1L (ABM) efficiently convert human fibroblasts into functional neurons. Recent findings demonstrated, that small molecules, which promote neuralization of human pluripotent stem cells, can also greatly enhance direct neuronal conversion. To screen for additional small molecules, we have developed an assay that allows for high throughput screening for small molecules, which enhance generation of induced neurons from human fibroblasts. Potential hits are identified based on conversion efficiency as well as based on morphological characteristics of emerging cells using an automated imaging system. By screening a series of annotated libraries, a total of 43 compounds could be identified to affect neuronal conversion efficiency and/ or morphological maturation of induced neurons. Performing dose- response experiments, a subset of these primary hits revealed a concentration- depended effect. Combinatorial screening of identified hits will allow for further increased neuronal conversion efficiency as well as provide mechanistic insight into signaling pathways involved in the direct fate conversion of a human fibroblast into an induced neuron.

F-2150
DE NOVO DNA DEMETHYLATION IS INDISPENSABLE FOR DIRECT LINEAGE REPROGRAMMING

Quan, Xiaoyuan, Kim, Jongpil
Dongguk University, Seoul, Republic of Korea

Dynamic changes in DNA methylation patterns are essential for the cell fate changes including direct reprogramming. While the direct lineage reprogramming of somatic cells is accompanied by demethylation and methylation of various genes, the functional importance of de novo DNA methylation and demethylation has not been clarified. Here, using loss-of-function studies, we generated directly induced cells from fibroblasts that were deficient in de novo DNA methylation and demethylation mediated by Dnmts and Tets respectively. These induced cells reactivated functional genes, underwent cell fate conversion, but showed restricted potentials as the functional cells that were rescued upon reintroduction of Dnmts and Tets. Thus, we conclude that de novo DNA methylation and demethylation is indispensable for direct lineage reprogramming of somatic cells.

F-2151
MODULATION OF TGF-BETA SIGNALLING IS CRUCIAL DURING INDUCTION OF PLURIPOTENCY

Ruetz, Tyson Joel, Kaji, Keisuke
MRC Centre for Regenerative Medicine, Edinburgh, United Kingdom

The generation of pluripotent cells from somatic tissue presented a major breakthrough for regenerative medicine, yet the reprogramming process lacks comprehensive understanding. It has been reported that blockade of TGFβ signaling enhances reprogramming of fibroblasts, through acceleration of the mesenchymal to epithelial transition (MET). However, here we show that inhibition of TGFβ signaling (Alki) increases reprogramming efficiency over 7-fold without altering MET. Instead, we identified one mechanism involved in the enhancement was bypassing of p19-initiated senescence. During the early stages of reprogramming, Alki suppresses transient up-regulation of p19. Moreover, fluorescence activated cell-sorting (FACS) analysis, using the reprogramming stage-specific surface markers ICAM1 and CD44, revealed that late reprogramming intermediates derived in the presence of Alki have an improved probability to successfully reprogram. This indicates that the Alki improves reprogramming through increasing both the quantity and quality of reprogramming intermediates. Seeking to understand the downstream mechanisms of Alki action, we applied a smad3 specific inhibitor (SIS3) during reprogramming. Surprisingly, we discovered that blockage of smad3 signalling inhibits progression of reprogramming in an intermediate stage specific manner, which is the opposite effect of the Alki. These results highlight the complex TGFβ signaling network as a crucial pathway for successful reprogramming, which requires precise modulation during induction of pluripotency.

F-2152
BIOPHYSICAL CONTROL OF NUCLEAR SHAPE CHANGE DURING HUMAN SOMATIC CELL REPROGRAMMING

Harkness, Ty[1], Cordie, Travis[1], Ashton, Randolph S.[1], Turng, Lih-Sheng[2], **Saha, Krishanu**[1]
[1]Biomedical Engineering, University of Wisconsin-Madison, Wisconsin Institutes for Discovery, Madison, WI, USA, [2]Mechanical Engineering, University of Wisconsin-Madison, Wisconsin Institutes for Discovery, Madison, WI, USA

Cellular reprogramming allows for the generation of a self-renewing and patient-specific cell source for drug discovery, cell therapies, and regenerative medicine through the generation of induced pluripotent stem cells (iPSCs). However, the complex signaling occurring among cells, factors in the media, and the surrounding proteinaceous matrix within reprogramming cell cultures is not well understood. The result is a reprogramming process that is notoriously variable and inefficient. Biophysical cues are known to be critical during differentiation but have been largely ignored in reprogramming studies. We utilized a biomaterials approach to probe biophysical regulation of somatic cell reprogramming. Human fibroblasts grown on micropatterned surfaces or aligned nanofibers were found to undergo dynamic changes in nuclear morphology during reprogramming. Nuclear morphology could be predictably controlled by rational alterations to the biophysical or biochemical environment, leading to enhanced reprogramming characteristics. Our data offer insights into the interplay of biophysics and biochemistry during reprogramming and present biomaterials-based systems to rationally control these cues.

F-2153
PASSING THROUGH A PRIMITIVE STREAK-LIKE MESENDODERM STATE AND MAKING A RESISTANT FOR REVERSION BACK TO A SOMATIC CELL FATE ARE HURDLES IN A MATURATION OF HUMAN REPROGRAMMING TOWARD PLURIPOTENCY.

Tanabe, Koji[1], Takahashi, Kazutoshi[2], Ohnuki, Mari[2], Nakamura, Michiko[3], Narita, Megumi[2], Aki, Sasaki[2], Yamamoto, Masamichi[4], Sutou, Kenta[5], Osafune, Kenji[6], Yamanaka, Shinya[7]
[1]Stanford University Institute of Stem Cell Biology and Regenerative Medicine, Palo Alto, CA, USA, [2]Kyoto University, Center for iPS Cell Research and Application, Kyoto, Japan, [3]Kyoto University, Center for iPS Cell Research and Application,, Kyoto, Japan, [4]Development Unit, Gunma University, Gunma, Japan, [5]CiRa, Dept.of Reprogramming Science, Kyoto, Japan, [6]Kyoto University, Kyoto, Japan, [7]Center for iPS Cell Research and Application, Kyoto, Japan

In this study, we found the new important reprogramming barriers toward pluripotent cell fate in induced pluripotent stem cell (iPSC) induction. Pluripotency is induced by over-expressing the combination of transcription factors, such as the OCT3/4, SOX2, KLF4 and c-MYC (OSKM). We selectively detected and analyzed nascent reprogrammed cells using TRA-1-60 antibody, one of the most specific markers of human pluripotent stem cells. We demonstrated that most human adult dermal fibroblasts (~20%) became TRA-1-60 (+) cells by receiving the OSKM on 7 days post-induction. However, only a small portion (~1%) of these nascent reprogrammed cells resulted in colonies of iPSC. These data indicates that maturation, not initiation, is the major roadblock during reprogramming toward pluripotency. We identified the major two reprogramming roadblock in maturation step of reprogramming. The majority of TRA-1-60 (+) nascent reprogrammed cells (~50%) turned back to be negative again in the maturation steps. We found that LIN28 increased the reprogramming efficiency by inhibition of the reversion, not like NANOG, p53shRNA and CyclinD1. Moreover, we found that nascent reprogrammed cells transiently resemble primitive streak-like mesendoderm during maturation of reprogramming. Marker genes for primitive streak, such as BRACHYURY (T), MIXL1, CER1, LHX1 and EOMES showed a transient activation in TRA-1-60 (+) cells. Single cell qRT-PCR and immunocytochemistry revealed that all of TRA-1-60 (+) cells expressed T on day 20. On the other hand, marker genes for other lineages, such as endoderm, mesoderm and neuroectoderm, did not show such transient changes. Actually, 5 out of 23 mesendoderm markers, such as FOXA2, FOXF1, FOXH1, LHX1 and T, significantly increased human reprogramming efficiency. In addition, the stage-specific activation of FOXH1 demonstrated that FOXH1 clearly facilitated the reprogramming efficiency in late stages. The proportion of TRA-1-60 (+) cells was reproducibly reduced by FOXH1 on day 7, but the proportion was increased again on days 11 and 15 compared to cells transduced with OSKM alone. On the other hand, knockdown of FOXH1 in ESCs significantly interfered with their differentiation into primitive-streak mesendodermal (PSMN) state. Depression of FOXH1 expression decreased reprogramming efficiency. Moreover, we previously reported that Glis1 increased reprogramming efficiency in iPSC induction. In this study, we found that GLIS1 was highly expressed in PSMN derived from human ESCs/iPSCs. We also found that mouse Glis1 is expressed in embryonic regions, including primitive streak. Forced expression of GLIS1 in human ESCs gave rise to PSMN features. These data demonstrated that GLIS1 has important roles in the PSMN lineage, which may contribute to its pro-reprogramming activity. These data demonstrated that human reprogramming cells go through a transient mesendoderm like state. The passing through that cell fate is one of reprogramming hurdles in human iPSC reprogramming.

F-2154

REDUCTION OF DNMT1 PROTEIN ENHANCES THE KINETICS AND EFFICIENCY OF IPSC REPROGRAMMING

TCW, Julia[1], Sindhu, Camille[1], Karnik, Rahul[1], Meissner, Alexander[2], Eggan, Kevin Carl[1]
[1]Harvard University, Cambridge, MA, USA, [2]Harvard University/Broad Institute, Cambridge, MA, USA

Reprogramming somatic cells into induced pluirpotent stem cells (iPSCs) by defined transcription factors holds great potential for biomedicine. However, reprogramming process retains slow kinetics and low efficiency. Reprogramming of somatic cells into iPSCs resets the epigenome to an embryonic-like state, which includes DNA demethylation of somatically-methylated pluripotentcy genes. We hypothesized that lowering the activity of DNA methyltransferase 1 (Dnmt1) gene might increase the reprogramming potential and perturb a blockade of iPSC reprogramming. Here, we have generated an allelic series of *Dnmt1* mouse embryonic fibroblasts (MEFs), which have gradually down-r egulated *Dnmt1* expression, and investigated their reprogramming efficiency and dynamics. At the epigenetic level, the global methylation level is reduced by 20% in hypomorphic *Dnmt1*MEFs, which reduces Dnmt1 expression to 10% of wild-type levels. Specifically, the methylation levels of *Oct4* regulatory regions are decreased by 50%, and the Oct4 protein binds to the genome with a higher affinity in hypomorphic *Dnmt1*MEFs as compared to the wild-type MEFs in the early stage of reprogramming. These results indicate that reducing *Dnmt1*unravels the repressive effect both in the global level and the *Oct4* region. We found that a 90% reduction in *Dnmt1* levels enhanced the efficiency of reprogramming over 4-fold. Importantly, hypomorphic allele ($Dnmt1^{chip}$) transduced line, which induces relatively low expression levels (20-40%) of DNMT1are reprogrammed to pluripotency with high efficiency and fast kinetics by directly activating Oct4 expression and bypassing the intermediate states. Furthermore, DNA demethylation synergized with histone acetylation and histone demethylation increased their conversion rates over 100-fold. Our findings demonstrate that DNA demethylation, by decreasing Dnmt1 expression levels, can increase the reprogramming potential of somatic cells. Collectively, our data offer new insights into the nature of epigenetic events inherent to cellular reprogramming.

F-2155

DIRECT REPROGRAMMING OF NG2 GLIA CELLS IN VIVO

Torper, Olof Anders[1], Rylander, Daniella[1], Lau, Shong[1], Parmar, Malin[2]
[1]Experimental Clinical Science, Lund University, Lund, Sweden, [2]Lund University, Lund, Sweden

In vivo reprogramming is an interesting alternative to traditional cell replacement therapy as it removes the need for an exogenous cell source. It aims to target a non-neuronal population of cells, resident in the brain that can be reprogrammed into neurons to replace cells lost due to disease or injury of the brain. There are many cells that would make good candidates for in vivo reprogramming like astrocytes, microglia and NG2 glia. We have previously shown the possibility to convert resident astrocytes in the striatum into neurons by injecting the conversion factors Ascl1, Brn2 and Myt1L into brains of adult mice. NG2 glia cells are almost as numerous as astrocytes with a remarkable self- renewal capacity. They serve as oligodendrocyte progenitor cells (OPC:s) and receive synaptic input from neurons indicating that they are part of the neuronal network. They are also anti-genetically different from any other macro-and micro glia cells present in the brain and can be identified by the expression of chondroitin sulphate proteoglycan (NG2). There are also reports of NG2 cells neurogenic potential when explanted to neurogenic zones in the brain or when cultured under neurological conditions. This apparent plasticity, their close proximity to neurons and their self-renewal capacity makes NG2 glia cells interesting targets for in vivo reprogramming. We here show the possibility to convert resident NG2 cells into neurons by injecting Cre inducible rAAV vectors coding for the transcription factors Ascl1, Lmx1a and Nurr1 (ALN), previously shown to convert mouse fibroblasts into dopaminergic neurons in vitro, into the striatum of NG2-Cre transgenic mice. By co-injecting GFP under the synapsin promoter together with the conversion factors, reprogrammed cells can be distinguished from resident neurons while GFP is not being expressed by unconverted NG2 cells. Converted cells display a typical neuronal morphology, express pan-neuronal markers and lose their NG2 expression indicating the full reprogramming into a neuronal lineage. Current experiments aim to assess the functionality of these cells in terms of electrophysiological properties and synaptic input by monosynaptic tracing using rabies virus.

IPS CELLS: DIRECTED DIFFERENTIATION

F-2157

DERIVATION OF RETINAL PROGENITORS FROM HUMAN INDUCED-PLURIPOTENT STEM CELLS IN A XENO-FREE DEFINED CONDITION.

Kaini, Ramesh Raj, Johnson, Anthony J., Burke, Teresa, Golden, Dallas J., Wang, Heuy-Ching H.
Ocular Trauma, USAISR, Fort Sam Houston, TX, USA

Recent advances made in stem cell research have instilled hopes for cell replacement therapy in many human diseases, including retina degeneration diseases and retinal trauma. For clinical application of stem cells derived bioproducts, they must be maintained and differentiated in xeno-free condition. In this study, we sought to derive clinical-grade retinal progenitors from human induced-pluripotent stem (iPS) cells in a xeno-free defined condition. Commercially available iPS cells, IMR90-1 and piPS, were maintained in VitronectinXF coated culture plates with xeno-free TeSR-E8 medium. iPS cells were dissociated and quickly reaggregated using Sumilon PrimeSurface96V culture plates in GMEM medium containing 20% KnockOut Serum Replacement XenoFree . To start the xeno-free 3D differentiation, VitronectinXF was added in the retinal differentiation medium from day 2 onwards to day 18. 3D cell aggregates were treated with Wnt inhibitor, IWR-1-endo, for the first 12 days. KnockOut SRGrowth Factor cocktail was added on day 12 and Smoothened Agonist (SAG) on day15. Aggregates were then switched to DMEM/F12-Glutamax medium with N2 supplement on day 18. Expression of different markers of eye field and retinalprogenitors were studied at different time points. iPS cells maintained in vitronectinXF coated plates with TeSR-E8 medium retained the expression ofpluripotent markers after five passages. Aggregates of xeno-free 3D differentiation started expressing different neural and eye field markers, including Otx2, Sox2, Rx, LHX2, Six6, PAX6, MITF, and CHX10 at different time points of differentiation. Retinal progenitor marker, CHX10 was observed on day 16 onwards. In this study, we observed that cells of neural retinal lineage can be derived from human iPS cells in a 3D culture system using xeno-free components. Developing stratified neural retina from iPS cells under xeno-free defined condition is in progress.

F-2158

NEURAL PROGENITOR CELLS CAN BE GENERATED EFFICIENTLY USING STEMDIFF™ NEURAL INDUCTION MEDIUM BY EITHER EMBRYOID BODY OR MONOLAYER CULTURE METHODS

Lee, Vivian[1], Blak, Alexandra[2], Eaves, Allen C.[2], Thomas, Terry E.[1], Louis, Sharon A.[1]

[1]Research and Development, STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]STEMCELL Technologies Inc, Vancouver, BC, Canada

Neural progenitor cells (NPCs) derived from human pluripotent stem cells (hPSCs), are extensively used for studying human central nervous system (CNS) development, modeling of neurological disorders and screening for therapeutic molecules. Current protocols for NPC derivation from hPSCs, including human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC), are not standardized and therefore can require significant optimization by researchers. We have previously reported that CNS-type NPCs can be produced efficiently using STEMdiff™ Neural Induction Medium (NIM) in an embryoid body (EB)-based protocol. When used in conjunction with AggreWellTM800 for EB formation from hPSCs, STEMdiff™ NIM induces up to 100% neural rosette formation in all the EBs harvested and replated on Corning® Matrigel® or poly-L-ornithine/laminin (PLO/L) coated plates. These neural rosettes can then be isolated using STEMdiffTM Neural Rosette Selection Reagent (STEMdiffTM NRSR) to obtain an enriched population of CNS-type NPCs for further studies. However, recent publications have reported that neural induction from hPSCs can also be accomplished using a single-step monolayer culture protocol. This method does not require EB formation thereby simplifying the experimental procedures. Here, we describe the efficient derivation of NPCs using STEMdiff™ NIM in a monolayer culture protocol. Briefly, hPSCs maintained in mTeSR1™ or TeSR™-E8™ were dissociated using Gentle Cell Dissociated Reagent (GCDR) into single cells, seeded at 200,000 - 250,000 cells/cm2 on Matrigel® or PLO/L coated plates, and cultured as a monolayer in STEMdiffTM NIM for up to 9 days. Neural induction was assessed qualitatively by immunocytochemistry at different time points (1, 3, 5, 7, and 9 days) for the presence of PAX6+OCT4- cells in culture, as well as, quantitatively by qPCR for PAX6 and OCT4 transcripts. The timing of induction varied between hPSC lines tested. hESC lines typically required 6 days for induction whereas hiPSC lines could take up to 9 days. Neural induction (> 95% PAX6+OCT4- cells detected in 8 random, non-overlapping areas on two coverslips per condition) was achieved by day 6 for hESC (H1, H9; n=6) and day 9 for hiPSC (WLS-4D1, STiPS; n=6) lines. Data from qPCR analyses corroborated the immunocytochemistry results and showed an increase in PAX6 expression during neural induction with a concomitant decrease in OCT4 gene expression. In parallel negative control cultures containing mTeSR1 or TeSR-E8 maintenance medium, OCT4 levels did not change. In contrast, OCT4 expression decreased over time in cells that were cultured in STEMdiff NIM with PAX6 levels upregulated, indicating that cells were undergoing neural induction. These data show that CNS-type NPCs can be rapidly induced from hPSCs maintained in mTeSR1™ and TeSR™-E8™ media using STEMdiff™ NIM in a monolayer protocol. In summary, we have demonstrated STEMdiff™ NIM can support efficient neural induction in a single step monolayer protocol. Because STEMdiff™ NIM is a versatile reagent that allows the efficient generation of NPCs from hPSCs using either the EB- or monolayer-based neural induction protocols, the functional characteristic of NPCs enriched from either protocol can be compared directly in the same medium.

F-2159

GENERATION, CHARACTERIZATION AND MULTILINEAGE POTENCY OF MESENCHYMAL-LIKE PROGENITORS DERIVED FROM EQUINE IPS CELLS.

Lepage, Sarah I. M.[1], Nagy, Kristina[2], Sung, Hoon-Ki[2], Kandel, Rita A.[2], Nagy, Andras[3], Koch, Thomas G.[1]

[1]Biomedical Sciences, University of Guelph, Guelph, ON, Canada, [2]Mount Sinai Hospital, Toronto, ON, Canada, [3]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada

In horses, mesenchymal stromal cells (MSCs) are used primarily in regenerative medicine studies to treat orthopedic injuries. However, these cells are limited in their expandability and differentiation capacity. Recently, the first equine induced pluripotent stem cell (eiPSC) lines were reported by Nagy et al. Induction of iPSCs into MSC-like cells is an attractive option to using MSCs derived from other sources, as a much larger population of patient-specific cells could be generated in hopes of treating large orthopedic defects. However, the differentiation capacity of eiPSCs and their potential for use in tissue engineering has yet to be explored. Multiple equine iPSC (eiPSC) lines were induced to differentiate into a MSC-like population via the removal of doxycycline and LIF and the addition of an ALK receptor antagonist to the culture media. This combination of factors, along with serial passaging of eiPSCs from their feeder layer to gelatin and finally to uncoated petri dishes, bypassed the need to generate embryoid bodies, which tend to produce heterogenous and irregular mesodermal cell populations. The differentiated cells were analyzed by morphology, gene expression, surface marker expression, and multilineage differentiation. One eiPSC line (H3-B) of the three tested was successfully induced into a semi-homogenous MSC-like population (termed eiPSC-MSCs). Upon induction, the H3-B eiPSCs lost their colony morphology and acquired a spindle-like phenotype, as well as the ability to adhere to plastic. This cell population demonstrated a downregulation of genes associated with pluripotency (Oct4, Sox2, Nanog, and Klf4) and an upregulation of MSC-specific genes (Sox5 and Sox6). As well, flow cytometry analysis revealed that eiPSC-MSCs uniformly expressed the same surface markers as equine MSCs derived from umbilical cord blood (CD29, CD44, and CD90), further confirming eiPSC-MSCs as a MSC-like population. We then observed the osteogenic and adipogenic potential of eiPSC-MSCs. After induction, histological staining of eiPSC-MSCs with von Kossa and Alizarin Red revealed mineralization characteristic of osteogenesis, and adipogenesis was observed using Oil Red O staining to show lipid droplet accumulation. We did not observe chondrogenesis of eiPSC-MSCs by TGFβ3 induction in 3D pellet culture; this is likely because eiPSC differentiation did not result in an increase in Sox9 expression, a master regulator of chondrogenesis. We demonstrate that eiPSCs can be directly differentiated into an intermediate MSC-like population that is capable of undergoing adipogenesis and osteogenesis, which is characteristic of putative MSCs. Though chondrogenesis is an ongoing effort, this work delivers valuable insight into the steps required for directed differentiation of eiPSCs down the mesodermal lineage. The potential of this novel cell type for large-scale derivation of MSCs is of great value for both veterinary and human regenerative medicine, as the horse serves as an excellent large animal model for translation to human orthopedic research.

F-2160

A SMALL MOLECULE APPROACH TOWARD GENERATION OF HEMATOPOIETIC STEM CELLS FROM PATIENT-SPECIFIC IPSCS

Li, Mo[1], Montserrat, Nuria[2], Suzuki, Keiichiro[1], Wu, Jun[3], Kim, Na Young[1], Benner, Christopher[1], Chang, Chan-Jung[1], Belmonte, Juan Carlos Izpisua[3]

[1]The Salk Institute for Biological Studies, La Jolla, CA, USA, [2]Centre de Medicina Regerativa de Barcelona, CMRB, Barcelona, Spain, [3]Salk Institute for Biological Studies, La Jolla, CA, USA

Human pluripotent stem cells (hPSCs), including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) can self-renew indefinitely and give rise to any cell type in the body. Patient-specific hiPSCs thus offer a new avenue of engineering immunocompatible tissues for cell therapy. Hematological diseases have been treated with traditional cell therapies, including blood transfusion and hematopoietic stem cell transplantation, in the clinic. However, allogeneic transplantation faces constant shortage of donors and risky immunological complications. We and others have generated iPSCs from monogenic blood diseases and demonstrated that these patient iPSCs can be genetically corrected. These proof-of-concept studies provide the grounds to develop methods to produce clinical-grade hematopoietic stem cells (HSCs) and mature blood lineages from hPSCs. hPSCs can be differentiated into hematopoietic progenitor cells (HPCs), from which major blood lineages can be derived. However, unlike somatic HSCs, iPSC-derived HPCs (iPSC-HPCs) have limited proliferative capacity, cannot achieve long-term engraftment and exhibit a strong bias to myeloid lineages in immunodeficient mice. These deficiencies likely reflect a failure to establish the authentic HSC transcriptional/epigenetic program during in vitro differentiation. Here we compare the transcriptome and epigenome of iPSC-HPCs with those of cord blood HPSCs and apply integrated bioinformatics analyses to identify pathways that are mis-regulated in iPSC-HPCs. Small molecules that target these pathways significantly increase the production of iPSC-HPCs. We show the specificity of the small molecules by neutralizing their effects on hematopoietic differentiation with antagonistic compounds. We additionally screened drugs that modulate other key developmental pathways and did not observe the same effects. Small-molecule treated HPCs are more colonogenic (especially more erythroid and mixed colonies) in colony forming assays. Directed differentiation into the erythroid lineage proves that small molecule treatment increases red blood cell production. We are in the process of evaluating the in vivo repopulating activity of HPCs treated with the small molecules.

F-2161

SOMATIC REVERSION IN THE WISKOTT-ALDRICH SYNDROME

Li, Xuan Shirley[1], Laskowski, Tamara J.[1], Van Caeneghem, Yasmine[2], Liao, Wei[1], Crane, Ana M.[1], Gandhi, Poojabahen[1], Ni, Zhenya[3], Kaufman, Dan S.[3], Candotti, Fabio[4], Vandekerckhove, Bart[2], Davis, Brian R.[1]

[1]Institute of Molecular Medicine - Center for Stem Cell and Regenerative Medicine, University of Texas Health Science Center Houston, Houston, TX, USA, [2]Laboratory for Experimental Immunology, Ghent University, Ghent, Belgium, [3]University of Minnesota, Minneapolis, MN, USA, [4]Disorders of Immunity Section, NIH National Human Genome Research Institute, Bethesda, MD, USA

Wiskott-Aldrich syndrome (WAS), an X-linked primary immunodeficiency disease, is caused by mutations in the WAS gene, an actin cytoskeletal regulator important in T cell receptor signaling, NK cell cytotoxicity, and cell migration. Somatic reversion, the spontaneous correction of a WAS gene mutation leading to restoration of WAS protein (WASp) expression, is observed in approximately 15% of WAS patients. We have identified a diversity of WAS genotypes, primarily insertions/deletions in proximity to the germ-line 1305 insG mutation, that restore reading frame in CD4+ or CD8+ T cells, and CD19+ B cells from a revertant WAS patient. Enrichment and persistence of revertant cells suggest a strong selective pressure in vivo for corrected WASp-expressing cells based on functionality. In fact, in other revertant WAS patients, we have demonstrated that primary revertant T cells recover functionality (e.g. anti-CD3 stimulated proliferation) absent in WASp deficient cells. Interesting questions remain regarding when and where in hematopoietic development the revertant mutations originate and how selection enables revertant cells to persist as a subpopulation. In order to develop an in vitro experimental model capable of addressing these issues, we generated iPSCs from fibroblasts of a WAS patient carrying the identical 1305 insG frame-shift mutation. Differentiation of mutant iPSC alongside WAS-2A-eGFP transgene corrected iPSC (cWAS) and WA01/WA09 ESC demonstrated NK and T cells were far more prevalent from cWAS and WA01/WA09 CD34+ progenitors than from WAS CD34+ progenitors. Differentiation to CD34+ CD43+ progenitors is not affected by WASp expression, indicating a period of development wherein WAS deficiency is not under selection. We propose to induce non-homologous end joining mutations, some of which are revertant, in WAS CD34+ CD43+ progenitors by introducing a double strand break with TALENs at the 1305 mutation site. Resulting pools of progenitors with varying degrees of reversion will be profiled by next generation sequencing, first as progenitors and then as terminally differentiated NK or T cells, to directly assay the selective pressures acting during differentiation. Titration of mutant WAS with revertant or wild-type WASp-expressing progenitors followed by differentiation will enable calculations of selective forces based on population genetics. Using WAS iPSC as a model system we hope to identify the mechanisms behind genotypic subpopulation dominance and loss in culture.

F-2162

DIRECTED DIFFERENTIATION OF MOTOR NEURONS AND ASTROCYTES FROM HUMAN INDUCED PLURIPOTENT STEM CELLS FOR THE STUDY OF SPINAL MUSCULAR ATROPHY

Loh, Sharon Jia Hui[1], Stanton, Lawrence W.[2]

[1]Stem Cell and Developmental Biology, Genome Institute of Singapore and Nanyang Technological University, Singapore, [2]Genome Institute of Singapore, Singapore

Spinal Muscular Atrophy (SMA) is a neurodegenerative disorder affecting spinal cord Motor Neurons (MNs), leading to muscle weakness. It has been reported that low expression level of SMN protein and other neuronal cell types cause MNs to degenerate in SMA. However, key downstream regulatory genes of SMN protein responsible for inducing MNs death and the roles of astrocytes in SMA pathology remain elusive. To study we successfully established methodologies that efficiently differentiate WT and SMA human induced pluripotent stem cells (hiPSCs) to MNs and astrocytes. Our differentiation methods generated MNs that expressed MNs specific markers (FOXP1, ISL-1, ChAT) and astrocytes that expressed astrocytic markers (GFAP, S100b, CD44). These MNs and astrocytes after being patterned by morphogens also achieved caudal ventral identity and expressed caudal ventral marker HOXB4. It's no surprise that dead cells started appearing only in the SMA MN culture during the second week of MN differentiation, suggesting that SMA iPSCs-derived MNs were degenerating. Interestingly, it was also observed

that SMA-iPSCs derived astrocytes expressed glutamate transporter less abundantly than WT-iPSCs derived astrocytes, implying that SMA astrocytes may have impaired glutamate uptake function that cause excitotoxicity to MNs. These preliminary findings suggest that SMA iPSCs-derived MNs and astrocytes exhibited abnormalities and these affected cell types obtained through directed differentiation from hiPSCs, as we have demonstrated, will serve as a valuable resource to study dysregulated molecular mechanisms in SMA.

F-2163
FC CHIMERIC PROTEIN-BASED BIOMATERIALS ARE SIGNIFICANTLY ADVANTAGEOUS FOR APPLICATION IN STEM CELL TECHNOLOGY AND REGENERATIVE MEDICINE

Nag, Kakon, Akaike, Toshihiro
Tokyo Institute of Technology, Yokohama, Japan

Designing a defined matrix for stem cell culture and differentiation requires special biomaterial that can provide simultaneous supports for cell adhesion, proliferation and differentiation. In fact, despite introducing many synthetic and semisynthetic biomaterials alone or as a blend as cell-culture substrate, successful stem cell culture condition with high pluripotency were rarely achieved. Therefore designing such a biomaterial remains as a difficult and rewarding task. We have generated cell recognizable Fc-chimeric protein, where Fc domain of IgG is fused with a target protein, as a highly effective novel class of defined biomaterials for stem cell technology. Our pioneering work has established several Fc-chimeric proteins such as epithelial cadherin (E-cad) and IgG Fc (E-cad-Fc), epidermal growth factor (EGF) and IgG Fc, (EGF-Fc), leukemia inhibitory factor (LIF) and IgG Fc (LIF-Fc), hepatocyte growth factor (HGF) and IgG Fc (HGF-Fc), vascular endothelial growth factor (VEGF) and IgG Fc (VEGF-Fc), neural cadherin (N-cad) and IgG Fc (N-cad-Fc) as novel cell-recognizable biomaterials in stem cell technology. Recently we have also introduced Fc-chimeric protein for adrenomedullin 1 (AM1) – a multipotent ligand for a G protein-coupled receptor (GPCR), namely, calcitonin receptor like receptor (CLR) in stem cell technology. We found that E-Cad-Fc can provide defined matrix to generate single-cell type stem cell culture, which is quite unique compare to the normal colony phenotype, and associated with higher transfection efficiency and proliferation capacity. One of the big issues in stem cell culture is the necessity of regular replenishment of media to provide required functional cytokines. Lif-Fc and HGF-Fc showed relevant sustained signaling and fittingly suggested that same type of chimeric cytokines can be used to eliminate the hassle of daily replenishment of media. Such sustained signaling for Lif-Fc and HGF-Fc resulted enhanced viability and differentiation of stem cells to neural and hepatic lineage, respectively. We further found that N-Cad-Fc can significantly promote the neural growth compare to any other known matrix reported so far. These advance biomaterials have shown tremendous potential for advancing stem cell technology closer to application in regenerative medicine and tissue engineering in multiple ways namely, feeder-cell free ESC culture, simplified and cost-effective culture system for stem cell, directed differentiation of stem cell, and on-site stress-free purification of target cells and deserve scientific recognition for practical application.

F-2164
DIFFERENTIATION PHASE-DEPENDENT FACTORS RESPONSIBLE FOR VARIATION IN HEMATOPOIETIC DIFFERENTIATION PROPENSITY AMONG HUMAN PLURIPOTENT STEM CELLS REVEALED BY GENOME-WIDE ANALYSIS OF GENE EXPRESSION AND DNA METHYLATION

Nishizawa, Masatoshi[1], Chonabayashi, Kazuhisa[1], Oishi, Akiko[1], Takei, Ikue[1], Nishikawa, Misato[1], Takaori-Kondo, Akifumi[2], Yamanaka, Shinya[1], Yoshida, Yoshinori[1]
[1]Department of Reprogramming Science, Center for iPS Cell Research and Application, Kyoto, Japan, [2]Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Objective: Hematopoietic differentiation from human induced pluripotent stem (iPS)/embryonic stem (ES) cells attracts much attention due to its huge potential for regenerative medicine. Reportedly, there is large variation in differentiation potential among pluripotent stem cell (PSC) lines, and the factor(s) responsible for the variations is still largely unknown. One of major study limitations of earlier papers is limited number of human PSC lines utilized in the studies although genetic difference among individual donors of iPS cells seems to be large. Although some papers reported about presence of epigenetic memories of parental somatic cells in iPS cells, amount of the influence on differentiation potential remains to be known. Furthermore, differentiation from PSCs to differentiated cells involves many steps for long time in culture; nevertheless, differentiation phase-dependent factors has not been taken into account in most of earlier papers discussing variations in differentiation propensity among human PSCs. To address these issues, we planned to investigate hematopoietic differentiation potentials of many PSC lines derived from many donors and many tissues, and assessed differentiation potential in early and late differentiation phase, separately. In addition, we planned to collect data on genome-wide gene expression and DNA methylation of these PSC lines. The final goal of this study is to create database about differentiation potentials of many PSC lines and molecular signatures such as gene expression and DNA methylation for clinical application of PSCs in the near future. Method: In the present study, we utilized 35 iPS cell lines derived from 5 types of somatic cells and 15 donors, as well as 4 ES cell lines derived from 4 donors. We assessed hematopoietic differentiation potential in early phase by counting generated CD43 (+) hematopoietic cell for the first 15 days after start of differentiation, and that in late phase by investigating colony forming potential of PSC-derived hematopoietic precursor cells, and erythroblast and megakaryocyte differentiation efficiency. In addition, we collected data on genome-wide gene expression and DNA methylation of these PSC lines, parental lines of iPS cells, PSC-derived hematopoietic precursor cells, and analyzed correlation of these data with result of the differentiation experiments. Result: Integrated analyses of differentiation experiments and these array data revealed that expression of some genes or factors were significantly correlated with hematopoietic differentiation potential of PSCs, and intriguingly, the factors affecting differentiation efficiency in early and late differentiation phase was absolutely different. Importantly, by combining several factors discovered in this analysis, we can predict hematopoietic differentiation potential of individual iPS/ES cell lines regardless of what parental cell lines iPS cells are derived or whether it is an iPS cell or ES cell. Conclusion: We have found out that the differentiation-phase determining factors responsible for difference in hematopoietic differentiation potential among PSC lines. We believe that our findings will contribute not only to understanding molecular mechanisms underlying diversity in differentiation potential among PSC lines, but also to clinical application of PSCs in the future.

F-2165

EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO CONTRACTING CARDIOMYOCYTES

Owens, D. Jason[1], Tang, Yixin[2], Aho, Joy L.[2]
[1]Stem Cell Department, R and D Systems, Inc, Minneapolis, MN, USA, [2]R & D Systems, Minneapolis, MN, USA

Functionally mature cardiomyocytes produced from a renewable cell source, such as human pluripotent stem cells (hiPS or hES), are in high demand for their potential contributions to developmental research, disease modeling, high throughput toxicity drug screening, and clinical therapies. Here, we introduce our StemXVivo™ Cardiomyocyte Differentiation Kit, which directs human pluripotent stem cells through the necessary cell fate decisions to recapitulate embryonic mesoderm development and produce functional cardiomyocytes. These differentiation reagents have been verified on multiple pluripotent cell lines, including both hES and hiPS lines. The differentiated cardiomyocytes have been verified by developmental and structural marker expression via immunocytochemistry and flow cytometry, as well as their performance in functional assays.

F-2166

IMPROVEMENT OF HEPATIC DIFFERENTIATION FROM HUMAN INDUCED PLURIPOTENT STEM CELLS BY REMOVAL OF UNDIFFERENTIATED STEM CELLS USING YM155

Kang, Seok-Jin, **Park, Young-Il**, Lee, Hyuk-Mi, So, Byung Jae, Kang, Hwan-Goo
Veterinary Drugs and Biologics Division, Animal and Plant Inspection Agency, Anyang, Republic of Korea

Human iPS derived hepatocytes are promising target cells for drug development of human ES and iPS cells. Recently, several protocols of multistep hepatic differentiation have been reported to recapitulate the in vivo hepatic development. In this study, to generate hepatic cells differentiated from human induced pluripotent stem cells (hiPSCs), we established differentiation protocol via three steps; definitive endoderm, hepatic endoderm and hepatic maturation. Under the condition, morphological changes and the expressions of lineage specific genes and proteins were observed at each stage. However, undifferentiated stem cells were still observed during the differentiation and consequently quality of hepatic population was poor due to differentiation of non-hepatic lineages originated from undifferentiated stem cells. Therefore, we hypothesized that it could improve the quality of hepatic population if undifferentiated stem cells were effectively removed during the hepatic differentiation. YM155 is inhibitor of surviving, which is generally overexpressed in pluripotent stem cells such as human ES/iPS and cancer stem cells. The compound has been used in clinical applications for cancer therapy and interrupt of teratoma formation. Therefore, we try to apply the compound in our hepatic differentiation for removal of the non-hepatic lineage cells derived from undifferentiated stem cells. For establishing the optimal concentration of YM155, differentiated cells were exposed from 1nM to 100nM (DMSO, 1, 5, 10, 50 and 100nM) determined at previous study. OCT4 and TRA-1-60 positive cells were decreased by dose-dependent manner. Also, the expressions of NANOG and OCT4 as pluripotent marker were decreased but those of endodermal markers such as CXCR4, SOX17 and FOXA3 were increased by dose-dependent manner. At the final stage of differentiation, non-hepatic lineage cells were disappeared regarding morphology, and the expressions of ectodermal and mesodermal genes were decreased but hepatic marker genes (ALB, AFP and HNF4a) were relatively increased as compared with non-treated differentiated cells. Also, Albumin positive cells were significantly increased in 5nM (74.6%) and 10nM of YM155 (69.1%) compared with DMSO (41.9%) and 1nM (47.2%). In conclusion, we suggested that YM155 could remove the undifferentiated stem cells during the hepatic differentiation and increase the portion of qualified hepatic cells from hiPSCs.

F-2167

TRANSCRIPTIONAL PROFILING OF CONTROL AND A53T-ALPHA SYNUCLEIN HUMAN IPS CELLS DIFFERENTIATED TOWARDS MIDBRAIN DOPAMINE NEURONS A WINDOW TO REGULATORY DEVELOPMENTAL PATHWAYS AND A53T-ASSOCIATED DYSFUNCTION

Prodromidou, Kanella[1], Vlachos, Ioannis[2], Kouroupi, Georgia[1], Era, Taoufik[1], Tsioras, Kostantinos[1], Hatzigeorgiou, Artemis[2], Matsas, Rebecca[1]
[1]Hellenic Pasteur Institute, Athens, Greece, [2]Biomedical Sciences Research Center Alexander Fleming, Athens, Greece

Human embryonic stem cells (HUES) and induced pluripotent stem cells (hiPS) are characterized by their ability to self-renew and their potential to differentiate into specialized cell types. The ability to generate large numbers of early precursors and stage-specific differentiated cells renders them a valuable source for studies in developmental biology, applications in regenerative medicine or for modeling human diseases. In particular, human pluripotent stem cells provide an unprecedented opportunity to study otherwise inaccessible human neurons and their precursors while the generation of patient-specific hiPSs can significantly contribute in our understanding of the molecular mechanisms underlying neurodegenerative diseases. We have recently generated midbrain dopamine neurons by directed differentiation of hiPS derived from fibroblasts of healthy individuals and Parkinsonian patients harbouring an autosomal dominant and highly penetrant A53T mutation in alpha-synuclein (A53T-αSyn). As a first step to identify novel differentiation-related critical genes and regulatory networks as well as to discover A53T-dysregulated pathways, we have launched whole transcriptome analysis of hiPS-derived cells by new generation sequencing of coding and non-coding RNAs (long non-coding RNAs and miRNAs) at specific stages of differentiation (hiPS, neuronal precursors NPCs and neurons). In parallel, we have complemented this analysis using HUES and HUES-derived differentiated cells. Total RNA was extracted from fibroblasts, hiPSs, NPCs and neurons and next generation sequencing was performed on polyA-selected transcripts (50nt, paired-end sequencing) and small-RNAs (50nt, signle-end sequencing) on an Illumina HiSeq sequencer. More than 1.5 billion reads were generated. Small-RNA-Seq reads were aligned against mature miRNAs, precursors and relevant genomic loci whilst RNA-Seq reads were aligned against the genome using a spliced aligner. Both miRNA and mRNA differential expression analysis was performed using DESEQ and Pathways controlled by significantly differentially expressed miRNAs were identified using DIANA-miRPAth v2.0. Using integrated mRNA-miRNA functional analyses we aim to identify major regulatory networks during differentiation of human neurons and A53T-dysregulated pathways.

F-2168
DESIGNER NUCLEASE-MEDIATED CORRECTION OF RS-SCID

Rahman, Shamim Herbert[1], Kühle, Johannes[2], Reimann, Christian[1], Maeder, Morgan L.[3], Riedel, Heimo[4], Cantz, Tobias[5], Rudolph, Cornelia[6], Joung, Keith J.[3], Schambach, Axel[2], Cathomen, Toni[1]

[1]Institute for Cell and Gene Therapy and Center for Chronic Immunodeficiency, University Medical Center Freiburg, Freiburg, Germany, [2]Institute of Experimental Hematology, Hannover Medical School, Hannover, Germany, [3]Molecular Pathology Unit, Mass. General Hospital Harvard Medical School, Charlestown, MA, USA, [4]Department of Biochemistry, Robert C. Byrd Health Sciences Center, West Virginia University, Morgantown, WV, USA, [5]Translational Hepatology and Stem Cell Biology, REBIRTH-Cluster of Excellence, Hannover Medical School, Hannover, Germany, [6]Institute for Cellular and Molecular Pathology, Hannover Medical School, Hannover, Germany

Severe combined immunodeficiencies (SCID) are the most severe form of inherited blood disorders. Failure of the adaptive immune response is due to the absence of T and/or B/NK cells. The NOD.SCID mouse is a valuable model for human SCID in general and for radiosensitive SCID (RS-SCID) in particular. The T-/B- immune phenotype is caused by a point mutation in exon 85 of the prkdc gene, which encodes for the DNA repair factor DNA-PKcs. To correct the RS-SCID underlying mutation, we generated zinc-finger nucleases (ZFNs) that target intron 84 of the prkdc locus with the aim to restore DNA-PKcs function by integrating a donor DNA encompassing a splice acceptor followed by a cDNA encoding exons 85/86. NOD.SCID-derived ear fibroblasts that underwent gene targeting at the prkdc locus revealed restored DNA-PK dependent signaling and reduced sensitivity to the radiomimetic drug bleomycin. Next, NOD.SCID-derived induced pluripotent stem cells (iPSCs) were generated. Selected clones contained an intact karyotype and gave rise to all three germ layers in teratoma assays. Upon ZFN-based correction, ~90% of iPSC clones showed targeted integration of exon 85/86 into intron 84. To assess the rescue of T cell development, a protocol to generate T cells from pluripotent stem cells was established. As expected, both corrected and uncorrected iPSC clones could be differentiated to DN2-stage T cells (CD44+/CD25+) in vitro, but only corrected cells with restored prkdc expression gave rise to double-positive CD4+/CD8+ T cells. The iPSC-derived T cells express TCRß and underwent DNA-PK dependent V(D)J T cell receptor recombination. In conclusion, our findings demonstrate that ZFN-based genome engineering can be applied to correct the RS-SCID phenotype in iPSCs by restoring DNA-PK dependent signaling and hence may present a paradigm for the generation of autologous cell therapeutics for adoptive transfer.

F-2169
PATIENT-DERIVED SKELETAL DYSPLASIA IPSCS DISPLAY ABNORMAL CHONDROGENIC MARKER EXPRESSION AND REGULATION BY BMP2 AND TGF-BETA1

Saitta, Biagio[1], Passarini, Jenna[1], Sareen, Dhruv[1], Ornelas, Loren[1], Sahabian, Anais[1], Krakow, Deborah[3], Cohn, Daniel H.[2], Svendsen, Clive[3], Rimoin, David L.[1]

[1]Cedars Sinai Medical Center, Los Angeles, CA, USA, [2]University of California Los Angeles, Los Angeles, CA, USA, [3]Cedars-Sinai Regenerative Medicine Institute, Los Angeles, CA, USA

Skeletal Dysplasias (SDs) are caused by abnormal chondrogenesis during cartilage growth plate differentiation. To study early stages of aberrant cartilage formation in vitro, we generated the first induced Pluripotent Stem Cells (iPSCs) from fibroblasts of an SD patient with a lethal form of metatropic dysplasia, caused by a dominant mutation (I604M) in the calcium channel gene TRPV4. When micromasses were grown in chondrogenic differentiation conditions and compared to control iPSCs, mutant TRPV4-iPSCs showed decreased expression by qPCR of COL2A1 (IIA and IIB forms), SOX9, Aggrecan, COL10A1 and RUNX2, which are all cartilage growth plate markers. We found that stimulation with BMP2, but not TGFβ1, significantly upregulated ($p<0.05$) COL2A1 (IIA and IIB) and SOX9 gene expression, only in control iPSCs. COL2A1 (Collagen II) expression data were confirmed at the protein level by Western blot and immunofluorescence microscopy. TRPV4-iPSCs showed only focal areas of Alcian blue stain for proteoglycans, while in control iPSCs the stain was seen throughout the micromass sample. Similar staining patterns were found in neonatal cartilage from control and patient samples. We also found that COL1A1 (Collagen I), a marker of osteogenic differentiation, was significantly upregulated ($p<0.05$) at the mRNA level in TRPV4-iPSCs when compared to control, and confirmed at the protein level. Collagen I expression in the TRPV4 model also may correlate with abnormal staining patterns seen in patient tissues. This study demonstrates that an iPSC model can recapitulate normal chondrogenesis and that mutant TRPV4-iPSCs reflect molecular evidence of aberrant chondrogenic developmental processes, which could be used to design therapeutic approaches for disorders of cartilage.

F-2170
DEEP RNA-SEQUENCING OF DIFFERENTIATING HUMAN CARDIOMYOCYTES REVEALS NOVEL LNCRNA, SPLICING, MIRNA AND TRANSCRIPTIONAL REGULATORY NETWORKS

Salomonis, Nathan[1], Spindler, Matthew[2], Nguyen, Trieu[2], Russell, Caitlin[2], Lizarraga, Paweena[2], Truong, An[2], So, Po-Lin[2], Conklin, Bruce R.[2]

[1]Department of Pediatrics, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, USA, [2]Gladstone Institutes, San Francisco, CA, USA

Induced pluripotent stems cells offer a promising avenue for understanding human cardiac development and modeling disease. Cardiac diseases can manifest during embryonic development or in a variety of cell types of the heart in adults, hence obtaining an accurate picture of the RNA species expressed at distinct phases of cardiac differentiation would provide a valuable resource for delineating normal cell fate decision programs and cardiac physiology. High-resolution transcriptional measurements over the time-course of human induced pluripotent (iPS) cell differentiation to cardiomyocytes (iPS-CMs) could allow for the integration of both global regulatory observations and corresponding gene-level biology. Until recently, this high-resolution analysis has not been possible due to inefficient and highly variable differentiation into iPS-CMs. To solve this problem, we developed a highly uniform differentiation assay that produced >75% CMs and conducted deep sequencing on 11 time-points over 60 days of differentiation. Rigorous evaluation of gene expression and alternative splicing for protein coding genes and non-coding RNAs (ncRNAs) from the RNA-seq data was achieved through an enhanced version of the AtlAnalyze toolkit. Applying combined algorithms for exon and reciprocal junction expression, we observed a high concordance between independent human iPS and embryonic stem (ES) cell-cardiac differentiation models. Using a new time-point specific marker prediction method, MarkerFinder, genes and splice variants largely restricted to each time-point of differentiation were identified. These marker genes can be used to predict differentiation success and identify the point where poor differentiations diverge. Comparison of expression profiles and biologically enriched targets revealed coordinated regulation by transcription factors (TFs), microRNAs,

splicing factors (SFs) and lncRNAs and their associated targets. These regulatory networks include enrichment of TF targets for alternatively spliced TFs. Additionally, regulated SFs with enriched targets include the cardiomyopathy associated RBM20 along with ESRP1, PTB, SFRS2, HNRPF and HNRH1, at distinct phases of differentiation. The same approaches can be used to analyze differentiation into any cell type of interest and this will build a detailed map of the molecular events that regulate cell fate decisions. In addition, we are developing tools to assess single cell gene expression and this will allow us to better understand the cellular heterogeneity present during iPS differentiation.

IPS CELLS

F-2171
GENERATION OF THERAPEUTICALLY APPLICABLE XENO- AND FOOTPRINT-FREE HUMAN IPSCS FROM NON-INVASIVELY OBTAINABLE URINE CELLS

Hwang, Dong-Youn
CHA University School of Medicine, Seongnam, Republic of Korea

The efficient generation of xeno- and integration-free iPSCs is a prerequisite for their use in clinical applications due to safety concerns. Furthermore, somatic cells that can be non-invasively obtained from patients, such as urine cells, have a significant advantage as a cell source for iPSC generation. Therefore, an efficient method to reprogram urine cells into therapeutically applicable (xeno- and integration-free) iPSCs would be of great value. We recently established a practical, simple, and convenient way to generate clinically applicable, safe (xeno- and integration-free) iPSCs that can be used for cell therapy in the near future. In this study, we sought to determine whether the method works well using urine cells, which can be obtained in a non-invasive manner. Urine cells were electroporated with EBNA1/OriP-based plasmids encoding reprogramming genes and were maintained in our xeno-free hPSC culture medium until iPSC colonies were formed. The urine-derived iPSCs were selected and expanded in the same xeno-free hPSC culture medium. The iPSCs derived from urine cells displayed characteristics that were very similar to those of ESCs and iPSCs generated from adipose-derived stromal cells (ADSCs). In conclusion, the xeno- and integration-free iPSCs derived from urine cells are a promising cell source for future immune-compatible cell therapies, and our method for clinically safe iPSC generation will provide an important platform for the generation of iPSCs from any type of human somatic cells for use in cell therapies. This work was supported by grants from the Stem Cell Research Program (2010-0020347) and 2012M3A9C7050130 from the MSIP, and A120254-1201-0000200 from the Ministry of Health and Welfare, Korea

F-2172
AUTOMATED, NON-INVASIVE, MORPHOLOGY-BASED EVALUATION OF INDUCED PLURIPOTENT STEM CELL CULTURE

Maddah, Mahnaz, Shoukat-Mumtaz, Uzma, Loewke, Kevin
Cellogy Inc., Menlo Park, CA, USA

As the use of iPSC technology continues to grow, there is a need for new technologies that can standardize the evaluation of iPSCs in order to allow objective comparison of results across multiple experiments and laboratories. Here, we present a non-invasive, fully-automated, and analytical method for morphology-based evaluation of iPSC cultures via time-lapse microscopy and image analysis. We collected time-lapse image data of 94 iPSC cultures (12 separate clones across 4 iPSC lines), with 6 observations of each culture. iPSCs were plated as small clusters of cells into 12-well plates, and imaged every 30 minutes for a period of 3-4 days until the cells reached near confluency. The collection of data was done over multiple experiments, and the majority of data was collected under standard environmental conditions. For 25% of experiments, non-ideal culture conditions such as low temperature and low CO2 levels were used to generate a broader range of iPSC quality. For each dataset, a time-lapse movie was generated, with optimal focus. Each movie of iPSC colony formation was then evaluated by an expert biologist and given a ranking of 'good', 'fair', and 'poor'. Data were segregated into two buckets; half were used for developing the algorithm and training the software, while the other half were used for testing the performance of the software. The images are automatically uploaded to the cloud. The analytical software then processes images, measures geometric- and texture-based features of iPSC colonies over time, and based on those features computes a set of 6 biologically-relevant features, including iPSC doubling time, degree of cell compaction, colony border spikiness, sensitivity to media change, and the prevalence of dead cells and spontaneously differentiated cells. After creating measurements of these 6 features for all training data, we computed their conditional probability distributions given class labels of 'good', 'fair' and 'poor'. Based on the probability distributions we defined a set of ranges for each feature that would result in the highest prediction accuracy on our training data. Per-position predictions were then summarized in a single prediction for each well, based on the majority rule. The features with highest predictive power are the degree of cell compaction and the doubling time, followed by the colony border spikiness and sensitivity to media change. We independently tested the software on the other half, achieving an overall classification accuracy of 80% per position and 89% accuracy per well. Our results show the following: 1) A higher percentage of full-compacted cells in large growing colonies is associated with high-quality iPSCs; 2) Slower doubling time is associated with lower iPSC quality; 3) Higher colony border spikiness and sensitivity to media change are associated with lower quality iPSCs; and 4) A higher prevalence of dead cells and differentiated cells was found in poor quality cultures. We believe that our results demonstrate the first fully-automated and objective assessment of iPSC cultures using non-invasive methods. Our method can be used and extended for a variety of applications, including characterizing the dynamic response of cells to stress and drugs, quality control during manufacturing of stem cell reagents, evaluating new protocols and testing changes to existing ones, developing new types of media and other reagents, reagent lot testing, and training new scientists and technicians on the aforementioned activities.

F-2173
LOW IMMUNOGENICITY OF MOUSE IPS DERIVED NEURAL STEM CELLS

Itakura, Go[1], Iwai, Hiroki[2], Nishimura, Soraya[2], Kawabata, Souya[1], Nishiyama, Yuichiro[1], Hori, Keiko[1], Ozaki, Masahiro[1], Iwanami, Akio[1], Toyama, Yoshiaki[1], Okano, Hideyuki[3], Nakamura, Masaya[1]
[1]Orthopedic Surgery, Keio University, Tokyo, Japan, [2]Keio University, Tokyo, Japan, [3]Keio University, School of Medicine, Tokyo, Japan

Objective: Previously, we reported the effectiveness of induced pluripotent stem cell-derived neural stem cells (iPSC-NSCs) transplantation for spinal cord injury in rodents. With the increase of the need for the allo-transplantation, several reports about immunogenicity of iPSCs have been published in recent years. According to their reports, even in the syngeneic settings, undifferentiated iPSCs are more immunogenic than their ECS counterparts and rejection will be occurred, immune rejection occur in allogeneic settings, but not occur in syngeneic settings, their immunogenicity have not been

adequately addressed. Furthermore, with respect to immunogenicity of iPSC derivatives, there are few reports and it remains one of the great obstacle for regenerative medicine. In this study, we evaluated the immunogenicity of mouse iPSC-NSCs in vitro and in vivo. Materials-Methods: Several neurospheres derived from iPSC lines (2A4F, 2A3F) and fetus NSCs of C57BL/6J mice were used in the present study. Molecular surface markers (MHC classI,II, CD40, CD80, CD86 etc) and immunological gene expression (Hormad, Zg16, Retn etc) of these NSCs were evaluated by flow cytometry and RT-PCR. These NSCs were transplanted into the intact spinal cord or subcutaneous lesion of C57BL/6J (syngeneic) and BALB/Ca (allogeneic) mice. In vivo bioluminescent imaging (BLI) was used to evaluate the survival of transplanted cells chronologically. Results: In vitro: NSCs present minimal expression of these surface markers and there were no expression of immunological gene expression. Immunogenicity of iPS-NSCs was low similar to that of fetus NSCs. In accordance with repeating the passage, these expressions slightly increased. in vivo: The NSCs, which were transplanted into spinal cord and subcutaneous tissue, survived in the syngeneic mice (graft survival rate was 100% at 28 d after transplantation). In contrast, all the NSCs grafted into the subcutaneous tissues were rejected in the allogeneic host (graft survival rate: 0%). Interestingly, the grafted iPSC-NSCs as well as fetus derived NSCs evaded the acute rejection even in the spinal cord of allogeneic host (graft survival rate: 60-80% at 28 d after transplantation). Conclusion: We evaluated the immunogenicity of mouse iPS-NSCs and mouse fetus NSCs in vitro and in vivo, and found that iPSC-NSCs exhibited low immunogenicity and well survived after transplantation in the allogeneic setting. These results suggest the feasibility of allograft of iPSC-NSCs for spinal cord injury with low dose immunosuppressant.

F-2174
INDUCED PLURIPOTENT STEM CELLS BEASED THERAPHY ON DYSTROPHY MODEL

Jeong, Jaemin[1,3], Kwon, Heechung[1,2]
[1]Division of Radiation Cancer Research, [2]Research Center for Radio-Senescence, Korea Institute of Radiological and Medical Sciences, Seoul 139-706, Korea, [3]BK21 Plus Project, Yonsei University College of Dentistry, Seoul 120-752, Korea

Mesenchymal stem cells (MSC) are very useful to repair and regeneration of damaged tissue. However, there are some difficulties of MSC isolation, like pain to patient, risk of infection and repetitive isolation. Here, we purify iMSCs from iPSCs with SB431542, and then apply to mdx mice for enhancing skeletal muscle regeneration of muscle dystrophy. Purified iMSCs, which is fibroblast-like shape, were represented CD29, CD33, CD73, CD90 and CD105, and formed three-dimensional spheroids. Moreover, they had differentiation capability into three lineages including adipogenic, osteogenic, and chondrogenic lineages. When they have been transplanted to tibialis anterior skeletal muscle of mdx mice, high levels of oxidative damages shown by anti-nitrotyrosin were diminished and dystrophin expressions were restored. Finally, purified iMSCs have a therapeutic effect in mdx mice, and iPSCs will be highly recommended another perpetual source of autologous MSC isolation.

F-2175
COMPARISON OF SOMATIC CELL NUCLEAR TRANSFER STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS

Johannesson, Bjarki[1], Sagi, Ido[2], Yamada, Mitsutoshi[1], Egli, Dieter[1], Benvenisty, Nissim[2]
[1]New York Stem Cell Foundation, New York, NY, USA, [2]The Hebrew University, Jerusalem, Israel

Recent advances have allowed the generation of human pluripotent embryonic stem cells (ESC) by somatic cell nuclear transfer (SCNT). ESC generated by SCNT (SCNT-ESC) hold potential as a source for cell replacement therapies and disease modeling, however it remains unclear how they compare to pluripotent stem cells generated with other methods e.g. induced pluripotent stem cells (iPSC). To date we have generated four diploid SCNT-ESC lines. Three lines were derived from the same culture of BJ fibroblasts and one line from skin fibroblasts of an adult type 1 diabetes (T1D) patient. In culture, SCNT-ESC lines efficiently form colonies that are positive for common pluripotency markers such as Tra-1-60, Tra-1-81, SSEA4, Nanog and Oct-4. Moreover, upon transplantation into NOD-SCID-GAMMA (NSG) mice, these cell lines formed teratomas containing cells of all three germ layers. To evaluate their differentiation potential, SCNT-ESC lines were further differentiated into PDX1-positive pancreatic precursors, insulin-producing autocrine cells and Tuj1 positive neuronal progenitors with an efficiency comparable to non-related iPSCs. For a more direct comparison of SCNT-ESC and iPSC reprogramming methods, we have further generated isogenic iPSC cell lines by four-factor mRNA transfection of the same BJ and adult fibroblasts cultures the SCNT-ESC were generated from. These isogenic cell lines will allow us to directly investigate how different methods of reprogramming affect parameters that define the pluripotent state (gene expression and DNA methylation), reprogramming-related defects (point mutations), and differentiation potential. A comparison of these cell lines using mRNA sequencing, DNA sequencing and methylation analysis is underway. Our initial studies indicate that stem cells generated by SCNT are comparable to iPSCs, however an in-depth characterization of isogenic stem cells generated by both methods is needed to fully determine this.

F-2176
STANDARDIZING GENERATION AND CHARACTERIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FOR DISEASE MODELING AND CELL THERAPIES

Uhm, Kyung-Ok, Kim, So-Jung, Choi, Hye Young, Jo, Eun Hee, Go, Gue Youn, Im, Young Sam, Yoon, Jeongsook, Ha, Hye-Yeong, Koo, Soo Kyung, **Jung, Ji Won**
National Institute of Health, Chungbuk, Republic of Korea

The reprogramming of a patient's somatic cells can be a potential autologous stem cell source for disease modeling and future regenerative therapy. However, a substantial variation among pluripotent cell lines could affect their utility and clinical safety. The differences among pluripotent cell lines should be better understood in biomedical research. In this study, we have generated human induced pluripotent stem cells (iPSCs) from various original cells by using several reprogramming methods available. These iPS cell lines contain genetic disease models such as Charcot-Marie-Tooth disease and genetic modification models such as TALEN mediated CCR5 knockout. We have characterized and analyzed those cell lines by global gene expression and compared with 3 previously derived human embryonic stem cell lines. We also have investigated the in vitro differentiation propensity of these cell lines. The findings will provide the comprehensive characterization of iPSCs from various source of original cells by using various reprogramming

methods and will further provide the insight into standardization of iPSC generation for disease modeling and cell therapy use.

F-2177
NOVEL TOOLS TO DISCERN THE PLURIPOTENCY GENE NETWORK ESTABLISHMENT

Chantzoura, Eleni[1], Skylaki, Stavroula[2], Johnsson, Anna[3], Linnarsson, Sten[3], Woltjen, Knut[4], **Kaji, Keisuke**[1]
[1]MRC Centre for Regenerative Medicine, Edinburgh, United Kingdom, [2]ETH Zurich, Zurich, Switzerland, [3]Karolinska Institute, Stockholm, Sweden, [4]Center for IPS Cell Research and Application (CiRA), Kyoto, Japan

Even though great studies have been attempting to investigate the reprogramming of somatic cells to induced Pluripotent Stem Cells (iPSCs) by exogenous transcription factors, yet the mechanism remains highly elusive. In this study we demonstrate a system to easily perform robust and reproducible systematic mechanistic analysis of reprogramming. First we targeted a single vector carrying both the reverse tetracycline-controlled transactivator (rtTA) and the doxycycline (dox)-inducible 2A-peptide-linked reprogramming factors to a novel genomic locus that assured optimal transgene expression for efficient reprogramming. One round of targeting with this vector makes any ES cell (ESC) line with different reporter genes or genotypes a source of dox-inducible reprogrammable cells/mouse. Using this system, we generated Nanog-GFP ESC lines with the 2A peptide-linked reprogramming factors in a different order (TNG MKOS/OKMS ESCs) so as to uncover new molecules involved in the reprogramming process. Interestingly, TNG OKMS MEFs generated from TNG OKMS ESCs, gave rise to ~10 times more colonies upon the addition of dox, but with poor activation of the Nanog-GFP reporter, compared to the TNG MKOS MEFs, indicating opposing effects of MKOS and OKMS cassettes on quantity and quality (homogeneity) of cells undergoing reprogramming. Reprogramming route analysis with E-Cadherin, CD44 and ICAM1 staining revealed that population changes in MKOS/OKMS reprogramming are different and there are clear roadblocks in OKMS reprogramming: cells are less efficient in 1. up-regulating E-Cadherin, and 2. the transition from a Nanog-GFP- to GFP+ state even after reaching an E-Cadherin+ CD44- ICAM1+ stage. To identify genes contributing to these differences, we performed RNA-sequencing analysis of the above MKOS/OKMS reprogramming intermediates. From this analysis we identified a gene, whose up-regulation is retarded in OKMS reprogramming and its overexpression can increase reprogramming efficiency. We also identified a gene, which is transiently up-regulated in MKOS, but not OKMS, reprogramming, and its knockdown increases iPSC colony number 5-6 times. These data demonstrate that different reprogramming cassettes cause different cellular responses during reprogramming while the emerging iPSCs are similarly pluripotent. Our system allows making a precise comparison of intermediate subpopulations, understanding reprogramming mechanisms, and improving reprogramming technology.

F-2178
EFFICIENT AND CONSISTENT CREATION OF FOOTPRINT FREE, FEEDER FREE, C-MYC FREE INDUCED PLURIPOTENT STEM CELLS USING A NOVEL EPISOMAL VECTOR SYSTEM AND PROPRIETARY MEDIA FORMULATION

Kamath, Anant, Ternes, Sara J., Moy, Alan B.
Cellular Engineering Technologies Inc, Coralville, IA, USA

For IPS cells to be fully realized as a cell therapy, safety concerns and manufacturing processes require non-viral and C-Myc-free cell reprogramming. The reprogrammed cells have to be grown under feeder-free conditions in a cost-efficient manner. However, the creation of footprint-free and C-myc-free IPS cells is inefficient in the absence of virally-mediated biohazardous agents such as retroviruses. Moreover, these viral agents are not only unsafe but could cause permanent perturbation of the reprogrammed cell in unintended ways by integrating into the cellular genome. These concerns greatly limit the use of IPS cells created by these techniques as screening tools but completely preclude them from use for clinical applications. In our technique, human IPS cells were created using an EBNA-1 (Epstein Barr) episomal vector containing the classical Yamanaka factors, Oct-4, Sox-2, Klf-4 but without the oncogene C-Myc. C-Myc has been implicated in a variety of tumors and cell cycle dysregulation and was deliberately deleted from the expression vector. The Yamanaka factors were optimized for better transcription and translation by molecular modification. The tissue culture media formulation was further optimized with a proprietary growth factor cocktail to increase cellular reprogramming efficiency to levels that exceed 95 percent of induced cells. Another innovation introduced was that reprogrammed IPS cells were plated on Vitronectin coated tissue culture dishes, rather than Matrigel, with media changes conducted once every 2 days. These approaches provide reproducibility, cost savings, and greater quality controls for cell manufacturing. At the end of 10 days, cells were enyzmatically passaged to new tissue culture dishes. Our technique allowed for the reprogramming and consistent passaging of human IPS cells derived from Human Foreskin Fibroblasts, Human Adipose Mesenchymal Stem Cells and Human Multipotent Unrestricted Stem Cells.

F-2179
REPROGRAMMING OF MOUSE SOMATIC CELLS INTO PLURIPOTENT STEM CELLS USING A COMBINATION OF SMALL MOLECULES

Kang, Phil Jun[1], Moon, Jai-Hee[1], Yoon, Byung Sun[2], Hyeon, Solji[1], Jun, Eun Kyoung[2], Park, Gyuman[1], Yun, Wonjin[1], Kim, Aeree[3], Whang, Kwang Youn[1], Koh, Gou Young[4], Oh, Sejong[5], You, Seungkwon[1]
[1]Korea University, Seoul, Republic of Korea, [2]Venture Incubation Center Korea University, StemLab, Seoul, Republic of Korea, [3]Korea University Guro Hospital, Seoul, Republic of Korea , [4]National Research Laboratory of Vascular Biology and Stem Cells, Daejeon, Republic of Korea, [5]Chonnam National University, Gwangju, Republic of Korea

Somatic cells can be reprogrammed to generate induced pluripotent stem cells (iPSCs) by overexpression of four transcription factors, Oct4, Klf4, Sox2, and c-Myc. However, exogenous expression of pluripotency factors raised concerns for clinical applications. Here, we show that pluripotent stem cells were generated from mouse somatic cells in two steps with small molecule compounds. In the first step, intermediate cells were generated from mouse astrocytes by Bmi1 and specific culture condition in the absence of other transcription factors. These cells called induced epiblast stem cell (EpiSC)-like cells (iEpiSCLCs) have several similarities to EpiSCs in terms of expression of specific markers, epigenetic state, and ability to differentiate into three germ layers. In the second step, treatment with MEK/ERK and GSK3 pathway inhibitors in the presence of leukemia inhibitory factor resulted in conversion of iEpiSCLCs into iPSCs that were more pluripotent and similar to mESCs, suggesting that overexpression of Bmi1 with specific culture condition are sufficient to reprogram astrocytes to pluripotency. Next, Bmi1 function was replaced with Shh activators (oxysterol and purmorphamine) in previous research, which demonstrating that combinations of small molecules can compensate for reprogramming factors and are sufficient to directly reprogram mouse somatic cells into pluripotent stem cells. The chemically induced pluripotent stem cells (ciPSCs) showed similar gene expression profiles,

epigenetic status, and differentiation potentials to mESCs. Our findings will enhance reprogramming strategies toward to generate desirable cells for clinical applications.

F-2180
HUMAN IPS CELL-DERIVED OLIGODENDROCYTE PRECURSOR CELLS CAN ENHANCE THE REMYELINATION OF DEMYELINATED AXONS AFTER SPINL CORD INJURY IN ADULT MICE

Kawabata, Soya[1], Takano, Morito[1], Numasawa, Yuko[2], Itakura, Go[1], Kobayashi, Yoshiomi[1], Toyama, Yoshiaki[1], Okano, Hideyuki[3], Nakamura, Masaya[1]

[1]Orthopaedic Surgery, Keio University, School of Medicine, Tokyo, Japan, [2]Pediatrics, Keio University, School of Medicine, Tokyo, Japan, [3]Physiology, Keio University, School of Medicine, Tokyo, Japan

Background: There have been many reports that transplantation of neural stem/progenitor cells (NS/PCs) promoted functional recovery after spinal cord injury (SCI) in rodents and non-human primates and stem cell therapy is becoming a reality to treat SCI patients. Potential mechanisms of functional recovery after NS/PC transplantation was not only reconstruction of neural circuits by grafted cells derived neurons but also remyelination of demyelinated axons by grafted cells derived oligodendrocytes. Recently, we have developed a new differentiation protocol, which enables to induce oligodendroglial differentiation of human iPSC-derived NS/PCs *in vitro*. In this study, we sought to determine whether human iPSC-derived oligodendrocyte precursor cells (hiPS-OPCs) can promote functional recovery after SCI. Methods: We induced hiPSC-OPCs from pre-evaluated safe iPS cell line, 201B7 and quantified differentiation rate of three neural lineages *in vitro*. Contusive SCI was induced at Th10 level as reported previously and hiPSC-OPCs were transplanted into the injured spinal cord of NOD-Scid mice. In the control group, phosphate buffered saline was injected instead of cells. Behavioral analysis using Basso Mouse Scale (BMS) was performed until 12 weeks after SCI, followed by histological analyses. We also performed immune-electron microscopic analyses. Results: The differentiation efficiency into oligodendrocytes significantly increased in hiPSC-OPCs compared to hiPSC derived NS/PCs *in vitro*. The transplantation group showed a better functional recovery in BMS score, compared to the control group. A large number of the grafted cells were survived and differentiated into oligodendrocytes predominantly as well as neurons and astrocytes. Interestingly grafted hiPSC derived mature oligodendrocytes migrated into the white matter of the injured spinal cord. Histological analysis of luxol fast blue staining revealed that myelinated areas in the transplantation group were significantly larger than the control group. Findings of immune-electron microscopy elucidated the remyelination of the demyelinated axons by the grafted hiPSC derived mature oligodendrocytes. Conclusion: hiPSC-OPC transplanted into the injured spinal cord contributed to the remyelination of demyelinated axons, resulting in restoration of motor function. These findings suggested that hiPSC-OPC is a useful source of cell therapy for SCI.

F-2181
A COMPARISON OF THE STRUCTURAL CHARACTERISTICS OF N GLYCANS DERIVED FROM MURINE INDUCED PLURIPOTENT STEM CELLS AND STEM CELL DERIVED CARDIOMYOCYTES

Kawamura, Takuji[1], Miyagawa, Shigeru[1], Fukushima, Satsuki[1], Kashiyama, Noriyuki[1], Itou, Emiko[1], Saito, Atsuhiro[1], Maeda, Akira[2], Eguchi, Hiroshi[2], Toda, Koichi[1], Miyagawa, Shuji[2], Sawa, Yoshiki[1]

[1]Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine, Osaka, Japan, [2]Division of Organ Transplantation, Department of Surgery, Osaka University Graduate School of Medicine, Osaka, Japan

Cell transplantation therapy using induced pluripotent stem cells (iPSCs) is a promising approach for tissue regeneration, and the immunogenicity of iPSCs and their derivatives has been extensively investigated. However, little is known about the production of glycans on the surfaces of such cells. In this study, N-glycans were isolated from iPSCs, iPSC-derived cardiomyocytes (iPSC-CMs), or C57BL/6 (B6) naive heart muscle cells (Heart; control group), and their structures were then analyzed using a mapping technique based on their elution following high performance liquid chromatography using diethylaminoethyl (DEAE), octadecyl silane (ODS) or amide columns, followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. The murine iPSCs were generated from B6 mouse embryonic fibroblasts by introducing Oct3/4, Sox2, Klf4 and c-Myc without viral vectors. IPSC-CMs differentiated from these iPSCs by Wnt signaling, and following purification using a glucose-free medium supplemented with lactate, consisted of troponin T-positive cell populations (>95% purity). DEAE column chromatography of N-glycans derived from iPSC, iPSC-CM and Heart resulted in three peaks based on increasing acidity: neutral, peak 1; mono-sialyl, peak 2; di-sialyl, peak 3. The peak areas (molar ratios) for the glycan fractions were as follows: 96.7%, 0.6%, and 2.7% for iPSC; 89.1%, 6.4%, and 4.5% for iPSC-CM, and 55.0 %, 19.5%, and 25.5% for Heart, respectively. N-glycans from iPSC comprised 14 neutral, 2 mono-sialyl and 5 di-sialyl glycans, the iPSC-CM N-glycans comprised 16 neutral, 16 mono-sialyl, and 6 di-sialyl structures, and those from Heart comprised 14 neutral, 12 mono-sialyl, and 8 di-sialyl structures. In total, 57 different N-linked glycans were isolated, and 52 of these were structurally characterized. Each preparation contained substantial amounts of high-mannose structures, but the proportion of these structures was quite different for iPSC, iPSC-CM, and Heart N-glycans (86.7%, 76.3%, and 46.1%, respectively). In contrast to the prevalence of high-mannose structures, in the hybrid and complex type structures, no type 1 N-acetyllactosamine (LacNAc) structures were detected. Under these conditions, most of the sialyl structures of iPSC or iPSC-CM were different from those of Heart. A few N-glycolyl neuraminic acid (NeuGc)-containing structures were detected in the iPSC or iPSC-CM samples, and the mRNA level of cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH), which catalyzes the conversion of N-acetyl neuraminic acid (NeuAc) into NeuGc, was very low in these samples. In contrast, the Heart sample contained numerous NeuGc structures with clearly higher CMAH mRNA levels. iPSC-CMs clearly produced NeuAc with both α2, 3 and α2, 6 sialyl structures, but this was not true of Heart, which contained NeuGc but not NeuAc with both α2, 3 and α2, 6 sialyl structures. Compared to Heart, IPSC-CM samples showed a relatively higher level of Gal β1, 4 GlcNAc α2, 6 sialyltransferase (ST6Gal-I) mRNA and a lower level of Gal β1, 3(4) GlcNAc α2, 3 sialyltransferase (ST3Gal-III) and Gal β1, 4(3) GlcNAc α2, 3 sialyltransferase (ST3Gal-IV) mRNA. The iPSC-CM-derived neural and mono-sialyl glycans showed several Gal α1, 6 Gal structures, which were rarely detected in the other cells. Our data

will be helpful in future studies examining the immunogenicity of iPSC or iPSC-CM.

F-2182
CONTROLLING PLURIPOTENCY AND EARLY CELL FATE DECISIONS OF HUMAN INDUCED PLURIPOTENT STEM CELLS ON A DENDERIMER-IMMOBILIZED SURFACE

Kim, Mee-Hae, Kino-oka, Masahiro
Osaka University, Suita, Japan

Understanding of the fundamental mechanisms that govern adhesive properties of human induced pluripotent stem cells (hiPSCs) to culture environments provides surface design strategies for maintaining their undifferentiated state during cell expansion. We used polyamidoamine dendrimer surfaces with first-generation (G1), third-generation (G3) and fifth-generation (G5) of dendron structure in cultures of hiPSCs with SNL feeder cells. Cells on the G1 surface formed tightly packed colony with close cell-cell contacts during division and migration; those on the G3 surface exhibited loose or dispersed colony pattern by enhanced migration. On the G5 surface, formation of aggregated colony with ring-like structures occurred spontaneously. We found that the substrate-adsorbed fibronectin and feeder cell-secreted fibronectin appeared elevated levels with the varied generation numbers of dendrimer surfaces. This subsequently resulted in cell migration and in activation of paxillin of hiPSCs. Location-dependent expression of Rac1 induced rearrangement of E-cadherin-mediated cell-cell interactions on dendrimer surfaces, and was associated with alterations in the cell and colony morphology, and migratory behavior. Furthermore, Caspase-3 occurred in apoptotic cells on dendrimer surfaces, concomitant with the loss of E-cadherin-mediated cell-cell interactions. Cells on the G1 surface were maintained in an undifferentiated state, while those on the G5 surface exhibited the early commitment to differentiation toward endodermal fates. We conclude that morphological changes associated with altered migration on the dendrimer surfaces were responsible for the coordinated regulation of balance between cell-cell and cell-substrate interactions associated with migration, thereby switching their transition from self-renewal state to early endoderm differentiation in hiPSCs.

F-2183
THE REPROGRAMMING FACTOR NR5A2 CAN NOT REPLACE OCT-4 FUNCTION IN THE SELF-RENEWAL OF EMBRYONIC STEM CELLS

Choi, Kyeng-Won, Oh, Hye-rim, Lee, Jaeyoung, Lim, Bobae, **Kim, Jungho**
Sogang University, Seoul, Republic of Korea

Although Oct-4 is one of the most intensively studied factors in mammalian development, no cellular genes capable of replacing Oct-4 function in embryonic stem (ES) cells have been found. Recent data show that the nuclear receptor Nr5a2 is able to replace Oct-4 function in the reprogramming process; however, it is unclear whether Nr5a2 can replace Oct-4 function in ES cells. In this study, the ability of Nr5a2 to maintain self-renewal and pluripotency in ES cells was investigated. Nr5a2 localized to the nucleus in ES cells, similar to Oct-4. However, expression of Nr5a2 failed to rescue the stem cell phenotype or to maintain the self-renewal ability of the ES cells. Furthermore, compared to Oct-4-expressing ES cells, the Nr5a2-expressing ES cells showed a reduced number of cells in S-phase, did not expand normally, and did not remain in an undifferentiated state. Ectopic expression of Nr5a2 in ES cells was not able to activate transcription of ES cell-specific genes and gene expression profiling demonstrated differences between ES cells expressing Nr5a2 and Oct-4. In addition, ES cells harboring Nr5a2 were not able to form teratomas in nude mice. Taken together, these results strongly suggest that the gene regulation properties of Nr5a2 and Oct-4 and their abilities to confer self-renewal and pluripotency of ES cells differ. The present study provides strong evidence that Nr5a2 cannot replace Oct-4 function in ES cells.

F-2184
TEMPORAL CONTROL OF REPROGRAMMING FACTOR STOICHIOMETRY

Kim, Shin-IL[1], Oceguera-Yanez, Fabian[1], Yamamoto, Takuya[2], Yamanaka, Shinya[2], Woltjen, Knut[2]
[1]Reprogramming Science, CiRA, Kyoto University, Kyoto, Japan, [2]CiRA, Kyoto University, Kyoto, Japan

Reprogramming cells by transcription factor overexpression represents an experimental paradigm where exogenous activators or repressors elicit changes in endogenous gene expression, ultimately altering cell fates. Expressed simultaneously, Oct3/4, Sox2, cMyc, and Klf4 (the "Yamanaka factors") are capable of re-wiring a somatic cell to achieve induced pluripotency (iPS cells). Yet, the temporal relevance of each factor throughout reprogramming stages remains inferred. We have developed doxycycline (dox)-inducible, combinatorial transgene systems for reprogramming mouse embryonic fibroblasts (MEFs), permitting documentation of unique reprogramming phenotypes in response to fixed factor stoichiometries. Moreover, composite systems that enable dynamic modulation of individual factors at the transcriptional or post-translational levels allow us to expose their temporal requirements in reprogramming. For example, control of early Klf4 protein levels reproducibly manifests disparate effects on cell morphology, proliferation, and cell-cell adhesion, by controlling the severity of the mesenchymal-epithelial transition (MET). Subsequently, we find that over- or under-expression of particular factors can either positively or negatively impact endogenous pluripotency gene expression and their associated transgenic reporters, reflecting apparent iPS cell quality. Our data implies discrete reprogramming factor requirements at each stage of the process, allowing more sophisticated experimental guidance of cell fates.

F-2185
RGD PEPTIDE-FUNCTIONALIZED NANOPATTERNED SUBSTRATES FOR ENHANCED MATURATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Carson, Daniel[1], Hnilova, Marketa[2], Nemeth, Cameron[1], Tsui, Jonathan[1], Yang, Xiulan[3], Murry, Charles[3], Tamerler, Candan[4], **Kim, Deok-Ho**[1]
[1]Bioengineering, University of Washington, Seattle, WA, USA, [2]Materials Science and Engineering, University of Washington, Seattle, WA, USA, [3]Center for Cardiovascular Biology, University of Washington, Seattle, WA, USA, [4]Mechanical Engineering, University of Kansas, Lawrence, KS, USA

Cardiovascular disease remains one of the leading causes of deaths in developed nations. Current therapies have found varying degrees of success, but fully functional cardiac tissue remains elusive. The extracellular matrix (ECM) of the heart is made up of aligned, nanoscale collagen fibers that facilitate a major role in the structural architecture of the overlying macroscopic myocardium. Advancements in nanofabrication techniques have made it possible to study the effect of substrate nanotopography on cardiomyocyte structure and function. In addition, the proteins of the basement membrane including fibronectin have been shown to strongly influence the adhesion of cardiomyocytes through integrin interactions. A specific repeating amino acid sequence, Arg-Gly-Asp (RGD), found in many native

adhesion proteins, has been shown to promote cell adhesion in vitro as well. Here, we present a platform in which we are able to study the effect of nanoscale structural cues as well as ECM biochemical signals on maturation of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs). Using a customized 4 x 4 island nanopatterned substrate, nanogroove widths ranging from 350nm to 2000nm were fabricated using UV-assisted capillary force lithography on polyurethane acrylate-based materials (PUA). We also present the synthesis and incorporation of a bifunctionalized peptide, PUA binding peptide - RGD (PUABP-RGD) into the platform to further study the effect of native ECM-like biochemical cues on the structural maturation of hPSC-CMs. Human induced pluripotent stem cell-derived cardiomyocytes (IMR90-CMs) were seeded on substrates coated with fibronectin (50ug/ml), PUABP1-RGD (100 μM), or PUABP2-RGD (100 μM). Quantitative analysis of cell attachment 24 hours post seeding showed that the cell attachment was similar across all dimensions and suggests the ability of PUABP-RGD to function as a viable alternative to more commonly used coating proteins such as fibronectin. Morphological analysis of samples at 3 weeks post-seeding indicates a possible intermediate range of nanogroove widths centered around 800nm that offer optimal conditions for maturation of structural characteristics. Immunofluorescent staining provided qualitative representations of cell morphology across dimensions. Statistical analysis was performed to determine significant variations between cell morphology on 350nm, 800nm, 1000nm, 1500nm, 2000nm, and flat controls. It was determined that cells on 800nm had significantly larger cell areas, larger perimeters, reduced circularity, and increased anisotropy compared to the other analyzed dimensions. It was also shown that sarcomere lengths of cells cultured on 800nm grooves was significantly greater than the other pattern dimensions. In summary, we present a platform in which different nanogroove widths can be screened for effects on hPSC-CM structural maturation. The study included evaluation of a bifunctional cell adhesion peptide PUABP2-RGD as an alternative to commonly used cell adhesion proteins such as fibronectin for hPSC-CM structural maturation. We demonstrated that nanopatterned dimensions have a significant influence on structural maturation of hPSC-CMs. Our ongoing work will evaluate functional maturation of hPSC-CMs using the platform described in the study.

F-2186
DERIVATION OF BCR-ABL-NEGATIVE INDUCED PLURIPOTENT STEM CELLS FROM PATIENTS WITH CHRONIC MYELOID LEUKEMIA

Klincumhom, Nuttha[1], Lueangamornnara, Usaneeporn[2], Wattanapanitch, Methichit[1], U-pratya, Yaowalak[2], Lorthongpanich, Chanchao[1], Laowtammathron, Chuti[1], Supokawej, Aungkura[3], Kheolamai, Pakpoom[4], Issaragrisil, Surapol[2]

[1]Siriraj Center of Excellence for Stem Cell Research, Faculty of Siriraj Hospital Medicine, Mahidol University, Bangkok, Thailand, [2]Department of Medicine, Division of Hematology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand, [3]Department of Clinical Microscopy, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand, [4]Department of Pre-clinical Science, Faculty of Medicine, Division of Cell Biology, Thammasat University, Pathumthani, Thailand

Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disease in a group of myeloproliferative neoplasms that is characterized by a chromosomal translocation between chromosome 9 and 22, t(9;22)(q34;q11), resulting in a *BCR-ABL* fusion gene known as Philadelphia chromosome. This fusion gene encodes excessive activity of tyrosine kinase which inhibits DNA repair causing genomic instability and blast crisis in CML. Tyrosine kinase inhibitor (TKI), such as imatinib mesylate, is a selective *BCR-ABL* tyrosine kinase inhibitor which used as a standard drug treatment for patients with CML. However, approximately 30% of the patients failed to imatinib treatment during the first 5 years. Allogeneic hematopoietic stem cell (HSC) transplantation provides a possible treatment for CML patients. Nevertheless, the treatment is limited by rare human leukocyte antigen (HLA)-matched donors and a high risk of graft-versus-host disease (GVHD), which occurs after transplantation. Induced pluripotent stem cells (iPSCs) can be generated by introducing pluripotency-associated transcription factors into differentiated somatic cells. The iPSC technology provides genetically identical pluripotent stem cells to the patient's which hold a great promise for future clinical applications. In this study, we generated iPSCs from dermal fibroblasts of a patient with CML by using retroviral vectors containing 4 reprogramming factors: OCT3/4, SOX2, KLF4, and c-MYC. Similar to human embryonic stem cell (hESC) counterparts, the established CML-iPSCs have pluripotent stem cell properties including expressions of stem cell surface markers, pluripotent markers and spontaneous differentiation potentials into 3 germ layers both *in vitro* and *in vivo*. The CML-iPSCs also exhibited a normal male karyotype. Moreover, the *BCR-ABL* fusion gene, a hallmark of CML, was not detected in the CML-iPSCs. Our established CML-iPSCs may be used as a renewable source of hematopoietic stem cells (HSC) for autologous transplantation for CML patients in the future.

F-2187
ANDROGEN RECEPTOR-MEDIATED APOPTOSIS IN BOVINE TESTICULAR INDUCED PLURIPOTENT STEM CELLS IN RESPONSE TO PHTHALATE ESTERS

Ku, Chia-Chen[1], Lin, Ying-Chu[1], Wang, Shin-Wei[2], Wu, Deng-Chang[1], Lin, Changsheng[3], Miyoshi, Hiroyuki[4], Nakamura, Yukio[4], Saito, Shigeo[1], Yokoyama, Kazushige[1]

[1]Kaohsiung Medical University, Kaohsiung, Taiwan, [2]Kaohsiung Medical University, Kaohsiung, Taiwan, [3]Gladstone Institute of Cardiovascular Disease-Ding, San Francisco, CA, USA, [4]RIKEN BRC, Tsukuba, Japan

The androgen receptor (AR) plays a critical role in promoting androgen-dependent and -independent apoptosis in testicular cells. However, the molecular mechanisms that underlie the ligand-independent apoptosis inducing activity of AR in testicular stem cells are not completely understood. In the present study, we generated induced pluripotent stem cells (iPSCs) from bovine testicular cells by electroporation of *OCT4*. The cells were supplemented with leukemia inhibitory factor and bone morphogenetic protein 4, which maintained and stabilized the expression of stemness genes and pluripotency. The iPSCs were used to assess the apoptotic activity following exposure to phthalate esters, including di (2-ethyhexyl) phthalates, di (*n*-butyl) phthalate, and butyl benzyl phthalate. Phthalate esters significantly reduced the expression of AR in iPSCs and induced a higher ratio of BAX/BCL-2, thereby favoring apoptosis, and increased expression of cyclin-dependent kinase inhibitor 1 ($p21^{Cip1}$) compared with control cells treated with dimethyl sulfoxide. The forced expression of AR and knockdown of $p21^{Cip1}$ led to the rescue of the phthalate-mediated apoptosis. Overall, this study suggests that testicular iPSCs are a useful system for screening the toxicity of environmental disruptors and examining their effect on the maintenance of stemness or pluripotency, as well as for identifying the iPSC signaling pathway(s) that are deregulated by these chemicals. Ref. Cell Death and Dis. 4, e907 (2013)

F-2188

PROFILING OF HUMAN INDUCED PLURIPOTENT STEM CELL LINES FOR PREDICTING THE DIFFERENTIATION PROPENSITY

Kuroda, Takuya[1], Tachi, Shiori[2], Yasuda, Satoshi[1], Kusakawa, Shinji[3], Sato, Yoji[1]

[1]National Institute of Health Sciences, Tokyo, Japan, [2]Nagoya City University, Aichi, Japan, [3]Foundation for Biomedical Research and Innovation, Hyogo, Japan

Human pluripotent stem cells (hPSCs) have the ability to differentiate into a variety of cells and to self-renew in vitro. Because of these two characteristics, they have been expected to provide new regenerative medicine/cell therapy. Recently, various in vitro differentiation protocols have been developed for the generation of various different functional cell types. For stable production of specific differentiated cells with reproducible quality, it is crucial to select the most suitable hPSC lines as starting materials. However, the large and unavoidable variations in the differentiation potentials of hPSC lines make it difficult to select appropriate cell lines for production of desired cells. Assays using biomarkers that accurately predict hPSC differentiation potentials would provide simple and economical experimental approaches for identification of promising hPSC lines for production of hPSC-processed therapeutic products. Here, we attempted to identify the biomarkers for predicting propensity to differentiate into three germ layers of human induced pluripotent stem cell (hiPSC) lines. We first examined spontaneous differentiation during embryoid body (EB) formation of ten iPSC lines without supplement with growth factors or serum. Transcriptional levels of 97 genes, which selectively express in three germ layer lineages, were assessed in EBs by quantitative RT-PCR. Using principal component analysis of these gene expressions, the first principal component scores were calculated as indices of the differentiation propensity of each hiPSC line into the three germ layers. Next, we performed the comprehensive transcriptome analysis of undifferentiated hiPSC lines. Then the correlations between signal intensities of the microarray data and the differentiation propensity were evaluated by calculating Spearman's rank correlation coefficients. We finally identified 308 mRNAs and 42 miRNAs expressed in undifferentiated hPSCs that significantly correlate with propensity to differentiate into at least one of the three germ layers. These results suggest that the expression levels of these mRNA and miRNA would contribute to predicting differentiation propensity of hPSCs into cell lineages of interest.

F-2189

CELL SURFACE MARKER EXPRESSION ANALYSIS TO TRACK PROGRESSION OF FIBROBLAST REPROGRAMMING

Lakshmipathy, Uma[1], Quintanilla, Rene H.[1], MacArthur, Chad C.[2], Asprer, Joanna[1]

[1]Life Sciences Solutions, Thermo Fisher Scientific, Carlsbad, CA, USA, [2]Thermo Fisher Scientific, Carlsbad, CA, USA

Pluripotent stem cells are appealing tools for drug discovery and in regenerative medicine. The process of somatic cell reprogramming is a three-week long process, and successful outcome is measured by the emergence of colonies positive for pluripotent markers. Methods to analyze reprogramming intermediates provide a powerful tool to track reprogramming and predict the potential successful outcome of the process at an early stage. We had earlier reported the utilization of global gene expression analysis of fibroblasts, reprogramming intermediates, and fully reprogrammed cells to identify CD44 as a negative marker of pluripotent cells. Further, depletion of CD44 cells during the second week of reprogramming eliminated unreprogrammed and partially reprogrammed fibroblasts, thereby producing cleaner reprogramming cultures. In this study, CD44 was used in combination with the pluripotent marker SSEA4 to measure reprogramming kinetics. Fibroblasts from different reprogramming mediated transduced by either CytoTune® and or CytoTuneTM-iPS 2.0 Sendai Reprogramming kits showed distinct patterns of expression of the two markers with during reprogramming progression. The proportion of cells that were CD44 negative and SSEA4 positive at 7-8 days of reprogramming correlated with reprogramming efficiency. Analysis of CD44 and positive pluripotent markers thus enables the definitive identification, monitoring, and enrichment of pluripotent stem cells in reprogrammed cultures.

F-2190

SECRETED EPHRIN RECEPTOR A7 PROMOTES SOMATIC CELL REPROGRAMMING THROUGH INDUCING ERK ACTIVITY REDUCTION

Lee, Joonseong[1], Koyama, May Nakajima[1], Koga, Makito[1], Ebisuya, Miki[2], Nishida, Eisuke[1]

[1]Department of Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University, Kyoto, Japan, [2]Developmental Biology, RIKEN, Kobe, Japan

Somatic cells can be reprogrammed to a pluripotency state (induced pluripotent stem (iPS) cells) by defined transcription factors (Oct4, Sox2, Klf4 and c-Myc). Although many studies have revealed a number of cellular and nuclear events that play a crucial role in somatic cell reprogramming, little is known about the role of cell-cell interactions and secreted molecules in reprogramming. Here we show that a truncated form of Ephrin receptor A7 (EphA7), which is secreted during reprogramming, promotes cellular reprogramming by inducing ERK activity reduction. A truncated, secreted form of EphA7 as well as full-length EphA7 is prominently upregulated during reprogramming. Knockdown of EphA7 results in marked reduction of the reprogramming efficiency. Interestingly, addition of truncated EphA7 is able to restore the reprogramming efficiency to its original level from the reduced level. Our results also demonstrate that ERK activity is reduced during reprogramming and that secreted, truncated EphA7 is responsible for the ERK activity reduction. Remarkably, treatment of EphA7-knockdowned MEFs with the MEK inhibitor restores the reprogramming efficiency. Moreover, we find that expression of truncated EphA7 is much higher in pre-reprogrammed cells than in reprogrammed cells. This suggests that pre-reprogrammed cells act as a functional niche for cells acquiring pluripotency by secreting truncated EphA7. Thus, our findings reveal the importance of secreted EphA7-induced ERK activity reduction in reprogramming.

F-2191

GENERATION AND CHARACTERIZATION OF IPS CELLS FROM EQUINE ADIPOSE-DERIVED STEM CELLS

Lee, Eun-Mi, Kim, Ah-Young, Lee, Eun-Joo, Min, Chang-Woo, Kang, Kyung-Ku, Kim, Sang-Hyeob, Lee, Myeong-Mi, Soo-Eun, Sung, Hwang, Meeyul, Jeong, Kyu-Shik

Department of Pathology, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea

The horse represents enormous value for the related companion and sports fields. Health of horse is a major concern to the horse industry. Accordingly, induced pluripotent stem (iPS) cells have been on the spotlight as a powerful tool for study of regenerative medicine and specific disease mechanisms of horse. For these reason, more study for equine the horse iPS cells is required, but so far only limited cell lines (neonatal fibroblasts or skin fibroblasts) were used for generation of

equine iPS cells. In this study, we report generation of iPS cells from equine adipose-derived stem cells (E-ADSCs) using polycistronic lentiviral vector composed by the combination of four transcription factors (Oct4, Sox2, Klf4, and c-Myc). Sixteen days after transduction, the first iPS colonies distinctly appeared with flat, tightly and well-defined structure, somewhat resembling primed pluripotent stem cells. The equine iPS cells stained positive for alkaline phosphatse, Oct4, Sox2, Nonog and SSEA1 by histochemical or immunofluorescence stain. RT-PCR of cells showed high-expression of endogenous Oct4, Sox2, Klf4, Nonog, Lin28 and Rex1. Furthermore, the equine-iPS cells showed in vitro embryoid bodies (EBs) formation and *in vitro* and formation of teratomas containing three embryonic germ layer *in vivo* grafting into immunocompromised mice. In conclusion, we consider that these established equine iPS cells from E-ADSCs pave the way for more understanding equine iPS cells technology and development of regeneration therapy in veterinary medicine.

F-2192
SIRTUIN 1 INTERACTS WITH DNA REPAIR MOLECULES DURING THE INITIATION PHASE OF REPROGRAMMING FROM MOUSE FIBROBLASTS TO MOUSE INDUCED PLURIPOTENT STEM CELLS

Lee, Yin Lau[1], Peng, Qian[2], Chen, Chun Hang[1], Ng, Ernest Hung Yu[1], Yeung, Willam SB[1]
[1]*Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong,* [2]*University of Hong Kong, Hong Kong*

The reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) involves chromatin remodeling. We have previously shown that a NAD+ dependent histone deacetylase, sirtuin 1 (SIRT1) positively regulated mouse iPSCs formation, partly through deacetylating p53 leading to altered Nanog and p21 expression (1). SIRT1 also deacetylates other proteins like PGC1α [2] and FOXO3 [3]. The present study aimed at identifying the SIRT1 interacting protein during the reprogramming process. Pure population of secondary mouse embryonic fibroblast (MEF) containing doxycycline (DOX) inducible OSKM factors were obtained from chimeric MEF by Fluorescence Activated Cell Sorting. The cells were cultured in the presence of DOX for the first 5 days (initiation phase of reprogramming). The interacting proteins of SIRT1 during reprogramming were isolated by co-immunoprecipitation. The immunoprecipitated proteins were visualized by silver staining, and identified by mass spectrometry. According to the pathway clustering analysis from the Kyoto Encyclopedia of Genes and Genomes (KEGG), a number of them were involved in repair of double strand break (DSB). Homologous recombination DNA repair genes are important for successful reprogramming [4]. We speculated that SIRT1 might promote DNA repair through binding to the complex of DSB repair proteins. Indeed, the levels of the SIRT1 interacting DSB repair proteins (SIRT1-DSB) were up-regulated in the MEF upon DOX induction. To this end, we tried to knock down two of these SIRT1-DSBs at the onset of reprogramming. The result showed that the down-regulation of SIRT1-DSBs suppressed the iPSC colony formation as demonstrated by lower colony number and reduced Nanog expressions in the colonies. These data supported that SIRT1 activated the SIRT1-DSBs in the initial phase of reprogramming process leading to increase in colony formation. Acknowledgement: This work is supported by General Research Fund (HKU775711M) and Seed Funding Scheme to Support Research Projects on Human Embryonic Stem Cells (ESC) and Induced Pluripotent Stem Cells (iPSC), Stem Cell and Regenerative Medicine Consortium (SCRMC), Li Ka Shing Faculty of Medicine, The University of Hong Kong. We thank Professor Andras Nagy (The University of Toronto) for his generous gift of primary iPSCs.

F-2193
PROTEIN-BASED HUMAN IPS CELLS ARE EFFECTIVE IN TREATING A RODENT MODEL OF HUNTINGTON'S DISEASE

Jeon, Iksoo[1], **Lee, Nayeon**[1], Kim, Hyun Sook[2], Chang, Da-Jeong[1], Choi, Chunggab[1], Oh, Seung-Hun[2], Lee, Hyunseung[3], Hong, Kwan Soo[3], Lee, Sang-Hun[4], Kim, Kwang-Soo[5], Song, Jihwan[1]
[1]*CHA Stem Cell Institute, CHA University, Seoul, Republic of Korea,* [2]*Department of Neurology, Bundang CHA Hospital, CHA University, Gyeonggi-do, Republic of Korea,* [3]*Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Republic of Korea,* [4]*Department of Biochemistry and Molecular Biology, Hangyang University, Seoul, Republic of Korea,* [5]*Department of Psychiatry, Harvard Medical School/ McLean Hospital, Belmont, MA, USA*

Huntington's disease (HD) is an autosomal-dominant neurodegenerative disorder that is caused by abnormal expansion of CAG repeats in the *huntintin* gene. People carrying HD mutation exhibit the progressive and selective degeneration of striatal medium spiny projection neurons (MSN). For this reason, cell transplantation strategies in HD have been aimed at restoring GABAergic striatal projection neurons in the stratum, thereby re-establishing the degenerated striatopallidal circuit. In this study, we examined the therapeutic potential of integration-free, protein-based human iPS cells (Pro1-iPSC) in quinolinic acid (QA)-lesioned rodent model of HD. We first observed that Pro1 cells can differentiate into forebrain-type neurons at early stages, and then into GABAergic and MSN-type neurons at later stages. Next, we transplanted Pro1-derived neural precursor cells (Pro1-NPC) into the contra-lateral side of striatum at 7 days after QA was injected to the rat brains unilaterally, and the transplanted animals were monitored up to 12 weeks using various behavioral tests. Interestingly, we observed that the rats receiving Pro1-NPC cells exhibited significant behavioral improvements in stepping, staircase, rotarod and apomorphine-induced rotation tests. To track the fates of transplanted cells in vivo, we employed 4.7T animal MRI, which visualized the migration of contra-laterally transplanted cells to the lesion site. Immunohistochemical analysis identified two distinct populations of cell types: cells remaining in the contra-lateral side mainly consisted of $Nestin^{HIGH}$ and $CXCR4^{LOW}$, whereas cells migrated to the injury site consisted of $NeuN^{HIGH}$. We also observed that transplanted animals exhibited a reduction of lesion size. To support this observation, we found that the transplanted Pro1-NPC cells gave rise to GABAergic and MSN-type neurons efficiently. They also promoted endogenous neurogenesis in the subventricular zone (SVZ) of QA-lesioned animal. Finally, we observed that the transplanted animals exhibited a significant reduction of inflammatory response (ED1), compared with sham controls. Taken together, these results strongly suggest that Pro1-NPC cells are effective in treating HD. This study was supported by a grant of the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012R1A1A2006827), Republic of Korea. We are grateful to the MRI facility in the Division of Magnetic Resonance, Korea Basic Science Institute, Ochang, Korea

F-2194
AN AUTOMATED MICROFLUIDIC CELL CULTURE SYSTEM FOR COMBINATORIAL SCREENING OF CELL FATE SELECTION CONDITIONS

Li, Nianzhen, Szpankowski, Lukasz, Fowler, Brian, Devaraju, Naga Gopi, Norris, Michael, Thu, Myo, Wong, Michael, Kemp, Darnell, Clerkson, Barry, Johnson, Christian, Leyrat, Anne, Wang, Xiaohui, Sun, Gang, West, Jay, Unger, Marc, Jones, Robert C.
Fluidigm Corp., South San Francisco, CA, USA

In vitro stem cell studies are essential for understanding developmental biology as well as for disease modeling and cell-based therapies. A major challenge is finding the optimal culture conditions for cell expansion, differentiation, and reprogramming. Since multiple genes, extracellular factors, and intracellular signaling pathways are involved in each cellular process, a combinatorial approach to screen different culture conditions is highly desirable. However, due to the complex experimental design, intense labor, and huge reagent and cell consumption required by traditional cell culture systems, results are often difficult to obtain and not robust. To overcome these limitations, we have developed an automated system that consists of a microfluidic chip and an automated controller instrument for cell manipulation and environmental control. Each chip has 32 culture microchambers and 16 reagent inlets. Each microchamber can be dosed separately with different combinations and ratios of the 16 reagents at various pre-defined time points. We demonstrate culturing cells for over three weeks and dosing cells with multiple combinations of different factors, including miRNAs, mRNAs, DNAs, proteins, viruses, small molecules, etc. Streamlined protocols have been developed to stain and image cells on chip or harvest live cells and lysate from individual microchambers for continued off-chip culturing, single-cell or bulk genomic analysis, and/or functional assays. Using this system, we have developed a non-integrating method for direct conversion of human BJ fibroblasts to neurons at high efficiency and cell viability using microRNA mimics (miR-9, 9*, 124) and synthetic mRNAs (NeuroD2, Ascl1, and Myt1L). We have also cultured human induced pluripotent cells (hiPSCs) on chip for over a week in various defined or conditioned media and have demonstrated that cells maintain pluripotency. hiPSCs were able to differentiate to neural progenitor cells or nociceptor neurons on chip after treatment with small molecules. Furthermore, using combinations of small molecules and signaling proteins in chemically-defined media we demonstrated directing hiPSCs into primitive lineages of all three germ layers on one chip within four days. Immunostaining of marker proteins and single-cell gene expression analysis of 96 to 192 genes were used in all experiments to profile the cell types. The results were consistent with published reports and confirmed in large well-dish format. In summary, the automated microfluidic platform employs precise control of microenvironment of cells, facilitates studies of multi-factorial combinations, and enables development of robust, reproducible, and chemically defined cell culture and fate selection conditions.

F-2195
C-JUN AS A GATEKEEPER TO PLURIPOTENCY

Liu, Jing, Han, Qingkai, Peng, Tianran, Chen, Jiekai, Pei, Duanqing
Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China

c-Jun is the first oncogene discovered to be a transcription factor. It promotes cell proliferation and inhibits apoptosis, both activities known to promote somatic cell reprogramming by defined factors. Here we show here that c-Jun potently inhibits the reprogramming of mouse embryonic fibroblasts (MEFs) by the Yamanaka factors. Dominant negative (DN) c-Jun enhances reprogramming robustly. Jdp2, a known c-Jun antagonist, also enhances reprogramming. Furthermore, both DN c-Jun and Jdp2 can replace Oct4 in Yamanaka factor-mediated reprogramming.

F-2196
GENE REGULATORY NETWORK PROMOTING MOUSE REPROGRAMMING PROCESS

Massumi, Mohammad[1], Mohammadnia, Abdulshakour[2], Yaqubi, Moein[2], Khazaei, Niusha[2], Fallahi, Hossein[3], Wheeler, Michael[1]
[1]Department of Physiology, University of Toronto, Toronto, ON, Canada, [2]National Institute of Genetic Engineering and Biotechnology, Tehran, Iran, [3]Department of Biology, Razi University, Tehran, Iran

Somatic cells could be reprogrammed to pluripotent stem cells called as induced Pluripotent Stem (iPS) cells through overexpression of reprogram inducing master transcription factors. This bioinformatics study is aimed to analyze gene regulatory network involved in stepwise reprogramming process by scrutinizing the high throughput gene expression profile of mouse fibroblast-derived DE cells. Data were downloaded from the GEO database and RMA and fold change algorithms were used to normalize raw data and finding differentially expressed (DE) genes using Flexarray software v.1.6.1. Gene regulatory network was constructed in Cytoscape software using the data of ChEA and BioGRID database. Active modules of the network identified using JActiveModules v1.8 plugin. Comparison of gene expression profiles of MEF to Thy1- cell population at day three of reprogramming revealed 1504 DE genes in which 802 were down-regulated and 702 up -regulated. Based on ChEA database 25 DE transcription factors regulate expression of 1345 DE genes. Biological process analysis showed that M phase of cell cycle, nuclear division and mitosis are three terms that have lowest p-value. Analysis of active modules of main network showed that Suz12, Klf2, Foxp2, Gata2, Ezh2, Nanog, Zic3, Cebpd, Egr1, Pparg and Wt1 transcription factors present in five top modules. Comparison between SSEA1+ cells to Thy1- cells at day three of reprogramming showed 56 DE transcription factor, SSEA1+ cells at day six versus SSEA1+ cells at day three showed 224 DE genes, SSEA1+ cells at day nine vs. SSEA1+ cells at day six showed 117 DE genes and finally Oct4-GFP cells vs. SSEA1+ cells showed 1378 DE gens. The results were in consistent with the previous reports highlighting two waves of transcriptional changes during reprogramming. The result of this study may provide a road map guide to reprogram of adult cells into iPS cells.

F-2197
PRISTEM - A PRIMATE RESOURCE FOR DEVELOPING STEM CELL THERAPIES

McCarrey, John R.[1], Navara, Christopher[1], Grow, Douglas[1], Shi, Qiang[2], VandeBerg, John L.[2]
[1]Biology, University of Texas at San Antonio, San Antonio, TX, USA, [2]Southwest National Primate Research Center, San Antonio, TX, USA

Stem cells and Regenerative Medicine offer a novel approach to the treatment of diseases and injuries that have otherwise been difficult to effectively treat with standard medical approaches. The advent of "induced pluripotent stem" cells (iPS cells) that can be derived from easily available adult somatic cells such as skin or blood offers an exciting alternative to the use of embryonic stem cells for this purpose, and the opportunity to develop "patient-specific" stem cells that are genetically and immunologically matched to each individual. Nevertheless, much research is still needed to optimize the efficacy and safety of stem cell-based therapeutic approaches before applying these to human patients. Nonhuman primates, such as baboons, are the most clinically relevant

yet still experimentally manipulable animal model for studies of stem cell-based therapeutic approaches. The objective of "PriStem" is to develop a comprehensive, user-friendly, non-profit/non-commercial resource providing nonhuman primate pluripotent cell lines and access to matched living animals to foster the development, testing and optimization of therapeutic protocols involving patient-specific iPS cells prior to initiating tests in human patients. Here we summarize the characteristics of available baboon stem cells including embryonic stem cells, induced pluripotent stem cells, and mesenchymal stem cells, plus results of directed differentiation of these cells to produce dopaminergic neurons, angioblasts and endothelial cells, osteoblasts, chondrocytes, and adipocytes. Our goal is to produce additional iPS cell lines from living animals to allow investigators to model patient-specific thereapeutic protocols involving autologous transplantation of cells originally derived from the same animal that will subsequently be treated. The Southwest National Primate Research Center in San Antonio is fully equipped to maintain living nonhuman primates and to perform experimental manipulations on these animals. PriStem is designed to function as a centralized, comprehensive resource that will complement work by researchers from many different institutions pursuing studies involving stem cells and their application to therapeutic treatments of diseases or debilitating conditions such as battlefield trauma. This resource is expected to accelerate the discovery, application and clinical translation of novel therapeutic approaches involving iPS and related types of stem cells.

F-2198
HDAC1 REGULATES MOUSE SOMATIC CELL REPROGRAMMING

Musheer Aalam, Syed Mohammed, Manian, Kannan V., Bharathan, Sumitha P., Velayudhan, Shaji R., Srivastava, Alok
Centre for Stem Cell Research (a unit of inStem, Bengaluru), Vellore, India

During somatic cell reprogramming genome wide chromatin reorganization occurs that results in decondensation of heterochromatin structure of somatic cells to a dispersed state similar to that of embryonic stem cells. This transition in the structure of chromatin is mediated by the activity of chromatin modifying enzymes, many of which act as facilitators and others as repressors of reprogramming. Histone deacetylases (HDACs) are recognized as the major class of histone modifying enzymes that are involved in the chromatin transition during somatic cell reprogramming. Perturbation of histone deacetylase activity during mouse somatic cell reprogramming with small molecule inhibitors such as valproic acid, trichostain A, SAHA or sodium butyrate have been shown to promote reprogramming and transition of pre-iPSCs to fully reprogrammed iPSCs. Besides, valproic acid is a major component of the chemical cocktail that was found sufficient to induce reprogramming of mouse somatic cells. It is also interesting to note that MEFs derived from Hdac2 knockout mouse could be efficiently reprogrammed by transfection of only microRNA miR302b-367 cluster. To understand the role of different classes of histone deacetylases in regulating mouse somatic cell reprogramming, we measured the mRNA expression levels of Hdac 1 to 11 in MEFs, R1ESCs, pre-iPSCs, iPSCs and SSEA1+ reprogramming intermediates. We previously showed that Hdac1 was expressed in significantly higher levels in R1ESCs, iPSCs and pre-iPSCs clones compared to MEFs. In contrast, higher expression of Hdac7 was observed in MEFs and pre-iPSCs compared to iPSCs and R1ESCs. Besides, it was also interesting to note that the pre-iPSC clones (n=3) which were undergoing reprogramming in serum free conditions had progressively downregulated the expression of Hdac7 to the levels similar to that of R1ESCs with the induction of endogenous Nanog and transgene silencing. These observations suggest that Hdac1 acts as a facilitator and Hdac7 as a repressor of reprogramming. To determine the stage specific roles of Hdac1 and Hdac7 during mouse somatic cell reprogramming, we exploited inducible shRNA constructs for perturbation of gene activity. The Hdac1 and Hdac7 shRNAs were induced constitutively (Days 1-15) and at three different time points (Days1-5, 5-10 and 10-15) during reprogramming with OSKM maintaining suitable uninduced controls. Consistent with observations reported elsewhere, no significant difference in alkaline phosphatase positive colony number could be observed with either stage specific or constitutive knockdown of Hdac7 in cells compared to uninduced controls. However, constitutive knockdown of Hdac1 resulted in reduction in alkaline phosphatase and Nanog positive colony number by three fold compared to uninduced controls. Two fold reduction in alkaline phosphatase and Nanog positive colony number could be observed with cells in which Hdac1 shRNA was induced between days 1-5 and 5-10 compared to uninduced controls, but no significant difference in colony number could be observed in days 10-15 Hdac1 shRNA induced cells and uninduced control cells, suggesting the possible role of Hdac1 in the early stages of reprogramming. Cell cycle progression and proliferation are considered important early events during somatic cell reprogramming and HDAC1 has been implicated to regulate these aspects in tumor cells. Our further studies will focus on highlighting these roles of HDAC1 during somatic cell reprogramming.

F-2199
INCREASED REPRESSION OF LINE-1 RETROTRANSPOSONS IN HUMAN STEM CELLS CONTRIBUTES TO LOWER GENOMIC DIVERSITY IN HUMANS COMPARED TO NON-HUMAN PRIMATES.

Narvaiza, Iñigo, Marchetto, Maria Carolina, Denli, Ahmet, Benner, Christopher, Muotri, Alysson, Gage, Fred H.
Salk Institute for Biological Studies, La Jolla, CA, USA

Long Interspersed Element-1 (LINE-1 or L1) retrotransposons comprise ~17% of the human genome. Full-length L1 elements have the ability to move from one location in the genome to another by a copy-paste mechanism called retrotransposition. Whereas most L1 sequences in the genome are unable to retrotranspose, 80-100 full-length L1 elements remained active and are potentially mobile in the human genome. Active L1 elements have been detected in both germline and somatic human tissues, and can impact genome integrity. As uncontrolled retrotransposition activity can be deleterious to the host, organisms have evolved mechanisms to control L1 mobility. Here we take advantage of the reprogramming technology and generated induce pluripotent stem cells (iPSCs) from humans, chimpanzees and bonobos to explore factors that could have contributed to primate evolution and diversity. Comparative gene expression analysis of human and non-human primates (NHP) iPSCs revealed differences in the levels of APOBEC3B (A3B) and PIWIL2, two regulators of L1 transposition. APOBEC3B is a member of the APOBEC3 family of cytidine deaminases that can inhibit L1 mobility in different cell types, including human embryonic stem cells (hESCs) and iPSCs, via a still unclear mechanism. PIWIL2 is an effector of the piRNA pathway involved in L1 silencing mainly in germ line. We observed decreased levels of A3B and PIWIL2 in NHP iPSCs compared to human iPSCs, which correlated with increased L1 mobility of both human and chimpanzee tagged-L1 elements in NHP iPSCs. Manipulation of A3B and PIWIL2 levels in iPSCs supported a causal inverse relationship between the levels of these proteins and L1 retrotransposition. When we analyzed endogenous L1 activity, we detected higher levels of endogenous L1 mRNA expression in NHP iPSCs. Furthermore, we

found increased copy numbers of species-specific L1 elements in the genome of chimpanzees compared to humans. And using divergence as a measurement of L1 age, we found that the most recent chimpanzee-specific L1s are responsible for the significantly higher number of L1 loci in the chimpanzee genome. These results support the idea that increased L1 mobility in NHPs is not limited to iPSCs in culture and may have also occurred in the germline or embryonic cells during primate evolution. Differential activity of L1 imposes significant changes to the genomic structure and function and could potentially impact adaptation. The human population has gone through one or more bottlenecks throughout evolution that might have contributed to decreased genetic diversity. Chimpanzees and bonobos, in contrast, have increased levels of genetic diversity when compared to humans. While it remains unclear what the main generators of the phenotypic differences between our closest relatives and us are, we propose that L1 mobility could be playing a role in differentially shaping the genomes of humans and NHPs, providing an extra layer of variability to the latter.

F-2200
DEVELOPMENT OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED CO-CULTURES OF CARDIOMYOCYTES AND ENDOTHELIAL CELLS

Natividad-Diaz, Sylvia[1], Jha, Amit K.[1], Marks, Natalie Corinn[1], Jackson, Wesley M.[1], Healy, Kevin E.[2]
[1]Bioengineering, UC Berkeley, Berkeley, CA, USA, [2]Bioengineering, Materials Science and Engineering, UC Berkeley, Berkeley, CA, USA

Cardiovascular disease is associated with myocardial infarction, arteriosclerosis, and cardiac arrhythmias, which leads to the segmental loss of functional beating cardiomyocytes. Numerous studies have investigated stem cell transplantation therapies using synthetic and natural protein matrices. However, most stem cell transplantation therapies have had limited success due to poor donor cell engraftment and low survival.1,2 One major contributing factor to the lack of success is the limited vascularization within the ischemic cardiac tissue and the stem cell transplant. In this study, we address this limitation by creating a co-culture of cardiomyocytes (CMs) and endothelial cells (ECs) derived from human induced pluripotent stem (iPS) cells by controlling Wnt and TGFβ/Activin/BMP pathways respectively.3,4 This co-culture was incorporated into an optimized hydrogel matrix for cell transplantation to promote the survival and vascular integration of donor cells. The Wnt pathway protocol differentiated human iPS cells into beating CMs that were positive for cardiac troponin (cTnT). The TGFβ/Activin/BMP differentiation protocol yielded CD31+ ECs from the same iPS cell line. These iPS cell-derived ECs developed into tubular networks in vitro. We used fluorescence microscopy and flow cytometry to determine the distribution of differentiated mesodermal cells using cell markers specific to progenitor ECs (CD105+, CD34+) and mature ECs (CD31+) and CMs (CTnT+). We then assessed the survival and proliferation of these iPS cell derived ECs and CMs by seeding them into a hydrogel system of hyaluronic acid (HyA), a commonly-used biological polymer, to promote a 3D co-culture system. This study demonstrates the viability of this co-culture of iPS cell derived ECs and CMs to integrate with each other and form working vascular networks for possible cardiovascular disease therapies.

F-2201
INVESTIGATION OF THE EFFECT OF CRYOPRESERVATION ON THE CHARACTER OF IPS CELL-DERIVED NEURAL STEM CELLS FOR SPINAL CORD INJURY

Nishiyama, Yuichiro[1], Itakura, Go[1], Kobayashi, Yoshiomi[1], Nishimura, Soraya[1], Iwai, Hiroki[1], Toyama, Yoshiaki[1], Okano, Hideyuki[2], Nakamura, Masaya[1]
[1]Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Japan, [2]Physiology, Keio University School of Medicine, Tokyo, Japan

Recently, we have reported the effectiveness of human iPS cell-derived neural stem cells (iPSC-NSCs) for spinal cord injury (SCI) in adult mice. However, before the clinical trial, there are still several hurdles, one of which is to investigate whether cryopreservation affects the viability and proliferation ability of iPSC-NSCs. The purpose of this study is to determine the effect of cryopreservation on the characters of human iPSC-NSCs *in vitro* and to determine the effectiveness of transplantation of cryopreserved iPSC-NSCs for SCI in adult mice. 201B7 iPSC-NSCs, which were considered safe clone-derived iPSC-NSCs, were used in the present study. Neurospheres of these cells were divided into two groups. One was a non-frozen (NF) group and the other was a frozen and thawed (FT) group. In the FT group, neurospheres (passage 3-5) were frozen in cellbanker3® by the slow-freezing method on 3 day *in vitro* after the last passage (div) or 10 div. First, we evaluated the viability of the cells that were frozen on 3 and 10 div using trypan blue exclusion test immediately after thawing. The survival rate of freezing on 3 div was 20.3%, which was significantly higher than 11.9% of freezing on 10 div. Therefore, we determined that we were going to use neurospheres frozen on 3 div. Then, we evaluated the cell viability, proliferation and differentiation ability of the following four groups: NF group on 3 div (NF3) and 6 div (NF6); FT group immediately (FT0) and on 3 days (FT3) after thawing. The cell viability of NF6, FT0 and FT3 were 211%, 20.3% and 92.4% respectively compared to that of NF3. This finding suggested that the process of cryopreservation decreased the cell viability and that additional 3 day-culture *in vitro* after thawing increased the cell number of iPSC-NSC to more than 90% of that before freezing. Differentiation assay revealed that iPSC-NSCs of both NF and FT groups differentiated into Tuj-1 positive neurons and GFAP positive astrocytes but not CNPase positive oligodendrocytes. There was no significant difference in differentiation efficiencies among them. Principal component analysis and hierarchical clustering revealed that the gene expression profile of FT0 was similar to NF3, and that there was a difference in gene expression profile between FT3 and NF3. This finding indicated that 3 day-culture after thawing would significantly influence various genetic expressions of iPSC-NSCs. *In vivo*, contusive SCI was induced at the Th10 level in NOD/SCID mice as reported previously, and iPSC-NSCs of NF3, FT0 and FT3 were transplanted into the injured spinal cord 9 days after injury. In the control group, phosphate buffered saline was injected instead of cells. Behavioral analysis using Basso Mouse Scale (BMS) was performed until 12 weeks after SCI. The transplanted group showed better functional recovery in BMS score, compared to the control group and there was no difference in motor function among NF3, FT0 and FT3. In conclusions, freezing and thawing of iPSC-NSCs decreased the cell viability and additional 3 days culture *in vitro* after thawing was needed for the number of iPSC-NSCs to recover to that before freezing. Furthermore, freezing and thawing did not influence their differentiation potential and therapeutic potentials for SCI.

F-2202

CONSIDERATIONS FOR CRITERIA FOR REFERENCE CELLS FOR STANDARDISATION OF IN VITRO HUMAN CELL-BASED ASSAYS FOR TOXICOLOGY

O'Shea, Orla[1], Healy, Lyn E.[2], Stacey, Glyn[1]
[1]UK Stem Cell Bank, Hertfordshire, United Kingdom, [2] National National Institute for Biological Standards, Hertfordshire, United Kingdom

SCR and TOX is a research project (www.scrtox.eu) co-funded by the European Commission and Cosmetics Europe as part of the SEURAT-1 cluster (www.seurat-1.eu), aiming at the reduction and replacement of animal models with *in vitro* stem cell models, for use in predictive systemic toxicology. The UK Stem Cell Bank is involved in the SCR and Tox programme to develop induced pluripotent stem cells (iPSCs) as candidate cells for development of reference preparations to assist in standardisation and control of acute and chronic toxicity assays. The current work in this project is aimed at identifying the key criteria of such reference cells and materials. Three hiPSC lines, were generated using mRNA reprogramming. Banks of these iPSCs and a diploid hESC lines (H9) were generated and quality controlled (identity, sterility, mycoplasma and blood borne viruses, karyotype). Phenotypic and genotypic analysis demonstrated that all cell lines displayed markers of self-renewal (Oct-4, Sox-2) the undifferentiated state (e.g. SSEA4, SSEA3 and TRA-160). All 3 iPSC cell lines displayed an abnormal karyotype and retained their ability to differentiate into all 3 germ layers via directed differentiation, thus demonstrating their potential as pluripotent cells. The iPSC lines were compared to the hESC line H9 cell line, commonly used in toxicology studies. These lines are now being examined for their expression of nrf-2 (a known indicator of oxidative stress) and other related downstream toxicology responsive markers such as 'Ho-1, NQO-1, GST'. We describe how these aberrant and diploid cells compare as potential test substrates for toxicological studies and provide a justification for the utilisation of karyotypically abnormal cells under controlled conditions and the scientific interpretation of toxicology data generated from such systems. We suggest that the use of cells with abnormal karyology may be useful tools in the study of certain toxicological activities and provide reliable readouts for compounds of unknown biological impact.

F-2203

DIRECT SCREENING OF HUMAN PLURIPOTENCY BY A FLUORESCENT SMALL COMPOUND FOR CLINICAL APPLICATION

Park, Myung Rae[1], An, Seong Ung[1], Lee, Hyunah[1], Chang, Young-Tae[2], Kim, Jeong Beom[1]
[1]Max Planck Partner Group-Molecular Biomedicine Laboratory, School of Nano-Bioscience and Chemical Eng, UNIST, Ulsan, Republic of Korea, [2]National University of Singapore, Singapore

Induced pluripotent stem cells (iPSCs) hold great promise as a cell source for regenerative medicine, but screening of pluripotency remains challenging for clinical application. Recently, there are methods for monitoring of reprogramming depends on their morphology or genetic modification with reporter gene. However, these classical methods are not suitable for further usage due to heterogeneity, precise timing for selection and host genome modification in terms of clinical applications. Chemical approaches have been widely applied in stem cell biology by promoting stem cell self-renewal, proliferation, differentiation and somatic cell reprogramming using specific small molecules. In our research, we developed a fluorescent compound for selectively monitoring human iPS cells, SHI5, as a reporting small molecule for rapid screening in reprogramming process. Among chemically modified several diversity-oriented fluorescence libraries, we discovered that SHI5 is a specific probe in human pluripotent stem cells. In addition, we verified the pluripotency of SHI5 positive cell via *in vitro* and *in vivo*. In addition the novel fluorescence molecule is specific for human pluripotent stem cell without any effect on cellular toxicity. Taken together, SHI5 based screening for human pluripotent stem cell opens probably unlimited possibilities of bioimaging probe for clinical trial via assures their safety issue. Our current research and applications of small molecules that can select reprogrammed stem cell with self-renewal and pluripotent state, as well as the application that uses stem cells as a tool for pluripotency screening during reprogramming.

F-2204

THE NINDS REPOSITORY COLLECTION OF FIBROBLASTS AND INDUCED PLURIPOTENT STEM CELLS DERIVED FROM NEURODEGENERATIVE DISEASE PATIENTS

Perez, Cristian A.[1], Heil, Stacey[1], Grandizio, Christine[2], Gandre-Babbe, Shilpa[2], Hodges, Kelly[3], Sutherland, Margaret[4], Corriveau, Roderick A.[4], Tarn, Chi[1]
[1]NINDS Repository, Coriell Institute for Medical Research, Camden, NJ, USA, [2]Stem Cell Biobank, Coriell Institute for Medical Research, Camden, NJ, USA, [3]Cell Culture, Coriell Institute for Medical Research, Camden, NJ, USA, [4]NIH/NINDS, Rockville, MD, USA

Induced pluripotent stem cells (iPSCs) reprogrammed from patient-derived primary fibroblasts have become an increasingly utilized and needed resource for the study of human disease and have proven especially valuable in studying neurodegenerative disorders for which disease models are difficult to establish. The National Institute of Neurological Disorders and Stroke (NINDS) Repository is a public resource established in 2002 aiming to provide a centralized and open collection of biological samples (DNA, lymphoblastoid cell lines, fibroblasts, iPSCs, biofluids such as plasma, serum, cerebrospinal fluids, and urine) and associated de-identified clinical data from a diverse population of patients and normal controls. Since 2011, the NINDS Repository has added to its web-based catalog (http://ccr.coriell.org/NINDS) close to 50 iPSC and 150 fibroblast lines. Most iPSC lines are contributed by investigators from the Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) or Huntington's disease (HD) NINDS-sponsored consortia funded by the American Reinvestment and Recovery Act (ARRA). By making available to the research community neurodegenerative disease iPSCs and fibroblasts, the NINDS Repository contributes to fulfill the NINDS mission of reducing the burden of neurological disease - a burden borne by every age group, by every segment of society, by people all over the world. To ensure the quality of these valuable resources, all iPSCs and fibroblasts submitted to the NINDS Repository by iPSC Consortia and other investigators undergo rigorous quality assessments (viability, pluripotency, karyotyping, differentiation status, gene expression analysis, sterility) prior to distribution by the NINDS repository. The results are summarized in a Certificate of Analysis and/or displayed on the web-based catalog along with the recommended culturing protocol. The NINDS Repository fibroblast and iPSC collections include mostly cell lines bearing specific genetic mutations associated with PD, ALS, HD, frontotemporal degeneration(FTD), or Alzheimer's disease (AD), as well as samples derived from neurologically normal controls. For certain affected individuals, the parental fibroblast, corresponding iPSC line, and whole blood DNA are available. The NINDS Repository serves as a unique and effective centralized resource where these iPSCs, fibroblasts and their critical phenotypic data, are available to basic and applied research investigators worldwide.

IPS CELLS: DISEASE MODELING

F-2205
FUNCTIONAL ANALYSES OF MOTOR NEURONS DIFFERENTIATED FROM ALS PATIENT-DERIVED IPSC WITH MUTATIONS IN THE FUS GENE

Naujock, Maximilian[1], Hermann, Andreas[2], Naumann, Marcel[2], Lojewski, Xenia[2], Frickenhaus, Marie[2], Storch, Alexander[2], Reinhardt, Peter[3], Sterneckert, Jared[3], Kim, Kwang-Soo[1], Wegner, Florian[4], Petri, Susanne[4]

[1]Molecular Neurobiology Laboratory, Harvard Medical School/ McLean Hospital, Belmont, MA, USA, [2]Division for Neurodegenerative Diseases, Dresden University of Technology, Dresden, Germany, [3]Max-Planck-Institute for Molecular Biomedicine, Muenster, Germany, [4]Department for Neurology, Hannover Medical School, Hannover, Germany

For the study of functional deficiencies in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), patient-derived iPSC can be used as in-vitro disease models. As proof of principle, it has been demonstrated that motor neurons (MNs) differentiated from such ALS-iPSC indeed recapitulate certain disease-specific abnormalities. These studies however, were limited to the study of TDP43- and C9ORF72-related ALS cases. The aim of the present study is to unravel pathophysiological mechanisms underlying ALS in MNs differentiated from iPSC lines carrying mutations in the FUS gene compared to iPSC derived from healthy controls. Differentiation was induced based on a novel approach starting from an expandable population of neural precursor cells generated from iPSC. Markers of differentiation were analyzed by qPCR and immunocytochemistry. Cells were characterized by patch-clamp analysis to demonstrate that ALS-iPSC can be differentiated into functional MNs with negative resting membrane potential, sodium-inward currents and action potentials. We successfully differentiated three different ALS-iPSC lines (n=93) and lines from three healthy controls (n=67) into Tuj1/Map2/SMI32/Islet1-positive MNs without observing major differences in differentiation efficiency as confirmed by immunocytochemistry. Cells from both conditions showed similar basic neural properties such as steady membrane potential and Na+K+ currents under patch clamp analysis. Also, no significant differences between FUS and control lines were observed in the percentage of cells that were spontaneously active and they similarly fired single action potentials upon stimulation. However, in healthy controls we observed higher frequencies of spontaneously occurring action potentials (controls: 3.3Hz; FUS 1Hz) and postsynaptic miniature events (controls: 0.72Hz; FUS 0.27Hz), both of which are physiological parameters for spontaneous activity. Furthermore, upon stepwise depolarization a higher percentage of control-cells responded with trains of action potentials (controls: 84.3%; FUS: 50%). Thus, our results indicate that ALS cells with mutations in the FUS gene present a hypoexcitability phenotype. Since we observe comparable basic electrophysiological properties but distinct differences in excitability patterns in ALS derived FUS mutant cells we can focus on the molecular mechanisms underlying the observed abnormality. How exactly this phenotype is related to the observed degeneration of MNs in ALS awaits further investigation.

F-2206
TRANSCRIPTOMIC ANALYSIS OF THE DEVELOPING ATAXIA-TELANGIECTASIA CEREBELLUM. RNA SEQUENCING OF IPSC DERIVED CEREBELLAR PROGENITORS

Nayler, Samuel P.[1], Vanichkina, Darya[2], Taft, Ryan[2], Kozlov, Sergei[3], Lavin, Martin[3], Wolvetang, Ernst[4]

[1]Stem Cell Engineering Group, Australian Institute of Bioengineering and Nanotechnology, Brisbane QLD, Australia, [2]Bioinformatics, Institute for Molecular Bioscience, Brisbane QLD, Australia, [3]Radiation Biology and Oncology, QIMR, Brisbane QLD, Australia, [4]Australian Institute for Bioengineering and Nanotechnology, Brisbane QLD, Australia

We set out to use patient derived iPSCs (induced pluripotent stem cells) to generate neuronal cell types resembling those that are affected in the rare neurological disorder Ataxia-Telangiectasia (A-T), namely Purkinje and granule cells of the cerebellum. Several groups previously demonstrated generation of cerebellar-like cells from murine ESCs. More recently one group reported similar success using human pluripotent stem cells. We successfully induced expression of mid-hindbrain markers EN1 and GBX2 and also transcription factors demarcating the two main cerebellar progenitor types, MATH1 (rhombic lip) and PTF1α (ventricular zone). These progenitors could be expanded to produce cells that express markers consistent with, and are morphologically similar to, developing granule cells. To gain insight into the early events which occur during the formation of the cerebellum and how this may be affected in the absence of ATM, RNA sequencing of neuronal progenitors after was performed. Over the course of the differentiation both control and A-T samples downregulated pluripotency genes and upregulated neural commitment and anterior/posterior patterning gene programs including a number of well characterized genes driving cerebellar development which were highly coordinately regulated, such as Isl1, Meis1, Shh, Reelin, Lhx9, Gpr177 and Nfix. Expression of members of the Hox gene family were also tightly regulated. We observed a number of non-coding RNAs and small miRNA precursors that are known to be expressed in the developing cerebellum in vivo, supporting the claim that we had generated cerebellar precursors. Pathway analysis predicted with high confidence gene expression patterns indicative of neurological disease, and in particular progressive motor neuropathy and cerebellar ataxia, major hallmarks of A-T. We were able to detect gene expression evidence that is congruous with a number of theories based on other cellular systems and animal models regarding the nature of the neurodegeneration in A-T. These include the commonly held theory that ROS levels are misregulated and adversely affect the cerebellum - Indeed in our dataset two of the most highly upregulated genes in A-T neuronal cells were GSTT1 and CATALASE, important factors in the clearance of damaging ROS intermediates. In further support of this, we noticed misregulation of a number of ERK1/2 substrates. This is significant because ERK1/2 is critical in maintaining redox balance, and is a known target of ATM. The equally prevalent notion that ATM maintains genomic fidelity of neural precursors and resulting progeny during development was represented in our dataset as well. We detected significant downregulation of a number of MYC substrates. MYC is required for activation of the ATM dependent DNA damage response and also critically required for neurogenesis. Further to this, we detected differential regulation of Tp53 and Pcna, two important transducers of DNA damage and regulators of cell cycle control. These data are a valuable resource for researchers to formulate and test hypotheses regarding the early developmental events that occur in the absence of ATM. It does however represent only a snapshot in time and ideally, further timepoints, clones and patients/ATM mutants should

be sequenced in order to garner more statistical confidence.

F-2207
GENERATION OF A COMPREHENSIVE PANEL OF PATIENT-DERIVED PLURIPOTENT STEM CELLS TO DISSECT OLIGODENDROCYTE DYSFUNCTION IN THE PEDIATRIC MYELIN DISORDER PELIZAEUS-MERZBACHER DISEASE

Nevin, Zachary S.[1], Karl, Robert T.[1], Hobson, Grace M.[2], Tesar, Paul J.[1]
[1]Genetics and Genome Sciences, Case Western Reserve University School of Medicine, Cleveland, OH, USA, [2]Nemours Biomedical Research, Alfred I. duPont Hospital for Children, Wilmington, DE, USA

Patient-derived induced pluripotent stem cells (iPSCs) have become integral tools for parsing the molecular biology of complex human genetic disorders. Pelizaeus-Merzbacher Disease (PMD) is a pediatric leukodystrophy affecting central nervous system myelin that results in severe motor impairment, intellectual delay, and premature death and has no available treatment. Though PMD is a single-gene disorder, it exhibits a wide spectrum of phenotypes and clinical severity attributed to over 200 different mutations in the essential myelin gene proteolipid protein 1 (PLP1). Protein misfolding and endoplasmic reticulum stress have been implicated in PMD pathogenesis in PLP1 overexpression transgenic models, but this may not hold true for the many various point mutations and whole gene deletions identified in patients. Detailed investigations of PLP1's normal function and the etiology of PMD are further complicated by a lack of access to primary human oligodendrocytes, the only cell type in which PLP1 protein is known to be expressed. To address this need, we have generated a large panel of patient-derived iPSCs from children with unique duplication, triplication, deletion, and point mutations in PLP1 that encompass the genotypic and phenotypic variation seen in patients. Using new methods optimized in our lab, we have derived pure populations of oligodendrocytes and oligodendrocyte progenitors from these iPSCs in order to dissect individual patient phenotypes at a cellular level. Access to this unique resource is now allowing us to identify the cellular pathways and processes disrupted by particular PLP1 mutations, which in turn will inform the pursuit of patient-specific chemical and genetic therapies.

F-2208
TRANSCRIPTOME PROFILING HIGHLIGHTS ENDOPLASMIC RETICULUM STRESS AS AN IMPORTANT COMPONENT OF SPINAL MUSCULAR ATROPHY

Ng, Shiyan[1], Hendrickson, David G.[1], Rodriguez-Muela, Natalia[1], Soh, Boon Seng[1], Rinn, John[1], Rubin, Lee[2]
[1]Harvard University, Cambridge, MA, USA, [2]Harvard University Department of Stem Cell and Regenerative Biology, Cambridge, MA, USA

Spinal Muscular Atrophy (SMA) is a genetic neurodegenerative disease caused by mutation of the SMN1 gene, resulting in a deficiency of functional SMN protein. Using induced pluripotent stem cells (iPSCs) from normal and SMA patients, we are able to generate an unlimited supply of motor neurons - the cells that are selectively lost in SMA patients. To unravel mechanisms of cell death in SMA motor neurons, we performed RNA-sequencing of purified HB9+ motor neurons from SMA and wild-type iPSCs. This revealed elevated endoplasmic reticulum (ER) stress in SMA motor neurons compared to wild-type motor neurons, with the extent of ER stress being dependent on the amount of SMN protein produced by the cells. We found that inhibition of ER stress by a variety of small molecules promoted SMA motor neuron survival, an effect similar to that achieved by increasing levels of SMN protein. In addition, ER stress inhibition resulted in healthier motor neurons with larger cell bodies in SMA cultures, but had no significant effects on cell survival or cell body size in wild-type motor neurons, indicating a treatment that is specific to SMA. We are currently carrying out in vivo studies of these ER stress inhibitors in a mouse model of SMA.

F-2209
GENE-EDITING OF RAG1 USING CRISPR/CAS9

Ott de Bruin, Lisa M.[1], Lee, Yu Nee[1], Musunuru, Kiran[2], Notarangelo, Luigi D.[1]
[1]Division of Immunology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA, [2]Department of Stem Cell and Regenerative Biology, Harvard University and Harvard Stem Cell Institute, Cambridge, MA, USA

Background: Mutations in the Recombination Activating Gene 1 (*RAG1*) can lead to a wide variety of clinical and immunological phenotypes in humans, ranging from complete absence of T and B lymphocytes to expansion of oligoclonal T cells or autoimmune manifestations. The mechanisms underlying such phenotypic heterogeneity remain poorly defined. Treatment for RAG1 deficiency is based on allogeneic hematopoietic stem cell transplantation, but suboptimal results have been observed for transplants from donors other than HLA-matched siblings. An alternative approach based on lentivirus-mediated gene transfer in *Rag1*$^{-/-}$ mice has failed to induce T and B cell reconstitution, due to inadequate expression of the transgene. There is a critical need to develop novel animal models to investigate phenotypic heterogeneity of the disease and to develop novel therapeutic approaches based on gene correction. The Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/Cas9 system is a powerful tool to perform gene-editing in embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). The Cas9 nuclease is directed to a specific DNA site by a guide RNA (gRNA), allowing introduction of a double strand DNA break (DSB). If a highly homologous single stranded DNA template (ssODN) is introduced at the same time, the endogenous genomic sequence can be replaced with that contained in the ssODN. Objectives: 1. To generate a novel mouse model of *RAG1* dependent autoimmunity using CRISPR/Cas9 to introduce the c.2512C>T (R838W) mutation (R841W in humans) in murine ESCs.2. To use CRISPR/Cas9 to correct *RAG1* mutations in patient-derived iPSCs. Methods: To introduce the c.2512C>T mutation in murine ESCs, cells were nucleofected with 5µg of one of two distinct gRNAs, introducing DSBs at position 2508 or 2514, respectively, along with 5µg of a Cas9-GFP plasmid and either 100bp or 200bp ssODN. After 48 hours, GFP+ cells were sorted and gDNA was used for next generation sequencing (NGS) with the Roche 454 platform. The remaining cells were plated at single-cell clonal density. Five days later, these clones were subcloned and analyzed by Sanger sequencing. Results: NGS showed different efficiency of *Rag1* targeting for the two gRNAs (gRNA-A: 57%; gRNA-B: 13%). Moreover, use of the longer ssODN resulted in improved gene-editing (1.87% of reads showed introduction of the c.2512C>T mutation when using the 200bp ssODN, compared to 0.47% when using the 100bp ssODN). Sanger sequencing of subcloned ESCs targeted with Cas9 combined with gRNA-A and 200bp ssODN showed 114 of 250 clones (46%) with an indel at the target site and 3 clones with the c.2512C>T mutation. Conclusions: Our data suggest that the *Rag1* locus can be efficiently targeted in murine ESCs, but multiple gRNAs must be screened to identify the ones that allow optimal targeting. Furthermore, use of a longer ssODN results in superior efficiency of gene-editing. We are currently using a similar approach to correct *RAG1* mutations in patient-derived iPSCs.

F-2212

MODELING TAUOPATHIES USING IPSC-DERIVED NEURONS OBTAINED FROM MAPT MUTATION CARRIERS FROM FAMILIES WITH FRONTOTEMPORAL DEMENTIA

Rasmussen, Mikkel[1], Holst, Bjørn[2], Clausen, Christian[2], Stummann, Tina Charlotte[3], Nielsen, Jørgen E.[4], Hall, Vanessa J.[1], Freude, Kristine Karla[1], Schmid, Benjamin[2], Hyttel, Poul[1]

[1]Department of Veterinary Clinical and Animal Sciences, University of Copenhagen, Frederiksberg, Denmark, [2]Bioneer A/S, Hoersholm, Denmark, [3]H. Lundbeck A/S, Valby, Denmark, [4]Danish Dementia Research Centre, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Frontotemporal dementia (FTD) is the second most prevalent neurodegenerative dementia, accounting for 20 percent of all pre-senile dementias. The disease pathology includes degeneration of the frontal lobes with post-mortem identification of insoluble tau inclusions termed neurofibrillary tangles. However, the underlying etiology of the disease is still unknown. Human neurons derived from patients with familial FTD would be highly useful for in vitro modeling of FTD and related tauopathies and for developing a potential cure. Induced pluripotent stem cell (iPSC) technology allows for generation of the affected neuronal cell type in vitro from families with a familial form of FTD and such a cell model has the potential to shed light on the underlying pathomechanisms. In this study, we investigated iPSC-derived neurons from two symptomatic individuals with a microtubule-associated protein tau (MAPT) gene mutation leading to a R406W or P301L amino acid change in the TAU protein, as well as one asymptomatic R406W carrier and a healthy control individual. Immunological analyses 10 days after neural induction with the dual SMAD inhibitors SB431542 and LDN193189, showed that all lines expressed the neural progenitor cells markers SOX2, PAX6, NESTIN and VIMENTIN as well as the proliferation marker KI67. Moreover, the pluripotency marker OCT4 was downregulated compared to the iPSCs. Five weeks after neural differentiation, bipolar neuronal cells containing small cell bodies and extended axonal projections were observed. Terminal differentiation was confirmed through the measurement of intracellular calcium changes. Stimulation with glutamate, GABA, or acetylcholine resulted in a considerable increase of the cytosolic calcium level in the measured lines. In vitro pathological investigations of MAPT mutation carriers and control lines, including phosphorylation of TAU, synaptic transmission and apoptosis, is currently ongoing. Results from these studies may help to shed light on the underlying disease mechanisms of FTD and to promote the development of a cure for FTD and related tauopathies.

F-2213

PLURIPOTENT STEM CELL MODELS OF RETICULAR DYSGENESIS AS A TOOL FOR ELUCIDATING THE POTENTIAL ROLE OF INTRACELLULAR BIOENERGETIC SYSTEMS ON CONTROLLING THE FATE OF HEMATOPOIETIC PROGENITORS

Saiki, Norikazu[1], Oshima, Koichi[1], Hirayama, Akiyoshi[2], Soga, Tomoyoshi[2], Tomita, Masaru[2], Nakahata, Tatsutoshi[1], Saito, Megumu K.[1]

[1]Center for iPS cell Research and Application, Kyoto University, Kyoto, Japan, [2]Institute for Advanced Biosciences, Keio University, Tsuruoka, Japan

Recent studies have linked bioenergetic regulation to cellular differentiation. Differentiated cells usually utilize mitochondrial oxidative phosphorylation (OXPHOS) as major energy source, while stem cells rely on anaerobic glycolysis. Metabolic plasticity of cytosolic to mitochondrial metabolism plays a critical role in differentiation process. However, the contribution of metabolic communication among subcellular components to the fate of progenitor cells remains unclear. Adenylate kinase 2(AK2) is an adenylate phosphotransferase through the reaction ATP + AMP $\leftrightarrow$ 2ADP localized in the mitochondrial intermembrane space. Although AK2 mutations in human can cause a severe combined immunodeficiency with neutropenia, named reticular dysgenesis (RD), underlying mechanisms have not yet been elucidated. To address relationship between mitochondrial phosphotransfer and differentiation disorder in RD, we established induced pluripotent stem cells (iPSCs) from two RD patients. We differentiated RD-iPSCs into hematopoietic to evaluate whether the in vivo phenotype of RD is recapitulated. Hematopoietic differentiation from RD-iPSCs was profoundly impaired compared to AK2-supplemented RD-iPSCs. Next, using a capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS)-based metabolome analysis, we found that RD-iPSCs have latent alteration of metabolic network caused by AK2 deficiency. For detailed description of metabolic flux distribution of energy metabolism, we performed 13C-labeled substance tracer analysis. The initial distribution of pyruvate, the end product of glycolysis, into the tricarbon acid (TCA) cycle appeared to be skewed into the reductive direction in AK2(-) clones, which indicated that AK2 mediates the intracellular metabolic communication through controlling the distribution of glycolytic metabolites into the mitochondrial TCA cycle. Although the physiological role of reductive TCA flow is not clear in PSCs, this may be useful to balance the amount of energy metabolites between cytosol and mitochondrial matrix through mitochondrial shuttles, thereby maintaining whole cellular energy status stable. Furthermore, we tracked the isotopomer labeling and found that aspartate was also labeled with 13C, thus indicating the possibility that the increased flux to malate and fumarate, the product of TCA cycle, in AK2(-) clones is actually derived from alternative pathway. Several studies have demonstrated important roles of mitochondria on maintaining energy homeostasis and pluripotency in stem cells. In PSCs, AK2-medieated putative phosphotransfer network that connects mitochondria and cytoplasm may also contribute to balance the intermediate metabolic networks such as glutamate and aspartate shuttle. Perturbation of intracellular bioenergetic homeostasis caused by differentiation may cause inappropriate optimization of their metabolic state during transition into OXPHOS.

F-2214

HUMAN HEREDITARY MULTIPLE EXOSTOSES IPS CELLS SHOW INCREASED MINERALIZATION IN VITRO

Schlieve, Christopher[1], Kim, Hannah[2], Nguyen, Trieu[3], Hayashi, Yohei[3], Taheri, Sahari[3], Yamanaka, Shinya[4], Hsiao, Edward[5]

[1]General Surgery, Mayo Clinic, Rochester, MN, USA, [2]Human Genetics, UCSF, San Francisco, CA, USA, [3]Gladstone Institutes, San Francisco, CA, USA, [4]Center for iPS Cell Research and Application, Kyoto, Japan, [5]University of California - San Francisco, San Francisco, CA, USA

Heparan sulfate glycoproteins (HSPGs) are crucial in establishing biological signaling gradients required for appropriate osteogenesis and chondrogenesis. By undergoing multiple side chain modifications, HSPGs confer biodiversity and establish unique cellular gradients that allow a multitude of distinct cell-signaling interactions. Disruption of these intricate signaling gradients can result in disease. Hereditary multiple exostoses (HME) is characterized by growth of benign cartilage-capped bone tumors around areas of active bone growth. HME arises from mutations in the exostosin genes EXT1 and EXT2 that produce a hetero-oligomeric Golgi-associated heparan sulfate polymerase that regulates heparin sulfate chain elongation. Mutations in EXT1/2 produce a truncated heparan sulfate polymerase that results

in the failure of side chain elongation. Thus, the ability to establish extensive biological gradients is abolished. In this study, we established human iPS cell lines from patients with HME as a way to develop a human disease in a dish model. Multiple iPS cell clones were successfully generated using retroviral transduction of four pluripotency-inducing factors (OCT4, SOX2, KLF4, and c-MYC) or by episomal integration-free induction of OCT4, SOX2, KLF4, LIN28, L-MYC, and p53 siRNA factors into skin fibroblasts of a HME patient carrying a classical EXT1 mutation. We confirmed that the HME iPS cells retained the original EXT1 mutation found in the fibroblasts, expressed pluripotency markers, and could differentiate into all three germ layers in teratomas. Culture of the HME iPS cells in mineralizing conditions showed significantly more robust mineralization as compared to wild type controls. These results provide a foundation for future studies using stem cell-based models to elucidate the biological functions of extracellular matrix in human osteogenesis and chondrogenesis and to find new therapeutic approaches for skeletal disorders.

F-2215
PATIENT SPECIFIC IPSC DERIVED HEPATOCYTES TO DECIPHER THE PATHOPHYSIOLOGY OF ALPHA1-ANTITRYPSIN DEFICIENCY

Segeritz, Charis-P.[1], Ordóñez, Adriana[2], Tan, Lu[2], Marciniak, Stefan J.[2], Lomas, David A.[3], Vallier, Ludovic[4]

[1]Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute, Anne McLaren Laboratory for Regenerative Medicine and Department of Surgery, University of Cambridge, Cambridge, United Kingdom, [2]Department of Medicine, University of Cambridge, Cambridge Institute for Medical Research, Cambridge, United Kingdom, [3]Wolfson Institute for Biomedical Research, The Cruciform Building, University College London, Gower Street, London, United Kingdom, [4]Cambridge Stem Cell Institute and Wellcome Trust Sanger Institute, Cambridge, United Kingdom

α_1-antitrypsin (A1AT) deficiency is one of the most common inherited metabolic disorders in the Caucasian population. This liver disease is caused by a point mutation in the A1AT gene *SERPINA1*, modifying the conformation of the corresponding protein. The resulting polymer aggregates are retained within the hepatic endoplasmic reticulum (ER), the main site of A1AT synthesis. This results in liver cirrhosis whilst the lack of secreted A1AT results in emphysema. The only curative treatment for individuals with cirrhosis is liver transplantation. Current challenges in developing new therapeutics are the lack of physiologically relevant models to study A1AT deficiency. Hepatocytes derived from A1AT-deficient patient hIPSCs therefore represent an opportunity to study the pathophysiological mechanisms of A1AT misfolding. Our lab has reprogrammed A1AT-deficient patient fibroblasts with Sendai virus vectors to generate hIPSC lines (A1AT-ZZ) and developed protocols for hepatic differentiation. Through genetic correction of the point mutation in A1AT-ZZ hIPSCs, an isogenic cell line was created (A1AT-RR) that faithfully recapitulated WT phenotypes. Comparisons of A1AT-ZZ and A1AT-RR derived hepatocytes showed that A1AT-ZZ, but not A1AT-RR, hepatocytes had an ER morphology that resembled walled-off, spherical cisternae of various sizes. Pulse-chase experiments showed reduced and delayed secretion of A1AT in mutant A1AT-ZZ hepatocytes, confirming impaired ER protein trafficking. Live-imaging FRAP (fluorescent recovery after photobleaching) of GFP-tagged ER inclusions in A1AT-ZZ hepatocytes did not recover florescence after a single photobleaching event, indicating that these structures are disconnected from the ER. However, ER morphologies in A1AT-RR hepatocytes exhibited a continuous ER phenotype, which showed rapid recovery of the fluorescent dye following bleaching, suggesting unimpaired protein diffusion. Similarly, FLIP (fluorescent loss in photobleaching) analysis revealed slow loss of fluorescence in inclusions in A1AT-ZZ hepatocytes, but rapid loss of fluorescence in A1AT-RR hepatocytes. These isolated ER structures in A1AT-ZZ hepatocytes were also confirmed by Z-stack imaging and in liver tissue samples from A1AT-deficient patients. Our data suggest that reorganisation of ER structures into walled-off inclusions limits accessibility of chaperones to enclosed misfolded A1AT that may contribute to unresolved ER stress and disease progression. We are now in the process of harnessing the value of patient-derived hIPSCs to closer detect this signalling pathway.

F-2216
MODELLING DUCHENNE MUSCULAR DYSTROPHY (DMD) WITH PATIENT DERIVED HUMAN INDUCED PLURIPOTENT STEM CELLS.

Shoji, Emi[1], Sakurai, Hidetoshi[2], Heike, Toshio[3], Awaya, Tomonari[4], Nakahata, Tatsutoshi[5], Sehara-Fujisawa, Atsuko[6]

[1]Center for iPS Cell Research and Application, Kyoto, Japan, [2]Center for IPS Cell Research and Application (CiRA), Kyoto, Japan, [3]Kyoto University Hospital, Ohtsu-City, Japan, [4]Department of Paediatrics, Kyoto University Graduate School of Medicine, Kyoto, Japan, [5]CIRA, Kyoto University, Kyoto, Japan, [6]Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

Duchenne muscular dystrophy (DMD) is characterized by progressive muscle atrophy, which results in a muscular depression. Although therapeutic approaches to DMD has been carried out extensively, the curative treatment has not been found yet. In this study, we aim to establish morphological and physiological comparison system using mature skeletal muscle cells from wild type (WT) and DMD-patient derived iPSCs. DMD is caused by mutations or deletions in Dystrophin gene, which encodes 79 exons producing a large muscle protein. Although, inflammatory responses due to the loss of Dystrophin protein are still unrevealed, excessive influx of Ca^{2+} has been reported as the major factor of inflammation in DMD. One of the promising treatments for DMD is exon skipping which also occurs naturally at low efficiency. Exon skipping restores dystrophin expression by skipping specific exons that leads to an expression of truncated but partially functional dystrophin protein. Currently, clinical trials using this technique are in progress and becoming an effective treatment. In our study, this exon skipping technique was applied to produce restored-DMD induced skeletal muscles to compare with those of WT and DMD. Moreover, since the genetic background is crucial to detect the differences in inflammation responses due to the loss of Dystrophin protein, comparing with restored-DMD is significant. Furthermore, in order to mimic *in vivo* pathological change, mechanical stress analysis such as electric stimulation was performed to study changes in physiological state by visualising signalling molecules, such as Ca^{2+}. Up until now, we have generated 3 types of adult skeletal muscle cells at high efficiency, WT, DMD, and restored DMD. We have observed the contraction of obtained skeletal muscle cells performing electric stimulation. We have established Ca^{2+} imaging system, which can detect the activity of intracellular Ca^{2+} and measure the concentration of Ca^{2+}responses to electric stimulation. Applying the system to induced skeletal muscle cells will allow us to measure differences in Ca^{2+} influx activities which leads to mimic DMD disorder specific phenotype. In conclusion, DMD modelling using hiPSCs can be achieved by characterising disease-specific changes and an establishment of pathological evaluation system. Moreover, this evaluation system is expected to be useful in terms of selecting anit-inflammatory or exon skipping promoting drugs.

F-2217
EPIGENETIC THERAPY FOR FRIEDREICH'S ATAXIA

Soragni, Elisabetta[1], Miao, Wenyan[2], Iudicello, Marco[3], Jacoby, David[4], Demercanti, Stefania[3], Clerico, Marinella[3], Longo, Filomena[3], Piga, Antonio[3], Ku, Sherman[1], Campau, Erica[1], Du, Jintang[1], Penalver, Pablo[1], Rai, Myriam[5], Madara, Joseph C.[1], Nazor, Kristopher[1], O'Connor, Melinda[2], Maximov, Anton[1], Loring, Jeanne F.[1], Pandolfo, Massimo[5], Durelli, Luca[3], Gottesfeld, Joel M.[1], Rusche, James R.[2]

[1]The Scripps Research Institute, La Jolla, CA, USA, [2]Repligen Corporation, Waltham, MA, USA, [3]Universita' di Torino, Torino, Italy, [4]BioMarin Pharmaceuticals, San Rafael, CA, USA, [5]Univeriste Libre de Bruxelles, Brussels, Belgium

The genetic defect in the neurological disorder Friedreich's ataxia (FRDA) is the hyperexpansion of a GAA·TCC triplet in the first intron of the FXN gene, encoding the essential mitochondrial protein frataxin. Histone posttranslational modifications near the expanded repeats are consistent with heterochromatin formation and consequent FXN gene silencing. Using a human neuronal cell model, derived from patient induced pluripotent stem cells, we characterized the 2-aminobenzamide histone deacetylase (HDAC) inhibitor 109/RG2833 that increases FXN mRNA levels and frataxin protein, with concomitant changes in the epigenetic state of the gene. Chromatin signatures indicate that histone H3 lysine 9 is a key residue for gene silencing through methylation and reactivation through acetylation, mediated by the HDAC inhibitor. A phase I clinical trial with 109 in FRDA patients demonstrated HDAC inhibition and increased H3K9 acetylation in PBMCs and increase of FXN mRNA in blood from patients treated with the drug. This study provides proof of principle that an orally dosed class I HDAC inhibitor can increase both FXN mRNA and acetylation of a key residue in the blood of FRDA patients and provides the first example of a study that bridges treatment evaluation in disease-specific patient neurons and drug exposure in a clinical trial.

F-2218
HEMOPHILIA B SPECIFIC IPSCS: CHARACTERIZATION AND GENETIC CORRECTION, TOWARDS THE PROOF OF CONCEPT FOR IPSC-BASED CELL/GENE THERAPY

Steichen, Clara[1], Luce, Eleanor[1], Dianat, Noushin[1], Goulinet-Mainot, Sylvie[1], Saravaki, Vincent[1], Burks, Deborah[2], Lambert, Thierry[3], Concordet, Jean-Paul[4], Weber, Anne[5], Nguyen, Tuan Huy[6], Dubart-Kupperschmitt, Anne[1]

[1]Inserm U972, Villejuif, France, [2]Centro de Investigacion Principe Felipe, Valencia, Spain, [3]Hôpital Bicêtre, Centre de Référence pour le Traitement des Hémophiles, Le Kremlin-Bicetre, France, [4]Institut Cochin, Inserm U567, Paris, France, [5]Inserm u972, Villejuif, France, [6]Centre pour la Recherche en Transplantation et en Immunologie, Inserm UMR 1064, Nantes, France

Hemophilia B is an X-linked genetic disorder characterized by a reduced activity of circulating clotting factor IX (FIX). Constraining and expensive treatments based on regular intravenous injections of recombinant FIX exist but they only target the symptoms of the disease and not its genetic cause. A gene therapy clinical trial published in 2011 by Nathwani et al. was really encouraging showing a significant increase of FIX plasmatic activity linked with an improved quality of life for most of the treated patients. However, long-term efficacy and safety remain to be demonstrated. The aims of our project are to use the potential of induced pluripotent stem cells (iPSCs) i) to generate a new cell model of hemophilia B with iPSC-derived hepatocytes and ii) to study the feasibility of ex vivo gene/cell therapy for hemophilia B. Using a lentivirus-based approach, iPSCs have been generated from skin biopsy of a patient affected with severe hemophilia B (FIX activity < 1%). These iPSCs were extensively characterized. The expression of stemness markers was validated by immunostaining, flow cytometry and RT-PCR. The pluripotency of these cells was demonstrated in vitro by generating embryoid bodies that expressed differentiation markers from the three germ layers, and in vivo by injecting the cells into immunodeficient mice leading to teratoma formation composed of derivative tissues from the three germ layers. Karyotype of the iPSCs was normal and DNA sequencing showed the presence in the HB-iPSCs of the original fibroblast mutation. The second step of our strategy was to correct the genetic defect by a genome editing approach. We constructed a correction cassette including the FIX cDNA under the control of the ApoA-II promoter (expressed during hepatocyte differentiation). The FIX cDNA was modified by addition of an intronic sequence (mini intron I), and a specific mutation called Padua (G>T transversion leading to R338L mutation) to enhance FIX production and activity respectively. Indeed, this mutation has been reported in an Italian family and was shown to lead to an 8-fold increase in FIX activity. This correction cassette was inserted between two sequences homologous to the genomic safe harbor AAVS1 (adenovirus-associated virus integration site 1) sequence. To target integration of the therapeutic cassette into AAVS1 genomic locus, we used TALENs or CRISPR-Cas nucleases-mediated genome editing approach. We are currently characterizing the puromycin resistant iPSCs obtained after co-transfection of the correction cassette together with either of the two nuclease-encoding constructs. We have set up conditions for hESC and normal hiPSC differentiation into hepatocytes under defined culture conditions. Non-corrected and genetically corrected iPSCs will be differentiated into hepatocytes, which physiologically secrete FIX. The efficacy of the correction approach will be evaluated by transplanting the two types of iPSC-derived hepatocytes into a Hemophilia B mouse model available in the lab, and by comparing FIX expression level as well as clotting ability in the two series of transplanted mice. This approach will pave the way for further use of corrected patient-specific iPSCs for liver cell therapy.

F-2220
FUNCTIONAL CHARACTERISATION OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED NEURONS AND MODELLING THE ROLE OF TAU PROTEIN IN NEURODEGENERATION

Stummann, Tina Charlotte[1], Madsen, Helena B.[1], Andersson, Christian R.[1], Vajhoej, Charlotte[1], Frederiksen, Kristen[1], Lassen, Anders C.[1], Rasmussen, Mikkel[2], Holst, Bjørn[3], Hyttel, Poul[2], Clausen, Christian[3], Nielsen, Joergen E.[4], Rosenqvist, Nina[1], Egebjerg, Jan[1], Fog, Karina[1]

[1]H. Lundbeck A/S, Valby, Denmark, [2]Copenhagen University, Faculty of Life Science, Frederiksberg, Denmark, [3]Bioneer A/S, Hoersholm, Denmark, [4]Danish Dementia Research Centre, Copenhagen University Hospital, Rigshospitalet,, Copenhagen, Denmark

Aggregation of microtubule-associated protein tau protein in the brain is a hallmark of several neurodegenerative diseases such as Alzheimer's disease and Frontotemporal dementia. The tau pathology has been suggested to spread in a prion-like manner from one neuron to the other. Neurons derived from human induced pluripotent stem cells (iPSCs) have potential to be developed into improved *in vitro* models for neurodegenerative diseases. In order to do so, a clear understanding of their functional matureness is crucial. In this study, iPSC derived neuronal cells were characterised for marker expression and functional properties. Furthermore, we investigated the potential of these cells to model neurodegenerative disease mechanisms. The iPSC derived neurons exhibited clear neuronal morphology with

neurite outgrowth after 4-5 weeks of differentiation. They continued to be proliferative for months although the proliferation was much lower than for undifferentiated iPSCs. Real-time PCR showed up-regulation of neuronal marker genes and immunohistochemistry demonstrated the appearance of βIII-tubulin, microtubule-associated protein 2, and tau positive cells. Western blot analysis demonstrated the presence of the embryonic tau isoform (3RN0) exhibiting an embryonic phosphorylation pattern. Intracellular calcium changes were detected using fluorescent probes. Depolarisation of the membrane potential by increasing the extracellular potassium levels caused an increase in the cytosolic calcium levels. Stimulation with GABA, NMDA or glutamate combined with glycine in the absence of extracellular magnesium resulted in a considerable increase in cytosolic calcium. This indicates the presence of gabaergic as well as glutamatergic receptors such as the NMDA receptors. Image analysis demonstrated the presence of spontaneous calcium spikes. Patch clamp recordings in whole-cell voltage-clamp mode showed the presence of voltage-gated sodium and potassium currents in some, but not all cells. Overall, the iPSC neurons did indeed exhibit neuronal marker expression and functional characteristics but encompassed a relatively immature neuron population. Moreover, we show that the iPSC neurons release endogenous tau into the cell culture media and that they can take up extracellular recombinant tau implying their applicability in modelling the cell-to-cell spread of tau that has been implicated in the pathogenesis of tauopathies.

F-2221
A MECHANISM OF PROGRESSIVE CARDIOMYOPATHY IN DUCHENNE MUSCULAR DYSTROPHY PATIENTS

Tsurumi, Fumitoshi, Baba, Shiro
Deptr of Pediatrics, Kyoto University, Kyoto City, Japan

Duchenne muscular dystrophy (DMD) is the most common and severe type of muscular dystrophy. Cardiomyopathy is one of the important complications of DMD. However, the mechanism of developing cardiomyopathy is not well known. It is reported that calcium overload is one of the mechanisms which causes skeletal muscle damage in mdx mice, an animal model of DMD. Human induced pluripotent stem cell (hiPSC) is a useful tool to investigate diseases mechanisms. Especially, hiPSC is powerful tool to examine cells or tissues which we can not obtain easily, like cardiomyocytes. Here, we generated hiPSC from a DMD patient with deletion in exon 44 of dystrophin gene and parents (controls) without the deletion. The hiPSCs had pluripotency and differentiated into all three germ layers including cardiomyocytes. The differentiated cardiomyocytes beat spontaneously and expressed cardiac troponin T assessed by immunostaining. We measured intracellular free calcium ion concentration by Indo-1 fluorescent indicator. Indo-1 fluorescence ratio was remarkably elevated in rest (R0), peak (R) and amplitude (R-R0) in the cardiomyocytes derived from DMD-hiPSCs compared with in those derived from control-hiPSCs. Moreover, calcium overload was observed much more clearly in cardiomyocytes derived from DMD-iPSCs than in cardiomyocytes derived from control-hiPSCs after compulsory stretching. These findings indicated that calcium overload is one of the mechanisms for cardiomyopathy in DMD patients. Reducing calcium overload will be one of the treatments for DMD cardiomyopathy patients.

F-2222
USING INDUCED PLURIPOTENT STEM CELL TECHNOLOGY TO MODEL AND TREAT IDIOPATHIC PULMONARY FIBROSIS

Vijayaraj, Preethi[1], Mehrabi, Mehrsa[2], Chung, Katherine[1], Kuo, Alan[1], Zhang, Kelvin[3], Darmawan, Kelly[3], Manze, Chase[1], Karumbayaram, Saravanan[4], Damoiseaux, Robert[1], Malone, Cindy[2], Gomperts, Brigitte[5]
[1]Pediatrics, University of California, Los Angeles, CA, USA, [2]Biology, California State University, Northridge, CA, USA, [3]University of California, Los Angeles, CA, USA, [4]Broad Stem Cell Research Center at UCLA, Los Angeles, CA, USA, [5]Mattel Children's Hospital UCLA, Los Angeles, CA, USA

Idiopathic Pulmonary Fibrosis (IPF) is a progressive and fatal interstitial lung disorder of unknown etiology, characterized by the development, proliferation and aggregation of myofibroblasts in the form of fibrotic foci. This results in the deposition of excessive extracellular matrix and impaired gas exchange, which eventually leads to respiratory failure and death. Due to the lack of a good model to study IPF, the cause of the disease remains unknown. Moreover, there is a large amount of heterogeneity between patients with IPF making it a challenge to study this disease precisely. IPF is an orphan disease and multiple clinical trials have been performed with new therapeutic candidates for IPF but none of them have shown any efficacy. Given the profound clinical, pathological and molecular heterogeneity of the disease, a highly individualized treatment approach is likely warranted. One of the major reasons for our poor understanding of the pathophysiology of IPF and lack of therapies for the disease is that the most widely used bleomycin lung injury rodent model for IPF does not truly represent the disease. This is because the fibrosis in this model is not progressive, fibrotic foci do not form, and the initial fibrosis resolves itself within a few weeks post-injury and is not progressive. In order to better study IPF, our lab has generated a human in vitro model of IPF using induced pluripotent stem cell (iPSC) technology. Our in-vitro model represents a significant advance for the field as it uses patient-specific cells that recapitulate fibrotic foci, which are the hallmark of the disease. This was done by using induced pluripotent stem cell (iPSC) generated from lung fibroblasts from familial IPF patients, which were then re-differentiated back into mesenchymal cells. Interestingly, we found that the patient derived mesenchymal cells formed fibrotic foci that mimicked the characteristics of the human IPF disease cells in the dish. These foci when characterized by markers for identity, proliferation, transcriptome analysis, collagen deposition, and activated TGF-β showed a remarkable resemblance to the foci seen in an IPF patient lung. Moreover, the model also shows resistance to Fas mediated apoptosis, which is also one of the characteristics of the IPF lung. In addition, the fibrotic foci showed high elastic moduli comparable to IPF patient lungs when measured with Atomic Force Microscopy, further strengthening our in-vitro disease model of IPF. This is therefore the first relevant in vitro model to phenocopy a fibrotic disorder in a dish. We are currently using our model for a high-throughput phenotype-based drug screen and have identified candidates capable of inhibiting fibrosis in a dish. These candidates are being processed for lead optimization and in-vivo efficacy studies.

F-2223
HIGH THROUGHPUT LIVE CELL PEROXISOME ANALYSIS REVEAL NOVEL CELL FUNCTION DEFECT IN HSP PATIENT-DERIVED OLFACTORY STEM CELL MODEL

Wali, Gautam[1], Sutharsan, Ratneswary[1], Fan, Youngjun[1], Fernandes, Johanna[1], Sue, Carolyn[2], Crane, Denis[1], Mackay-Sim, Alan[1]
[1]Eskitis, Griffith University, Brisbane, Australia, [2]Kolling Institute of Medical Research, Sydney, Australia

Hereditary spastic paraplegia (HSP) is a neurodegenerative disorder that leads to progressive gait disturbances with lower limb muscle weakness and spasticity. Mutations in *SPAST* gene (encoding Spastin) are a major cause of adult-onset, autosomal-dominant HSP. Spastin is a microtubule-severing protein, enriched at the distal end of the corticospinal motor neurons that is degenerated in HSP. We recently described a patient-derived olfactory stem cell model (ONS cells) for *SPAST* HSP. Based on our observations in HSP patient ONS cells involving 50% reduced spastin, 50% reduced stabilized microtubule levels, dysregulated microtubule specific gene expression and also altered peroxisome and mitochondria distribution we hypothesised that organelle (peroxisome) trafficking is altered in HSP patient cells. Peroxisome trafficking was assessed by time-lapse imaging of peroxisomes for 2 minutes and analysis using IMARIS. 10 cells were imaged from each cell line (6 HSP patients v/s 6 controls), capturing around 6,000 peroxisomes per group. ONS cells were differentiated to cells with axon-like processes by disrupting actin with Cytocholasin D. These processes help study microtubule specific peroxisome trafficking, specific to axons. We describe various defects in HSP patient peroxisome trafficking. a) HSP peroxisomes had slower speed: Speed of peroxisomes from healthy control ONS cells had a range from 0.00 to 1.20μm/s (N=5935). Quantile regression analysis of peroxisomes at 90th percentile, which represent microtubule based fast moving peroxisomes showed that patient peroxisomes moved slower than control peroxisomes (β= -0.011, p-value <0.001). This difference in patient-control peroxisome movement was amplified in neuron-like cells (β= -0.10, p-value <0.001). b) Lower percentage of HSP peroxisomes perform fast transport: Also the percentage of peroxisomes exhibiting microtubule based fast travel (>0.15μm/s) was lower in patient than controls (13.36% v/s 16.21%, p-value< 0.001). c) HSP peroxisome movement quality not affected: Analysis of 100 randomly selected individual peroxisomes from control and patient ONS cells for their quality of peroxisome travel shows no difference i.e. the frequency of fast movement (p-value: 0.22); duration of fast movement (p-value: 0.52); ability to attain maximum speed (p-value: 0.93) and ability to retain at maximum speed (p-value: 0.56). d)Rescue of HSP peroxisome movement: Treating patient cells with Comp D, a microtubule binding drug which increases the stabilized microtubule levels rescued the peroxisome defect. Quantile regression analysis of patient peroxisomes at the 90th percentile showed Comp D treated patient peroxisomes move faster than untreated patient peroxisomes (β= 0.019, p-value< 0.001). When treated with Comp D, the percentage of fast travelling peroxisomes in patients increased to control peroxisomes level (Treated HSP: 16.31%, Untreated HSP: 13.23%; p-value<0.001). We conclude that peroxisome trafficking defects are present in HSP patient-derived stem cells. The patient-control defect being amplified in cells with axon-like processes, like axons which depend only on microtubule for organelle transport and also the rescue of patient-control differences by microtubule-binding drugs, suggest that peroxisome defect in HSP cells is microtubule specific. This is the first evidence for quantified peroxisome trafficking deficits in HSP patient-derived cells.

F-2224
IDENTIFICATION OF BIOMARKERS FOR THE ARTHROPATHY IN CINCA SYNDROME USING CHONDROCYTES DERIVED FROM PATIENT-SPECIFIC IPSCS

Watanabe, Makoto[1], Yokoyama, Koji[2], Ikeya, Makoto[3], Yamamoto, Rie[1], Tanaka, Takayuki[2], Nishikomori, Ryuta[4], Saito, Megumu[5], Umeda, Katsutsugu[6], Heike, Toshio[7], Sato, Taka-Aki[1], Toguchida, Junya[8]
[1]Shimadzu Corporation, Kyoto, Japan, [2]Kyoto University, Kyoto, Japan, [3]Kyoto University, Kyoto, Japan, [4]Gradate School of Medicine, Kyoto University, Kyoto, Japan, [5]Center for iPS Cell Research and App, Kyoto University, Kyoto, Japan, [6]Graduate School of Medicine, Kyoto University, Kyoto, Japan, [7]Kyoto University Hospital, Ohtsu-City, Japan, [8]Institute for Frontier Medical Sciences Kyoto University, Kyoto, Japan

Chronic infantile neurological cutaneous articular (CINCA) syndrome is an autoinflammatory disease, characterized by urticarial rash, central nervous system disorders including meningitis and deafness, and articular symptoms. The gene coding for Nod-like receptor pyrin domain-containing protein 3 (*NLRP3*) is responsible for CINCA syndrome. NLRP3 protein forms inflammasomes and regulates the release of proinflammatory cytokines, such as interleukin-1β (IL-1β) by caspase-1 in response to extrinsic pathogens and intrinsic danger signals. In CINCA patients, secretion of mature IL-1β is deregulated and accelerated because of constitutive active mutants of *NLRP3*. *NLRP3* is expressed in chondrocytes, and the arthropathy of patients results in the deformity of epi-metaphases of long bones with abnormal proliferation of calcified chondrocytes. These observations suggest the involvement of NLRP3 protein in the growth and differentiation of growth plate chondrocytes. To elucidate the molecular mechanisms of arthropathy, we generated induced pluripotent stem cells (iPSCs) with and without mutant *NLRP3* from CINCA patients with mosaicism (n = 2). Chondrogenic progenitors were induced from each type of iPSC. The extracted proteins from chondrogenic progenitors were subjected to proteome analysis using two-dimensional electrophoresis. Proteins with altered expression levels between mutant and wild-type were subjected to protein identification by mass spectrometry. Proteome analysis identified 125 and 155 CINCA-related proteins in each patient. The CINCA-related proteins included those that interacted with caspase-1. To gain further insights, the CINCA-related proteins were then subjected to pathway analysis to identify significantly associated biofunctions and protein networks. The findings in the present study uncovered proteins and protein networks that were related to the disease. In addition, these observations might provide useful markers for elucidating disease pathogenesis and for developing treatment for arthropathy.

F-2226
MODELING HIV LATENCY USING HUMAN PLURIPOTENT STEM CELLS

Xie, Wen[1], Lee, Tun-Hou[2], Essex, Max[2], Daley, George[1]
[1]Boston Children's Hospital, Boston, MA, USA, [2]Immunology and Infectious Diseases, Harvard School of Public Health, Boston, MA, USA

The failure of Highly Active Anti-Retroviral Therapy (HAART) to cure AIDS due to the existence of latent HIV reservoirs highlights the importance of understanding host restriction of viral replication. In this study, we modeled HIV latency and reactivation using pluripotent stem cells. We generated human induced Pluripotent Stem Cell (iPSC) colonies harboring a single-copy, envelope-defective HIV genome via reprogramming of HIV- infected fibroblasts. We found that the envelope-defective HIV genome was transcriptionally silenced during reprogramming, and that the Nef protein of the HIV genome promoted

the latency of HIV in human pluripotent stem cells via interactions with several host factors:NMT1, NMT2, ACOT8, Hck and PAK2. Deleting Nef from the HIV proviral genome resulted in a transcriptionally active provirus, while over-expressing Nef in human pluripotent stem cells infected with a Nef-defective HIV genome led to proviral silencing. We further documented enrichment of the repressive H3K9me3 mark and a lack of the permissive H3K4me3 mark on the HIV proviral genome, depending on Nef status. Differentiation of HIV infected pluripotent stem cells towards either the fibroblast or blood lineages led to partial reactivation of the HIV proviral gene, suggesting that stem cells harbor factors that promote long-term HIV latency. Loss of function screens for factors that re-activate the HIV genome hold promise for identifying molecular targets that might reactivate otherwise latent proviral reservoirs that are responsible for long term persistence of HIV infection.

F-2227
A PATIENT-SPECIFIC IPSC MODEL FOR STUDYING THE PATHOPHYSIOLOGY OF HIRSCHSPRUNG'S DISEASE

Yung, Jasmine S.[1], Tse, Hung-Fat[2], Tam, Paul KH[1], Ngan, Elly[1]
[1]Department of Surgery, University of Hong Kong, Hong Kong, [2]Department of Medicine, University of Hong Kong, Hong Kong

Hirschsprung's (HSCR) disease is a congenital neurocristopathy in which some enteric ganglia are absent due to incomplete colonization of neural crest cells (NCCs) in the hindgut, causing chronic constipation. A significant number of HSCR patients also clinically present with other NC-associated disorders, such as ventricular and atrial septal defects (VSD/ASD). A hypomorphic allele or SNP of a major gene, RET, causes or imparts susceptibility to HSCR. In particular, SNP (rs2435362) residing in the intron 1 of RET gene was found to be highly associated with HSCR and lead to reduced RET expression. However, the molecular basis of HSCR associated VSD/ASD is largely unclear. With the use of a iPSC-based HSCR model, we illustrate the pathophysiology of a specific HSCR patient. Three iPSC clones from a syndromic HSCR patient, carrying the RET risk allele in rs2435362 were generated. We used different caudalizing cues to differentiate iPSCs into NCCs with unique HOX expression patterns, corresponding to anterior cranial or posterior vagal NCCs. Consistently, the patient iPSCs displayed similar capacities in generating NCCs at all axial levels, marked by HNK1 and p75NTR. Nevertheless, the patient NCCs and their derivatives exhibited severe migration and/or differentiation defects in making neurons and smooth muscle cells. In particular, HNK1+p75NTR+ posterior NCCs derived from patient-iPSCs were less migratory compared to the control NCCs, while no obvious migration defect was observed in their cranial counterpart, indicating that the migration defect was only restricted to the more posterior NCCs. These patient NCCs were also less capable in generating neurons and readily biased toward generating glial cells, which pinpoints the fine balance between neurogenesis and gliogenesis. Intriguingly, the neural differentiation defects were restricted to NC lineage. The capacity of patient iPSCs to make various types of CNS progenitors and neurons was comparable to that of the control iPSCs, nicely recapitulating the patient's phenotype where only enteric neurons, but not CNS progenitors were affected. Subsequent expression analysis revealed that patient NCCs express lower level of RET which is known to be regulating enteric NCC migration and differentiation. Together, our patient-specific model endow a reliable platform to decipher the underlying pathogenesis in other HSCR patients.

IPS CELLS: EPIGENETICS

F-2230
HIGHLY SPECIFIC MONOCLONAL ANTIBODIES AND KITS TO STUDY DNA HYDROXY-METHYLATION IN THE GENOME

Squazzo, Sharon[1], Goval, Jean-Jacques[2], Panteleeva, Irina[2], Mazon, Ignacio[1], Hendrickx, Jan[2], Poncelet, Dominique[2]
[1]Diagenode Inc., Denville, NJ, USA, [2]Diagenode s.a., Seraing, Belgium

In recent years, studies suggest that the mammalian genome undergoes various epigenetic modifications during development and embryonic stem cell (ESC) differentiation. Understanding the mechanisms that control these epigenetic signals is essential in deciphering stem cell biology and the patterns of gene expression during cellular differentiation. ESC gene expression relies heavily on transcriptional factor networks and epigenetic signals such as histone variants, histone modifications, DNA methylation, and non-protein-coding RNAs that give stem cells their ability to differentiate into different cell types. 5-methylcytosine (5-mC) is an important epigenetic modification involved in development and cancer (in which this modification is frequently altered). 5-mC can be enzymatically converted to 5-hydroxymethylcytosine (5-hmC), and 5-hmC modifications are known to be prevalent in DNA of embryonic stem cells and neurons. Since 5-hmC is present in mammalian DNA at physiologically relevant levels and in a tissue-specific manner there is an important need to determine how 5-hmC can be distinguished from 5-mC and unmodified cytosine.Here we present highly-specific monoclonal antibodies and kits for the differential study of the functions of 5-hmC, 5-mC, and unmodified C, with new data from Diagenode's 5-hmC antibody, hMeDIP kits, and MeDIP kits. Our kits and reagents represent the most compelling set of validated products available for the study of 5-hmC and 5-mC. We provide data showing the specificity of 5-hmC versus both 5-mC and unmodified C using dot blots, Dual MeDIP and hMeDIP. In situ cellular staining on interphase ES cells (data not shown) indicates distinct 5-hmC staining, both in overall brightness and in binding patterns, from that obtained with the Diagenode 5-mC antibody. Our data are consistent with distinct roles of 5-hmC and 5-mC. Our new kits and antibodies open the door to novel epigenetics studies and clarify the role of 5-hmC in differentiation, displacing methyl-binding proteins, regulating DNA repair, recruiting chromatin modifiers, and other important functions.

F-2231
EPIGENETIC AND SIGNALING PATHWAYS COLLABORATE IN THE ACQUISITION OF PLURIPOTENCY

Sridharan, Rupa
University of Wisconsin-Madison, Madison, WI, USA

Transcription factor mediated reprogramming of somatic cells to induced pluripotent stem cells represents a profound change in cell fate. While this process is accompanied by major changes in the epigenome and transcriptome, how these events culminate in the activation of the pluripotency regulatory network remains unknown. Using reprogramming intermediates that are stalled at the stage before achieving complete pluripotency, we found that addition of both ascorbic acid (AA) and 2i (MAP kinase and GSK inhibitors) synergistically allowed the acquisition of a fully reprogrammed state at a very high efficiency. However, minimal conversion occurred with either component alone. When uncoupled, AA treatment had to strictly precede 2i and mediated perturbation of the epigenome. Among the AA-dependent activities, there was critical early requirement for histone

demethylase effects while Tet-mediated 5 hydroxymethylation was required throughout the conversion to iPSCs. Under hypoxic conditions the concentration of AA required for enhanced 5-hydroxymethylation and iPSC conversion was considerably reduced. Transcriptional response to the individual stimuli was almost completely divergent and both components were required for the rewiring of the pluripotency network. AA driven activation of pluripotency genes such as Nanog and Zfp42 was complemented by 2i-mediated activation of Tcfcp2l1 and general transcription machinery, and both stimuli were required for the activation of Esrrb. In addition, 2i was the major driver of repression of cell differentiation and proliferation genes that are downregulated in reprogramming. Taken together our results provide important insights into changes in the epigenome and transcriptome and the pathways that lead to the acquisition of pluripotency.

F-2232
THE INHIBITORY ROLES PLAYED BY LSD1 DURING CELL FATE CONVERSIONS VIA H3K4 DEMETHYLATION

Zheng, Hui
Guangzhou Institutes of Biomedicine and Health, Chinease Academic of Sicence, Guangzhou, China

The critical roles played by lysine-specific demethylase 1 (LSD1) during the generation of induced pluripotent stem cells (iPSCs) and the regulation of pluripotency have been reported extensively and involve multiple factors like p53, DNMT1, miR302 and so on. However, the current studies have identified an additional role of LSD1 during the reprogramming of mouse embryonic fibroblasts (MEFs). Although over-expression of LSD1 did not affect the reprogramming efficiency, down-regulation of LSD1 or LSD1 inhibitor significantly increased the efficiency in a dose-dependent manner. In addition, inhibiting LSD1 activity increased efficiency in the early phase during reprogramming rather than in the later phase without significant influence on cell proliferation, global DNA methylation and miR-302 expression. Additional studies with CHIP-seq and immune-precipitation suggested that by interacting with Oct4, LSD1 introduced the histone 3 lysine 4 demethylation onto the promoter of Oct4 downstream targets, which subsequently promoted the reprogramming efficiency. Furthermore, the current mechanisms were also applicable during the differentiation from iPSCs to neural stem cells and from the neural stem cells to functional neurons. Therefore, these studies on LSD1 should provided new information and viewpoint for understanding the cell fate conversions including reprogramming, differentiation and even trans-differentiation.

CHROMATIN IN STEM CELLS

F-2233
CHD1 IS ESSENTIAL FOR THE HIGH TRANSCRIPTIONAL OUTPUT AND RAPID GROWTH OF THE MOUSE EPIBLAST

Guzman, Marcela[1], Sachs, Michael[2], Ming Koh, Fong[1], Lin, Chi-Jen[1], Wong, Priscilla[1], Onodera, Courtney[3], Song, Jun[3], Ramalho-Santos, Miguel[1]
[1]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, UCSF, San Francisco, CA, USA, [2]UCSF, San Francisco, CA, USA, [3]Institute for Human Genetics, UCSF, San Francisco, CA, USA

Pluripotent cells in the mammalian pre-gastrulation embryo undergo unusually fast cell proliferation that is essential for development. This rapid embryonic growth is expected to generate a high transcriptional demand to sustain biosynthesis. However, the molecular mechanisms that regulate the transcriptional output of pluripotent cells, and how it relates to development, remain unknown. Chromodomain helicase DNA binding protein 1 (Chd1) is a chromatin remodeler that can bind to the activating histone mark H3K4me3 and has been associated with transcription in several species. Here we report that genetic deletion of mouse Chd1 leads to an embryonic arrest at the onset of gastrulation. Chd1-/- mutants initiate but do not sustain the expression of markers of the pluripotent epiblast, which is smaller than controls at E5.5 and does not grow, become patterned or initiate gastrulation. Deletion of Chd1 using Sox2/Cre indicates that Chd1 is specifically required in the epiblast for its growth. We show that the developmental arrest of Chd1-/- embryos is due to a combination of cell cycle defects and increase in apoptosis in the epiblast. To dissect the mechanism of action of Chd1, we generated tamoxifen-inducible Chd1 mutant ES cells. Chd1-/- ES cells have normal viability and can be expanded in the undifferentiated state, but have a self-renewal deficit. Mutant cells have lower levels of H3K4me3 and elongating RNA Pol II (phospho-S2) at the bodies of highly expressed Chd1 target genes associated with cellular growth, including ribosomal proteins. In agreement with these findings, Chd1-/- ES cells have a reduced transcriptional output of all mRNAs tested when normalization is carried out for cell number instead of RNA amount. We further found that rDNA is a direct target of Chd1, that mutant ES cells have reduced levels of H3K4me3 at rDNA and express lower levels of pre-rRNA. These defects correlate with an abnormal nucleolar morphology observed uniformly at the single cell level both in ES cells and in the epiblast. The combination of our in vivo and in vitro genetic data indicates that Chd1 promotes a high transcriptional output that underlies rapid growth of the pluripotent epiblast.

F-2234
EPIGENOMIC GROUND STATE OF A NEWLY DERIVED NAÏVE HUMAN ES CELL LINE

Hawkins, David[1], Battle, Stephanie Lauren[1], Ahmed, Faria[1], Nagulapally, Abhinav[1], Hesson, Jennifer[2], Ware, Carol B.[2]
[1]Medicine and Genome Sciences, University of Washington, Seattle, WA, USA, [2]Comparative Medicine, University of Washington, Seattle, WA, USA

Human embryonic stem cells (ESCs) are derived from the inner cell mass of the blastocyst in a manner similar to mouse ESCs. Although human and mouse share expression of several key regulators, there are distinct differences in morphology, growth conditions and overall expression and metabolomic profiles. In addition, human ESCs share a number of properties with post-implantation blastocyst derived mESCs (EpiSCs), including epigenetic similarities. This suggests that human ESCs do not exist in the naïve pre-implantation state. Here, we present the epigenomic ground state of a new human ESC line derived entirely under conditions conducive to the naïve state (two small molecule inhibitors plus LIF: 2iLIF). Derivation of this line is recently described elsewhere (Ware et al., PNAS, Accepted), and hereafter referred to as ELF1. To understand how the naïve ground state is regulated compared to typical hESCs, we have generated comprehensive epigenomic profiles. Using ChIP-seq we have constructed histone modification maps of five informative modifications that reveal information about promoters, enhancers and heterochromatin (H3K4me3, H3K4me1, H3K27ac, H3K27me3 and H3K9me3). Using MethylC-seq (BS-seq), we have generated genome-wide, nucleotide resolution maps of 5-methylcytosines, and genome-wide, nucleotide resolution maps of 5-hydroxymethylcytosine are in progress. RNA-seq was used to identify mRNA, lncRNA and miRNA transcripts. The epigenomic landscape is important for understanding transcriptional regulatory differences between cellular states. A comparative analysis with H1 hESCs reveals dramatic epigenomic differences in the naïve state. There is an overall reduction in repressive epigenomic modifications in the naïve state. A

substantial number of naïve ESC genes and human epiblast genes show ELF1(naïve)-specific epigenomic patterns. IL6ST, SOX6, DUSP1 and TFE3 show ELF1-specific enhancer usage, while SMYD2, AHNAK, IL6R, and TBX3 exhibit a lack of H3K27me3 at promoters and/or gene-proximal enhancers in ELF1 hESCs. ELF1 is a new human ES cell line derived under naïve growth conditions, 2iLIF. We have generated the most comprehensive epigenomic maps to date in human naïve ESCs. Our analysis provides a general overview of epigenetic differences consistent with other recent findings, including a general reduction in repressive epigenetic modifications. We also determined epigenomic changes that signify how the naïve state is transcriptionally regulated. Understanding how the epigenome contributes to control of the naïve versus non-naïve ES cell state will provide important insights on the regulation of pluripotency, generation of truly pluripotent iPSCs, and control of cellular differentiation.

F-2235
QUANTIFICATION AND CHARACTERIZATION OF DNA DAMAGE IN HUMAN INDUCED PLURIPOTENT STEM CELLS FOR SAFETY ASSESSMENT

Hursh, Deborah[1], Goehe, Rachel[2], Lynch, Patrick J.[3]
[1]Food and Drug Administration, Rockville, MD, USA, [2]FDA/CBER, Bethesda, MD, USA, [3]FDA, Bethesda, MD, USA

Accumulation of genetic mutations during derivation and culture of hiPSC-based medical products are a safety concern that remains incompletely understood. Global epigenetic reorganization and enhanced proliferation kinetics that accompany cellular reprogramming may negatively affect DNA replication fork progression, thus increasing the risk of genomic abnormalities. To improve our understanding of genome stability in hiPSCs, we quantified DNA damaging events in hiPSCs and their foreskin fibroblast founder cells by assaying phosphorylated histone H2A.X (γ-H2A.X), which localizes to sites of DNA damage. Analysis of γ-H2A.X levels by confocal microscopy and flow cytometry revealed that hiPSCs exhibit a significant increase in γ-H2A.X levels in comparison to progenitor fibroblasts. Furthermore, field volume analysis of γ-H2A.X, which is indicative of the size of the DNA break site, demonstrated that hiPSCs possess larger sites of DNA breakage when compared to foreskin fibroblasts. Cell cycle studies coupled with γ-H2A.X quantification demonstrated that the DNA damage events occur within the S and G2/M cell cycle phases in hiPSCs. 3-D confocal microscopy revealed an increase of coincident foci of γ-H2A.X and 53BP1, a protein that localizes to double stand breaks, in hiPSCs over foreskin fibroblasts. Quantitative 2-D microscopic analysis showed that while most foci of 53BP1 were intracellularly co-localized with γ-H2A.X, significant numbers of γ-H2A.X foci were present that did not colocalize with 53BP1. One explanation for this is that γ-H2A.X is localizing to elevated numbers of single strand DNA breaks unrecognized by 53PB1. Consistent with this view, alkaline comet assays showed the majority of damage present was due to DNA single strand breaks in hiPSCs. Furthermore, phosphorylation levels of RPA32 serine33, a single-stranded DNA-binding protein which becomes phosphorylated in response to DNA damage, were elevated in hiPSCs in comparison to foreskin fibroblasts. Collectively, our data suggest that elevated levels of γ-H2A.X may reflect a significant increase in the frequency of bona fide single-strand DNA break events in hiPSC compared to their somatic founder cells. These results have important implications for the assessment of genome stability and ultimately the safety of hiPSC-derived medical products.

F-2236
DNA METHYLTRANSFERASE INHIBITOR INCREASES IMMUNE MODULATION ACTIVITY AND MIGRATION CAPACITY OF MESENCHYMAL STEM CELLS

Lee, Seunghee[1], Kim, Hyung-Sik[2], Rho, Kyoung-Hwan[1], Yoo, Ju-Mi[1], Kim, Yu-Lee[1], Lee, Mi-Hye[1], Kang, Kyung-Sun[2], Seo, Kwang-won[1]
[1]Institute for Stem Cell and Regenerative Medicine in Kang Stem Biotech, Seoul, Republic of Korea , [2]Seoul National University, Seoul, Republic of Korea

DNA methyltransferase(DNMT) inhibitor regulates target gene expressions through modification of methylation status of promoter regions. However, the effect of DNMT inhibitor to immune modulation capacity of MSCs has not been discussed. In this study, we treated 5-azacytidine, one of the DNMT inhibitors, on hMSCs and confirmed that mixed lymphocyte reaction (MLR) activity was significantly decreased by 5-azacytidine treated hMSCs. To identify the immune modulation factors which are increased by 5-azacytidine treatment in hMSCs, we investigated the changes of promoter methylation patterns in 150 genes which are up-regulated in inflammatory environment using methylation array. We found that methylation statuses of 66 genes were changed and 30 genes were hypomethylated among the 150 genes by 5-azacytidine treatment in hMSCs. We found that immune modulation factors; COX2, PTGES, IDO and IL-6 and migration/adhesion related factors; ICAM, VCAM, CXCR2, CXCR4 and CXCR6 were increased through realtime qPCR. Moreover, we found that the promoter regions of COX2, PTGES, CXCR2, CXCR4 and CXCR6 were hypomethylated after 5-azacytidine treatment. We found that inhibition of COX2-PGE2 pathway before 5-azacytidine treatment could not decrease MLR activity comparing with control hMSCs indicating that COX2-PGE2 pathway is one of the main pathways in increasing immune modulation activity of hMSCs through 5-azacytidine treatment. We also confirmed that migration ability of hMSCs was increased by 5-azacytidine treatment. Taken together, 5-azacytidine increases immune modulation activity and migration capacity of hMSCs through changes of methylation statuses of target gene promoters.

F-2237
EVOLUTIONALLY DYNAMIC KAP1-MEDIATED CONTROL OF LINE-1 ENDOGENOUS RETROELEMENTS IN HUMAN EMBRYONIC STEM CELLS

Castro-Diaz, Nathaly, Turelli, Priscilla, Kapopoulou, Adamandia, Yazdan-Panah, Benyamin, Marzetta, Flavia, Friedli, Marc, Raclot, Charlene, Trono, Didier
Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland

KRAB-ZFPs are tetrapod-restricted, sequence-specific DNA binding transcriptional repressors encoded in the hundreds by higher vertebrates, which have been subjected to intense positive selection during recent evolution. Through their cofactor KAP1, KRAB-ZFPs govern the locus-specific docking of a chromatin-modifying complex comprising histone deacetylases (HDACs), histone methyltransferases, DNA methyltransferases (DNMTs) and other heterochromatin-inducing factors. KRAB/KAP1-mediated repression is key to several events essential for early embryogenesis, including the maintenance of imprinting marks, the silencing of endogenous retroviruses, and the establishment of site-specific DNA methylation patterns. Close to half of the human genome is derived from endogenous retroelements, of which only LINE-1 repeats are currently active. These genetic invaders constitute important evolutionary forces but are also a threat to genomic integrity through transcriptional perturbation and insertional mutagenesis. LINEs are thus controlled from the earliest

stages of embryogenesis by chromatin modifications and DNA methylation, but what determines the target-specificity of this process is poorly defined. By performing RNAseq and ChIPseq analyses, we found that KAP1 controls a discrete subset of L1 subfamilies in human embryonic stem (hES) cells. The recruitment of KAP1 seems to be evolutionally dynamic: the corepressor is not detected at most ancient, degenerated L1 elements (e.g. L1MA lineage), while it is highly enriched at more recent L1 subfamilies (e.g. L1 primate-specific PA4 and PA5). This pattern could reflect the host's need for controlling newer - potentially active - but no longer older - inactive - elements. However, members of the most recent, human-specific L1Hs subfamily are not bound by KAP1, likely because the corresponding sequence-specific DNA binding recruiters - i.e. KRAB-ZFPs - have not yet emerged. Noteworthy, KAP1 binding correlates with the presence of the H3K9me3 histone mark, supporting the repressive action of the KRAB-KAP1 complex on L1s. Furthermore, KAP1-binding L1 sequences can act as cis-repressors when placed upstream of a reporter cassette, correlating with KAP1 recruitment and H3K9 trimethylation at the cis-acting L1 sequence, as well as high levels of CpG methylation at the adjacent promoter. In contrast to hES, KAP1 is absent from most L1s in somatic cells. However its deletion acts synergistically with HDAC and DNMTs inhibition to relieve LINE-1 repression, consistent with ongoing KAP1-mediated control of some L1s in differentiated tissues. In summary, our results indicate a progressive take-over of the epigenetic control of LINEs by KRAB-ZFPs and their cofactor KAP1 during primate evolution. Furthermore, our data suggest the dynamic control of LINEs by KRAB/KAP1-mediated repression from early embryogenesis to somatic cell differentiation.

EDUCATION AND OUTREACH, ETHICS AND PUBLIC POLICY, HISTORY AND SOCIAL ISSUES

F-2239
FREEDOM, FUNDING AND OTHER MOTIVATIONS FOR PROFESSIONAL MOVEMENT IN STEM CELL RESEARCH

Jacob, Karen Jane, Longstaff, Holly, Sihoe, Aaron, Illes, Judy
National Core for Neuroethics, Vancouver, BC, Canada

Brain drain is a significant concern for governments around the world including international bodies such as the Organization for Economic Co-operation and Development, yet little empirical research exists on the perspectives of individual researchers. We examined the brain drain phenomenon for both established stem cell researchers and trainees at the graduate and postdoctoral levels. To examine the variables involved in decision-making by established investigators, we created a database of over 2000 experts from four geographic hubs of stem cell research – Canada, Germany, the US and the UK – and invited them to participate in a semi-structured interview about their professional movement and the perceived impact that these moves had on their careers. The objectives of these interviews were to determine and characterize the relative impact of different variables in decision making, including: source of stem cells, availability of funding, public perception of stem cell research, legal and regulatory restrictions, and opportunities for commercial advancement. To examine trainee movements and motivation, we mined a database of approximately 1600 trainees of the Canadian Stem Cell Network for movement patterns and career outcomes. We also conducted focus groups with trainees to examine in depth the patterns about career decisions emerging from the database analysis including: resiliency of lab funding, funding for postdoctoral work, size of lab, lab culture, personal and family considerations, and attitudes about future academic employment opportunities. We will use the data as the basis for guidance aimed at future policies for research planning, program development and funding, and for recruiting and retaining highly qualified personnel in stem cell research.

F-2240
THE STEM CELL "HYPE" AFTER GERON: MEDIA PORTRAYAL OF THE "READINESS" OF STEM CELL THERAPIES

Kamenova, Kalina, Caulfield, Timothy
Health Law Institute, University of Alberta, Edmonton, AB, Canada

Scientists have often been wary of the ways in which the news media portray important medical and health policy issues, particularly the tendency of sensationalism in medical reporting and miscommunication of scientific data, which may diminish the ability of the public to participate as knowledgeable participants in policy debates. This study examines how the clinical translation of stem cell research has been portrayed in the mass media before and after the biotech company Geron announced on November 15, 2011 that it was halting its pioneering stem-cell program and discontinuing the first FDA-approved clinical trial of hESC-derived treatment for spinal cord injuries. The Factiva database was used to collect two data sets of news reports published in major newspapers in Canada, the United States and the United Kingdom - the first one including press coverage over the two-year period immediately preceding Geron's decision and the second one focusing on news stories in the two-year period after. Content analysis of media texts was conducted to identify major frames and themes in representing advances in the field of stem cell research and the clinical promise associated with stem cell therapeutics. We contrast and compare the tone of media coverage to identify differences in representations after the significant setback for stem cell research caused by Geron's decision. Our analysis focuses on how journalists continue to "hype" discoveries in the field and to make exaggerated claims regarding the "readiness" stem cell therapeutics.

F-2241
NETWORK ENRICHMENT ANALYSIS: A NOVEL METHOD FOR FUNCTIONAL INTERPRETATION OF 'OMICS' DATA IN GLOBAL NETWORKS OF FUNCTIONAL COUPLING

Alexeyenko, Andrey
Science for Life Laboratory, Bioinformatics Infrastructure for Life Sciences, Stockholm, Sweden

Several years ago we developed FunCoup, an optimised Bayesian tool to predict global gene interaction networks by multi-facetted data integration. It used evidence from multiple species to reconstruct global networks in eukaryotic organisms, including the human. Further, to enable statistically sound network analysis to test biological hypotheses, we proposed a new method of network enrichment analysis where topology was employed to evaluate functional impact of experimentally determined genes and gene sets. Apart from hypothesis-driven research, this analytic paradigm can assist in exploring molecular landscape in hypothesis-free manner. The method extends the gene set enrichment analysis into the network domain, and was applied to e.g. validation of candidate genes in Alzheimer's disease and characterization of cancer transcriptomes and experimental results in cell lines. Based on network analysis of transcriptome data from recent research in mouse embryonic stem cells, we report genes and signaling pathways involved in the patterning in the embryonic brain. Depending on specific requests, I will try to exemplify the method with novel applications (e.g. identification of disease-causing genomic variants among GWAS hits) and present relevant on-line tools

and other software.

F-2242
ANALYSIS OF MEDIA DISCOURSES ON STEM CELL RESEARCH AND REGENERATIVE MEDICINE IN JAPANESE NEWSPAPERS

Shineha, Ryuma[1], Yashiro, Yoshimi[2], Tanaka, Mikihito[3]
[1]The Graduate University for Advanced Studies, Kanagawa, Japan, [2]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [3]Waseda University, Tokyo, Japan

The rapid progress of stem cell research (SCR) and regenerative medicine (RM) has provided us with not only an abundance of biomedical knowledge but also a variety of debates on ethical, legal, and social issues (ELSI). In the course of the debates about ELSI regarding SCR and RM, the mass media have influenced the frames of discussions and the public's attention. In Japan as well as in other countries, there is a huge amount of news being reported about SCR and RM, particularly following the development of human ES cells. This situation was accelerated by the development of human iPS cells. However, in regard to Japan, the changes in media attention and their discourses concerning SCR and RM have not been examined. This lack of insight related to media attention is a hurdle for thinking about the current status and future direction of communication concerning SCR and RM in society. What kinds of topics have attracted media attention? What kinds of frames have dominated media discourses, and what is the time line of changes in media trends? To further discussions about SCR and RM in society, it is essential to describe the features, characteristics, and changes of discourse and framing concerning SCR and RM. Thus, in this study, we will focus on media discourse and the time line of changes regarding SCR and RM, considering the relationship between media discourse and social discussions. We collected over 4,000 news articles from three major newspaper sources and conducted quantitative analysis: co-word network analysis and content analysis. Co-words and content share have been regarded as indicators of semantic maps of document sets, which enable the mapping of the dynamics of meaning. Thus, these quantitative methods can evaluate connections between key words and concepts. In addition, we can identify different semantic structures and time lines of changes of dominant topics and framing appearing in texts by comparing maps and data. As the result of our media analysis, we gained several insights. Firstly, there was no significant increase in media attention when mouse ES cells and mouse iPS cells were developed. Media attention increased with the beginning of policy making related to the success of human ES/iPS cell research. In other words, Japanese media tend to focus on the social impacts rather than the scientific breakthroughs related to research. Secondly, we observed a change in co-word network structures and contents such as "ethics" in discourses found in the Japanese media from the 1990s to 2013. For example, after the establishment of human-induced pluripotent stem cells in 2007, the number of connections of key words related to ethical aspects decreased rapidly. This result implied a change in position and treatment of the ethical aspects of stem cell research, and suggested the peripheralization of ELSI in the Japanese media. We have to think about the meaning of this peripheralization of ELSI on SCR and RM. Is this peripheralization of ELSI in the media sphere beneficial to stem cell scientists, policy makers, journalists, and the public? This situation also carries a potential risk of damaging the communication about the social aspects of SCR and RM between experts and journalists. We have to consider our next steps and the architecture required to promote discussions among related actors based on data. We hope our analysis will benefit future discussions.

F-2243
THE DISCUSS PROJECT: INDUCED PLURIPOTENT STEM CELL LINES FROM PREVIOUSLY COLLECTED RESEARCH BIOSPECIMENS AND INFORMED CONSENT

Isasi, Rosario[1], Lomax, Geoffrey[2], Hull, Sara Chandros[3], Lowenthal, Justin[4], Rao, Mahendra[5]
[1]Centre of Genomics and Policy, McGill University, Montreal, QC, Canada, [2]CIRM, San Francisco, CA, USA, [3]NHGRI Bioethics Core, National Institutes of Health, Bethesda, MD, USA, [4]Department of Bioethics, National Institutes of Health, Bethesda, MD, USA, [5]NIH Center for Regenerative Medicine, Bethesda, MD, USA

Stem cell banks to date have focused predominantly on obtaining prospective consent from contemporaneous cell/tissue donors, culminating in the drafting of harmonized protocols covering a broad range of foreseeable scientific, therapeutic, and commercial applications. However, there are also large stocks of previously collected specimens of scientific value, and questions have emerged regarding consent for the utilization of these existing specimens for stem cell derivation. Concerns have been expressed about certain features of iPSC research (ubiquitous source material and pluripotency); the rapid growth in translational initiatives and commercial partnerships. The "DISCUSS Project: Deriving Induced Stem Cells Using Stored Specimens" - a partnership between the USA's National Institutes of Health, the California Institute for Regenerative Medicine and the International Stem Cell Forum - was established to address these concerns. A major goal of the DISCUSS Project is to develop consensus among the research community for the utilization of previously collected specimens for iPSC derivation, banking and distribution. The project employed a two-step process involving the development of draft recommendations. Subsequently to publication, A series of seminars and workshops were held to receive comment and recommendations from international .stakeholders. This presentation will report our final recommendations based on consensus developed by international partners.

F-2245
DEVELOPING A CENTRAL "POINT-OF-TRUTH" DATABASE FOR SHARING INFORMATION ABOUT EXISTING STEM CELL LINES

Pearse, Richard VanDyke
Clinical and Translational Science, Harvard Medical School, Boston, MA, USA

With rapidly advancing technologies, allowing researchers to create collections of stem cell lines faster than ever, it's become increasingly difficult to keep abreast of the lines that have already been developed. While proposals are swimming in for funding the development of thousands of new lines, it's entirely unclear which of these are proposing to generate lines that have already been developed. We propose the creation of a centralized "point-of-truth" database that would serve as an authoritative description of all stem cell lines that are available for potential sharing. A qualified platform should: 1) allow researchers to edit and update their own information, 2) be openly discoverable by traditional internet search engines, 3) publish information as linked open data (i.e. xml, rdf) to be compatible with third party applications such as Pubmed "linkouts" that automatically link journal articles to relevant resources contained therein, 4) be open source and open access to allow individual institutions free access to customize and modify functionality as required, 5) be driven by a centralized Ontology to force consistent and accurate description of stem cells, and 6) allow seekers to directly request the resource (when available) from the most appropriate source whether that is a bio-repository or

an individual researcher. We are using the eagle-i research-resource sharing platform (which meets the above criteria) to support this effort. We've started with a collection of iPS and ES cells from the Harvard Stem Cell Institute, from individual researchers across Harvard University, and from the New York Stem Cell Foundation. We have also used the eagle-i platform to coordinate iPS cell and ES cell ontological descriptions with input from researchers who are actively generating stem cells from patients. Here we demonstrate the features of the eagle-i platform that are relevant to the stem cell information sharing project, the ontology that allows accurate description of stem cells, and the future developments that will lay the groundwork for building the single "point-of-truth" source for stem cell information.

F-2246
TRANSPARENCY IN TRANSLATION: WHAT EVIDENCE DO WE REALLY NEED AT THE BENCH TO JUSTIFY HUMAN STUDIES?

Robert, Jason S.
School of Life Sciences and Lincoln Center for Applied Ethics, Arizona State University, Tempe, AZ, USA

Failures to translate preclinical research with non-human animals into therapeutics that work in humans are legion. For every successful attempt, there are hundreds if not thousands of failures. While the failures are expected - humans are, after all, different from non-human animals in all sorts of relevant ways - researchers and research institutions, including funders, have been seeking ways to improve translational success rates and so reduce the absolute number and the relative proportion of failures. This presentation focuses on the dynamic relationship between preclinical success and clinical failure, with a special focus on model animals and animal models in stem cell research. I assess epistemological and methodological dimensions of stem cell research with non-human animals, and evaluate the ethical and other non-scientific values at stake in debates about how to maximize knowledge translation from preclinical to clinical contexts. In particular, I explore how to justify an expansion in preclinical stem cell research with non-human animals and in in vitro human systems, so as to generate a more adequate evidence base for translation to human patients. I argue that genuine transparency about the translational gaps between bench and bedside is morally required as a condition of the success of translational stem cell research.

F-2247
NEW HEALTH REGULATION TO TRANSPLANT STEM CELLS IN PATIENTS IN MEXICO: OUR EXPERIENCE IN 137 CASES.

Tagle, Jorge Mario
Director, Institutdo De Terapia Celular, Tijuana, Mexico

The presentation's objective is to illustrate the different ways in which our national health legislation here in Mexico, which is overseen by COFEPRIS (Mexican FDA), has modified throughout the past 5 years its criteria for both the authorization of stem cell transplantation, as well as for the authorization of stem cell handling/cultivation by Banks for Organs, Tissues, and/or Cells. -We will illustrate how these changing regulations of the past 5 years have shaped our experiences in treating 137 different patients with stem cells drawn from various sources such as peripheral blood, umbilical cord, bone marrow, and adipose tissue (fat). -We will also discuss these regulations' effect in the expansion from solely autologous stem cell transplants to the allogeneic stem cell transplants allowed currently under the law. -Finally, we will describe changes in the regulations for exportation and importation of stem cells to and from countries which have a health regulation collaboration agreement with Mexico. Our presentation includes a chronological study of the benefits that medical science in our country has reaped thanks to the support of federal legislators and their commitment to not delay or be an obstacle to the development of this important, growing, and promising field that is cell therapy/regenerative medicine. We feel that a description of these regulations, which have been effective for the control and the sanitary regulation of stem cells in Mexico, could in the future serve as a basis or starting framework for other countries, once these respective countries authorize stem cell treatments in humans.

NEURAL CELLS

F-3001
HUMAN ES CELL-BASED MODELING OF PEDIATRIC GLIOMAS BY K27M MUTATION IN THE H3.3 HISTONE VARIANT

Funato, Kosuke[1], Major, Tamara[1], Lewis, Peter W.[2], Allis, C David[3], Tabar, Viviane[1]
[1]Memorial Sloan-Kettering Cancer Center, New York, NY, USA, [2]University of Wisconsin, Madison, WI, USA, [3]Rockefeller University, New York, NY, USA

Human pluripotent stem cells (hPSCs) have been the subject of intense study in the past decade, raising hopes for translational applications, disease modeling and greater understanding of human development. Here we explore a novel aspect of hPSCs, namely the ability to model human tumors *in vitro* and *in vivo*. Diffuse intrinsic pontine gliomas (DIPG) are an aggressive and highly fatal form of pediatric brain tumors that arise in the brainstem of young children. Recent data suggest that a single amino acid substitution mutation in the H3.3 histone variant, known as K27M, occurs at high frequency in DIPGs, along with mutations in the *PDGFRA* and *TP53* genes. Here we differentiate human ES cells (H9) into neural precursors (NPC) using the dual Smad inhibition protocol. Lentiviral constructs encoding for a constitutively active form of PDGFRA, shRNA for p53 and K27M were introduced into the NPCs singly or in combinations, and maintained under selection. Our data show that expression of K27M was sufficient to induce some increased proliferation, compared to mock or WT H3.3 expression. The oncogene combination of PDGFRA and sh-p53 also resulted in increased proliferation, but the combination of all 3 factors resulted in a substantial increase in the Ki-67 index (32.4 ± 1.2 % *vs* 15.2 ± 3.6 % in control NPCs, $p<0.001$). Under low density culture conditions, cells in all groups had poor survival and expansion, but cells expressing the 3-factor combination exhibited robust growth (>5-fold, $p<0.04$). Additional *in vitro* testing demonstrates an increase in migration and matrigel invasion by the PDGFRA/sh-p53/K27M group, compared to the rest (>1.9-fold, $p<0.007$). These data provided convincing evidence of transformation *in vitro*. *In vivo* evidence was obtained by injecting the different cell groups into the brainstem of NOD-SCID mice. This led to the emergence of infiltrative tumors of human origin in the pons and the surrounding subarachnoid cisterns that resemble human DIPGs (in 58% of animals receiving cells with all 3 factors *vs* 11% for the combination of PDGFRA, sh-p53 and WT H3.3; mock infected cells did not lead to any tumors). Genomewide analysis indicates global changes in the chromatin landscape, and enrichment of the H3K27me3 marks at several master regulator gene sites. Furthermore, comparison with the reported data from DIPG patients shows a shared expression signature between our transformed cells and patient tumors

with K27M mutation. Overall, these data support a transformative role for the K27M mutation on neural precursors. We also demonstrate that human ES cells represent an excellent platform for the modeling of human tumors *in vitro* and *in vivo*, which could potentially lead to the elucidation of the molecular mechanisms underlying neoplastic transformation and the identification of novel therapeutic targets.

F-3002
THYMOQUINONE PROTECTS CULTURED HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONS AGAINST AMYLOID BETA-INDUCED NEUROTOXICITY.

Alhebshi, Amani[1], Odawara, Aoi[1], **Trujillo, Michael**[2], Gotoh, Masao[3], Suzuki, Ikuro[1]
[1]Graduate School of Bionics, Tokyo University of Technology, Tokyo, Japan, [2]Alpha MED Scientific, Berkeley, CA, USA, [3]Tokyo University of Technology, Tokyo, Japan

Thymoquinone (TQ) is the major active component of the medicinal plant Nigella Sativa (also known as the Black Seed) seed's oil. This natural antioxidant has recently received considerable attention for its potent protective properties and has demonstrated several neuropharmacological attributes. Using cultured human induced pluripotent stem cell (hiPSC)-derived neurons, the present study aimed to investigate whether TQ could provide protection against amyloid beta (Aβ)-induced neurotoxicity, a peptide considered to play a major role in the pathogenesis of Alzheimer's disease (AD). Cultured hiPSC-derived neurons were treated simultaneously with Aβ (1-42) and TQ for 48 h. The results showed that co-treatment with TQ efficiently attenuated Aβ-induced cell death, as evidenced by the improved cell viability using the CellTiter-Glo assay. In addition, TQ protected hiPSC-derived neurons against apoptosis induced by Aβ. Moreover, TQ ameliorated glutathione deficits, which was depleted by Aβ and inhibited the reactive oxygen species generation caused by Aβ. Using a multielectrode array (MED64), we further demonstrated that the treatment of hiPSC-derived neurons with Aβ induced a reduction in spontaneous firing activity, and the co-treatment with TQ partially reversed this loss. These results indicate that TQ holds potential for neuroprotection and may be a promising approach for the treatment of neurodegenerative disorders including AD.

F-3003
ASSESSMENT OF NEURONAL REPLACEMENT AFTER TRANSPLANTATION OF HUMAN IPS CELL-DERIVED CORTICAL PRECURSORS IN A RAT STROKE MODEL

Prieto, Daniel Tornero, Lindvall, Olle, Kokaia, Zaal
Lund University. Stem Cell Center, Lund, Sweden

Cell therapy for cortex repair after stroke is currently being explored using human induced pluripotent stem (iPS) cells. We have performed a priming step to promote differentiation of human iPS cell-derived long-term expandable neuroepithelial stem (NES) cells into mature cortical phenotype. Neuronal precursors generated in vitro from human iPS cells survived and gave rise to mature neurons two months after intracortical transplantation into injured brain, leading to bilateral recovery of impaired motor function. Pyramidal morphology and localization of cells expressing the cortex-specific marker TBR1 in a characteristic layered pattern provided further evidence supporting the cortical phenotype of the grafted cells. It is unlikely that the behavioral improvement observed already at two months was due to neuronal replacement and reconstruction of circuitry by the transplanted cells. Modulation of inflammatory processes is one possible mechanism underlying the observed early improvement. Conceivably, though, functional integration of the grafted mature neurons occurring several months later, could lead to further improvement. In line with this idea, our electrophysiological data from acute brain slices, obtained at five months after transplantation, showed that the grafted cells had acquired the properties of mature neurons and received synaptic inputs from host cells. We are now studying to what extent morphological and functional integration of grafted cells occurs in stroke-damaged brain and leads to further improvement of motor and cognitive function at later time-points. To address the contribution of neuronal activity to functional recovery, remote and reversible manipulation of electrical properties with high spatio-temporal precision would be an ideal approach. One such tool uses small molecules to primarily activate G protein-coupled receptors that in turn activate or inhibit neuronal firing. These so-called "designer receptors exclusively activated by designer drugs" (DREADD) are a family of evolved muscarinic receptors that increase or decrease neuronal activity following administration of an otherwise inert synthetic ligand, clozapine-n-oxide. When packaged into viral vectors or expressed in transgenic mouse models, these tools allow cellular activity to be controlled in a defined spatial and temporal manner. We have generated human iPS cells carrying those switches, which will be used for transplantation in the stroke model. Neuronal activity of the grafted cells will be activated or silenced remotely and the contribution of the functional integration of the new neurons to recovery will be characterized and evaluated.

F-3004
MODELING AND CHARACTERIZATION OF HUMAN MOTOR NEURON (MN) DEVELOPMENT USING A HIGH-EFFICIENCY DIFFERENTIATION METHOD FROM HUMAN PLURIPOTENT STEM CELLS

Qu, Qiuhao, Li, Dong, Louis, Kathleen R., Sun, Qinyu, Yang, Hong, Tsang, Stephanie, Peng, Sha, Cox, Charles L., Wang, Fei
University of Illinois at Urbana Champaign, Urbana, IL, USA

Efficient derivation of MNs from human pluripotent stem cells is central to the understanding of human neural development, modeling of MN disorders in vitro and development of cell-replacement therapies, but effective strategies are still limited. By applying high-efficiency induction of neural progenitor cells (NPCs), followed by tightly controlled temporal modulation of neural patterning at a previously undefined primitive neural progenitor stage and fully optimized extracellular matrix (ECM)/medium conditions for neuronal maturation, we developed a method for rapid (~20 days), efficient (~70%) and high-yield (>250%) differentiation of mature and functional MNs from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) in chemically defined adherent cultures. Specially, we utilized this new MN differentiation method to study Islet-1 (ISL1) function in human motoneuron development. RNAi and rescue experiments demonstrated that human ISL1 acts as a key regulator of human motoneuron development and functions including the formation of HB9+ positive neurons, neurotransmitters and neuromuscular junctions. Unlike the murine and *Drosophila* mutant models, in which ISL1 defines MN identity by suppressing the V2a inter neuron (V2a INs) fate, the conversion from MNs to V2a INs was not observed in our human MN development model when ISL1 was depleted, indicating distinct regulatory mechanisms in humans. Thus, our method is an invaluable tool to study and model human MN development and neurological diseases.

F-3005

UP-REGULATION OF GLIAL GENE EXPRESSION BY ALTERATION OF THE GENOMIC METHYLATION STATUS IN HUMAN FETAL NEURAL STEM CELLS

Roig-Lopez, Jose Luis[1], Rivera-Serrano, Alexandra M.[1], Landrau-GIovannetti, Solymar[1], Cirino-Escobar, Sixdaira N.[1], Alvarez, Derry[1], Pardo, Sherly[2]
[1]School of Science and Technology, Universidad Del Este, AGMUS, Carolina, PR, USA, [2]UPR-School of Medicine, Caguas, PR, USA

There are different mechanisms for the regulation of gene expression, such as genomic DNA methylation of cytosines. Studies have emonstrated that the presence of 5-methylcytosines plays an important role in regulating the gene expression of neural and glial genes, specifically genes used as lineage markers during *in vitro* differentiation. Analysis of the methylation status of the 5'-upstream region of the transcription start site (5´ URTSS) is essential for the interpretation of the role of the methylation in regulating gene expression. The objective of this work was to study the effect of the drug 5-aza-2-deoxycytidine in the methylation status within the 5'-URTSS of glial and neural genes, and correlate them with expression profiles. Primers were designed using reference genomic sequences from the NCBI database. Genomic DNA was isolated and bisulfite converted using the Ez-Methylation it. Topo-TA Cloning kit was used to clone small fragments of gDNA, transformed into competent cells and sequences were analyzed using GeneDoc. Expression profiles were done using Real-Time PCR with Syber Green Master mix and relative quantification in a 7300 System from Applied BioSystems. Our sequencing results showed changes in nucleotide base pairs of cytosines to thymidine, which tells us that we successfully achieved bisulfite conversion of the gDNA. Furthermore, we could observe differences at the CpG sites between control samples and those exposed to 5-aza-2-deoxycytidine. The changes observed in the methylation pattern correlate with the expression profiles of glial, neural and neural stem cell markers. In the presence of 5-aza-2-deoxycytidine, glial lineage related genes were up-regulated whereas neural lineage related genes were down-regulated, and neural stem/progenitor cell related genes remained fairly constant. Human fetal NSC commited mtowards a glial lineage after exposure to 5-aza-2-deoxycytidine. Their DNA de-methylation status was confirmed by bisulfite sequencing. These results will help predict changes in the commitment and differentiation of human neural precursor cells under different *in vitro* conditions.

F-3006

DROSHA-MEDIATED POSTTRANSCRIPTIONAL MODIFICATIONS REGULATE FATE COMMITMENT IN MOUSE HIPPOCAMPAL NEURAL STEM CELLS

Rolando, Chiara, Erni, Andrea, Taylor, Verdon
University of Basel, Basel, Switzerland

Self-renewing and multipotent neural stem cells (NSCs) reside in the dentate gyrus (DG) of the adult mammalian hippocampus. DG NSCs are a heterogeneous cell population fated to become granule neurons. The intrinsic mechanisms that regulate NSC maintenance and fate decisions have not been fully elucidated. The Microprocessor, a multimeric complex of the ribonuclease Drosha and the RNA binding protein DGCR8, binds and cleaves double-stranded hairpins in miRNA primary transcripts in the nucleus to release a precursor miRNA that is exported to the cytoplasm and further processed. The Microprocessor also has miRNA-independent functions, directly targeting and cleaving stem-loop hairpin structures of mRNAs and destabilizing the transcripts. We recently showed that Drosha targets the mRNAs encoding the proneural factor Neurog2, thus preventing NSC differentiation in the embryonic forebrain. We hypothesised that the Microprocessor may regulate NSC activity in the adult nervous system, therefore we deleted Drosha from DG NSCs using the Hes5::CreERT2 allele. Drosha conditional knockout (cKO) reduced proliferation and the number of Sox2+ cells in the DG. Moreover, neurogenesis from Drosha-deficient NSCs was impaired and the number of neuroblasts decreased suggesting exhaustion of the NSC pool. The remaining NSCs in Drosha cKO failed to reactivate after kainate-induced seizures suggesting that they become unresponsive to pathological stimulation. Moreover, three months after Drosha deletion, mitotic NSCs were absent and the remaining radial cells expressed the differentiated parenchymal astrocyte marker S100β. Interestingly, Drosha-deficient NSCs generated oligodendrocytes, a cell type that is normally not produced in the adult DG. In a complementary approach, we performed stereotactic injections of adenovirus GFAP::Cre virus into the adult DG of Droshalox/lox mice which increased oligodendroglial 10-fold compared to controls. Clonal analysis revealed that Drosha-deficient NSCs fail to expand and confirmed that they differentiate into oligodendrocytes in vitro. We have investigated possible targets of Drosha in the differentiation of NSCs towards oligodendrocytes by Drosha-CLIP (cross-linking and immunoprecipitation). We found that Drosha binds the mRNAs of critical oligodendrocyte and gliogenic transcription factors. Further in silico analysis of hairpin containing mRNAs revealed conserved hairpin loops in these putative Drosha targets. We confirmed the regulation of the key oligodendrocyte and gliogenic transcription factors by conditional ablation of Drosha from NSCs in vitro. Taken together our findings reveal a new miRNA-independent action of the Microprocessor in the maintenance of adult NSCs and control of oligodendrocyte differentiation.

F-3007

SPINAL CORD EPENDYMAL CELLS ARE FUNCTIONALLY HETEROGENEOUS WITH PROLIFERATING PROGENITOR CELLS AND QUIESCENT STEM CELLS

Sabelstrom, Hanna[1], Stenudd, Moa[1], Stahl, Fredrik[1], Goritz, Christian[1], Basak, Onur[2], Clevers, Hans C.[2], Brismar, Hjalmar[3], Barnabé-Heider, Fanie[4], Frisen, Jonas[1]
[1]Cell and Molecular Biology, Karolinska Institute, Stockholm, Sweden, [2]Hubrecht Institute, Utrecht, Netherlands, [3]Science for Life Laboratory, Karolinska Institute Science Park, Stockholm, Sweden, [4]Neuroscience, Karolinska Institute, Stockholm, Sweden

The adult spinal cord is a non-neurogenic region with limited regenerative capacity. Neural stem cells from the adult mouse spinal cord can be propagated *in vitro* and will promote functional recovery when transplanted into an injured spinal cord. The neural stem cell potential of the adult spinal cord is contained within the ependymal cell population, which only self-duplicate under physiological conditions. Ependymal cells are activated after injury and generate both glial scar astrocytes and remyelinating oligodendrocytes. Several studies have indicated that ependymal cells are heterogeneous in morphology and marker expression, however it has remained unknown if this heterogeneity exists on a functional level or if all spinal cord ependymal cells have stem cell properties. We have identified two functionally distinct subpopulations of ependymal cells using genetic fate mapping. One transgenic line (Glast-CreER) allowed specific recombination of a population of restricted progenitors. These ependymal cells proliferate under physiological conditions, have limited self-renewal capacity *in vitro*, but do not give rise to progeny after spinal cord injury. Another subpopulation of ependymal cells can be genetically labeled in Troy-CreER mice. These cells are quiescent under physiological conditions, but display stem cell properties with extensive self-renewal *in vitro* and give rise to both scar tissue and oligodendrocytes *in vivo* after

spinal cord injury. We conclude that ependymal cells are functionally heterogeneous and that the neural stem cell potential in the adult spinal cord is confined to a subpopulation of ependymal cells, which are completely quiescent in the intact spinal cord, but proliferate and give rise to a large number of cells both *in vitro* and in response to injury.

F-3008
UNCOVERING MOLECULAR DISEASE MECHANISMS WITH INDUCED NEURONS

Schaffer, Ashleigh[1], Eggens, Veerle[2], Caglayan, Ahmet[3], Reuter, Miriam[4], Scott, Eric[1], Coufal, Nicole[1], Silhavy, Jennifer[1], Xue, Yuanchao[1], Kayserili, Hulya[5], Rosti, Rasim Ozgur[1], Abdellateef, Mostafa[1], Weterman, Marian[2], Cantagrel, Vincent[1], Cai, Na[1], Zweier, Chistiane[2], Tuysuz, Beyhan[6], Yalcinkaya, Cengiz[6], Caksen, Huseyin[7], Fu, Xiang-Dong[1], Trotta, Christopher[8], Gabriel, Stacey[9], Reis, Andre[4], Gunel, Murat[3], Baas, Frank[2], Gleeson, Joseph[1]

[1]University of California, San Diego, La Jolla, CA, USA, [2]University of Amsterdam, Amsterdam, Netherlands, [3]Yale University, New Haven, CT, USA, [4]Institute of Human Genetics, Erlangen, Germany, [5]Istanbul University, Istanbul, Turkey, [6]Cerrahpaşa Medical School, Istanbul, Turkey, [7]Meram Medical School, Konya, Turkey, [8]PTC Therapeutics, Inc, South Plainfield, NJ, USA, [9]Broad Institute, Cambridge, MA, USA

Neurodegenerative diseases can occur so early as to affect neurodevelopment. From a cohort of over 2000 consanguineous families with childhood neurological disease, we identified a founder mutation in four independent pedigrees in *cleavage and polyadenylation factor I subunit (CLP1).* CLP1 is a multifunctional kinase implicated in tRNA, mRNA and miRNA phosphorylation-dependent maturation. Kinase activity of the human recombinant CLP1 mutant protein was defective, and a *clp1* germline zebrafish null mutant showed cerebellar neurodegeneration that was rescued by wild type but not mutant human *CLP1* expression. Patient induced neurons displayed both depletion of mature tRNAs and accumulation of unspliced pre-tRNAs. Transfection of partially processed tRNA fragments into patient cells exacerbated an oxidative stress-induced reduction in cell survival. Our data links tRNA maturation to neuronal development and neurodegeneration through defective CLP1 function in humans.

F-3009
EFFICACY, PHARMACOLOGY, AND TOXICOLOGY PROFILE OF HUMAN NEURAL STEM CELLS IN A NON-HUMAN PRIMATE MODEL OF PARKINSON'S DISEASE

Semechkin, Ruslan[1], Gonzalez, Rodolfo[1], Garitaonandia, Ibon[1], Ostrowska, Alina[1], Andrew, Crain[2], Abramihina, Tatiana[1], Wambua, Gerald[1], Poustovoitov, Maxim[1], Noskov, Alexander[1], McEntire, Caleb R.S.[3], Chu, Tiffany[1], Laurent, Louise[4], John, Elsworth[3], Snyder, Evan Y.[2], Redmond, D. Eugene[3]

[1]International Stem Cell Corporation, Carlsbad, CA, USA, [2]Sanford-Burnham Medical Research Institute, La Jolla, CA, USA, [3]Yale University School of Medicine, New Haven, CT, USA, [4]UCSD, La Jolla, CA, USA

Clinical studies have shown that cell based therapies hold great promise in the treatment of Parkinson's disease (PD). Fetal neural transplants have provided very long-term symptomatic relief (up to 20 years) in some PD patients without medication. This is a significant improvement over standard medication, where effects begin to diminish after 5 years and disease progression is not halted. Unfortunately the source of fetal tissue is limited (6-8 fetuses are needed per patient) and ethically controversial. Human pluripotent stem cells (hPSCs) offer a better alternative for cell replacement therapy because they provide an unlimited source of homogenous tissue. We have previously reported that neural stem cells (NSCs) derived from human parthenogenetic stem cells (hPSCs created from unfertilized oocytes) survive long term, successfully engraft, increase brain dopamine levels, and induce stable behavioral scores and no adverse effects in rodent and non-human primate models of PD. Here we report the interim results of a comprehensive long-term pharmacology, toxicology, and efficacy study of NSCs in MPTP-lesioned African green monkeys with moderate to severe clinical Parkinsonian symptoms. NSCs were manufactured under cGMP conditions and injected bilaterally into the striatum and substantia nigra of the primates. Behavioral changes and motor movements were evaluated against sham vehicle control based on a Parkinsonian summary score, as well as necropsy, histopathology and biodistribution to determine the safety profile of the implanted NSCs.

F-3010
IN VITRO ASSESSMENT OF NEUROTOXICITY HAZARDS OF ENVIRONMENTAL COMPOUNDS USING HUMAN IPSC-DERIVED NEURONS

Sirenko, Oksana[1], Behl, Mamta[2], Ryan, Kristen[3], Parham, Fred[3], DeLaura, Susan[4], Cromwell, Evan F.[1], Tice, Raymond[3]

[1]Molecular Devices, Sunnyvale, CA, USA, [2]NIH/NIEHS, Kelly Government Solutions, RTP, NC, USA, [3]NIH/NIEHS, RTP, NC, USA, [4]Cellular Dynamics, Madison, WI, USA

The developing brain is more vulnerable to injury caused by chemicals compared to the adult brain due to the fact that very complex processes of cell development and maturation take place within a tightly controlled time frame. However, a large number of environmental agents remain inadequately tested for developmental neurotoxicological (DNT) effects. In an effort to develop and characterize an *in vitro* model system for DNT screening, we used human iPSC-derived neurons and high content imaging methods in a neurite outgrowth assay to monitor the potential toxic effects of chemicals on neuronal development. We exposed iPSC-derived neurons to a diverse set of 80 environmental chemicals representing different classes of toxic chemicals (drugs, pesticides, heavy metals, flame retardants, aromatic hydrocarbons, etc) and their assessed effects on the development of neural networks and mitochondria integrity. Compounds were screened across a 7-point concentration range (generally 0.3 to 100 μM). Effects of chemicals on neurite outgrowth were monitored after 72h of treatment, and on mitochondria membrane potential after 60 min of treatment with calcein AM or JC10 fluorescence dyes, respectively. Automated image analysis methods were developed and optimized to characterize subtle changes in neuronal phenotypes. A number of phenotypic parameters of neurite development, such as total and average neurite outgrowth, number of branches and processes, average process length, number of viable cells, as well as the area and intensities of intact mitochondria, were measured and analyzed. For each parameter, concentration-response profiles were evaluated using logistic modeling to derive a benchmark concentration (BMC) point-of-departure value. BMC values were used for estimation of potential neurotoxicity effects independent of overt cell toxicity. Several compounds in the test-set, which selectively inhibited neurite development, include but were not limited to drugs (colchicine), pesticides (dieldrin, rotenone, carbaryl) and other environmental chemicals. Interestingly, some of these compounds also decreased mitochondrial membrane potential at 60 minutes. These data suggests that environmental chemicals have the ability to selectively affect neurodevelopmental processes in vitro, some of which may be due to early effects on mitochondrial function. This approach provides a reliable screening method that appears useful for prioritizing potential developmental neurotoxicants for *in vivo* hazard characterization and mechanistic follow-up studies.

F-3011

NEUROEPITHELIAL STEM CELLS OPEN THE BACK DOOR TO NEUROGENESIS IN DOWN SYNDROME

Sobol, Maria[1], Shahsavani, Mansoureh[2], Dyachok, Oleg[3], Dahl, Niklas[1], Falk, Anna[2]

[1]Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden, [2]Neuroscience, Karolinska Institute, Stockholm, Sweden, [3]Medical Cell Biology, Uppsala University, Uppsala, Sweden

To create a relevant in vitro system for the identification of cell characteristics and molecular pathways associated with neuropathogenesis in Down syndrome (DS). Primary fibroblasts from patients with full trisomy of chromosome 21 were reprogrammed to induced pluripotent stem cells (iPSC) followed by the generation of neuroepithelial-like stem (NES) cells and neuronal cells. Reprogramming was performed using Sendai virus mediated over-expression of Oct3/4, Sox2, c-Myc and KLF4. iPSC were characterized by immunofluorescence staining, RT-qPCR, karyotyping and embryoid body formation. NES cells were generated by introducing N2, B27, DAPT, LIF, CHIR and SB factors into iPSC cultures followed by culturing in NES specific medium containing N2, B27, FGF2 and EGF. Spontaneous neural differentiation was induced by removal of EGF and FGF2 from the NES cell culture. Flow cytometry, immunofluorescence staining and RT-qPCR were applied to confirm NES and neuronal status of cells. Functional study of neuronal cells was performed by analyzing of calcium signaling measured with the fluorescent dye fura-2. iPSCs were successfully established from primary fibroblast cultures of two patients with DS. iPSC colonies showed embryonic stem cell-like morphology and expressed characteristic stem cell markers (NANOG, Tra1-60, Tra1-81). iPSCs derived from both patients showed stable 47,XX,+21 and 47,XY,+21 karyotypes, respectively, with the ability to differentiate into all three germ layers in vitro during embryoid body formation. Two iPSCs lines at passages 12 and 17, respectively, were differentiated to NES cells by replacing iPSCs culture medium with neural differentiation culture medium for 9 days. The differentiated cells were replated and maintained in NES specific medium. CD133 was used as marker to confirm NES cell status and up to 90% of NES cell populations derived from two iPSC lines were CD133 positive during proliferation. Furthermore, NES cells have a neuroepithelial character and express Nestin, SOX2, Pax6, and other neuronal progenitor markers. Proliferative activity of DS NES cells had been studied using long-term live cell imaging and EdU staining. Migration potential of DS NES cells was analysed by tracing of NES cells during long-term live cell imaging. Preliminary analysis revealed reduced growth and migration capacities in DS NES cells when compared to controls. Spontaneous differentiation of DS and control NES had been traced up to day 60. After 10 days of spontaneous differentiation the majority of NES cells acquire neuronal cell morphology. This was accompanied by progressive down-regulation of the stem cell marker CD133. At the same time differentiated cells gained expression of postproliferative neuronal markers as wells as glia-cell markers (Tuj1, MAP2, GABA, GFAP). Differentiated cells became excitable which was verified by depolarization. Neuronal cells responded to 50mM K+ with pronounced [Ca2+]i increase due to influx of ions through the voltage-gated Ca2+ channels. Taken together differentiation analysis data show the capacity of NES cells to produce various subtypes of functional neuronal cells indicating neuronal progenitor state of derived NES cell lines. Preliminary data indicate specific phenotypes in NES cells and neurons of DS patients compared to control cells. The NES cell lines provide a unique in vitro system for the recreation of key events during early stages of neuropathogenesis of DS.

F-3012

MODIFICATION OF ANXIETY BEHAVIOR IN ADULT MICE BY TRANSPLANTATION OF INHIBITORY INTERNEURONS

Sorrells, Shawn Fletcher[1], Rubenstein, John[2], Alvarez-Buylla, Arturo[2]

[1]Neurosurgery, University of California, San Francisco, San Francisco, CA, USA, [2]University of California, San Francisco, San Francisco, CA, USA

The translation of aversive experiences into innate defensive responses occurs in a part of the limbic system called the amygdala. Changes in amygdala circuitry can also facilitate the learning of new information about dangerous environments. While this can have adaptive benefit for animals in learning to respond effectively to danger cues, it can also be incorrectly augmented in pathological circumstances giving rise to debilitating fear and anxiety, as is seen in post-traumatic stress disorder. Many of the local circuit interneurons in the amygdala are derived from a part of the embryonic telencephalon called the medial ganglionic eminence (MGE). We transplanted young inhibitory interneuron progenitors from the E13.5 MGE into the amygdala of adult mice and examined whether these animals had alterations in amygdala-dependent behaviors. We found very few behavioral effects of MGE transplantation compared with heat-attenuated dead MGE cells or naive mice that received no surgery. MGE recipients had normal nociception, locomotor activity, and learning and memory. Depending on where the cells were transplanted, however, host animals exhibited either increased or decreased anxiety (indicated by time spent in the open arms) in both the elevated plus and elevated zero mazes. These findings suggest that inhibitory interneuron transplantation is a possible strategy to modify extant host circuitry and could be further developed as a way to modify specific animal behaviors that rely on proper excitatory-inhibitory balance within certain neural circuits.

F-3013

OPTOGENETIC DISSECTION OF HUMAN EMBRYONIC STEM CELL-DERIVED DOPAMINE GRAFT FUNCTIONALITY

Steinbeck, Julius Alexander[1], Choi, Sejoon[2], Mrejeru, Ana[2], Ganat, Yosif[1], Deisseroth, Karl[3], Sulzer, David[2], Mosharov, Eugene[2], Studer, Lorenz[1]

[1]Center for Stem Cell Biology, Memorial Sloan Kettering Cancer Center, New York City, NY, USA, [2]Department of Neurology, Columbia University, New York City, NY, USA, [3]Department of Bioengineering, Stanford University/HHMI, Stanford, CA, USA

Over the last few years, a number of transplantation studies in animal models of neurological disease have shown convincing evidence of graft-induced, long-term behavioral recovery following transplantation of pluripotent stem cell (PSC)-derived neural progeny. Such promising preclinical data have prompted a major push towards human translation with clinical trials being actively planned (e.g. in Parkinson's disease, PD) or already ongoing (e.g. macular degeneration). Despite this move towards clinical translation there remains a remarkable lack of knowledge about the specific mechanisms of graft function. In order to effectively refine grafting approaches and to prevent potential side effects it is critical to define the mechanism(s) of action in a given paradigm. In Parkinson's disease (PD) for example, trophic support has been suggested as a contributing factor in models of graft-induced functional recovery while full recovery may require the integration of grafted dopamine neurons into host circuits. However, due to the lack of appropriate methods, it has to date not been possible to assess the specific roles of network integration or trophic support on host behavior and on the rescue of Parkinsonian symptoms. Optogenetics enables the functional manipulation of genetically and spatially defined circuits at unprecedented precision. The ability to control the

activity of specific neurons can link circuit activity to animal behavior in real time in freely moving animals. Despite its transformative role in neuroscience, optogenetic tools have had only limited impact in human stem cell biology so far. Some of the reasons for the slow adoption of optogenetics relate to the fact that human PSC-derived neurons initially exhibit immature functional properties and may not synapse efficiently across species boundaries in the adult brain, factors that could make them less suitable for optogenetic studies Here we show that electrophysiological and neurochemical properties of human PSC-derived mesencephalic dopamine neurons expressing halorhodopsin can be modulated by exposure to light in vitro and in vivo. We demonstrate that selective interference with dopamine graft function in successfully recovered animals rapidly and reversibly re-induces PD-like behaviors. Our study reveals that graft function is dependent on activity-induced dopamine release and that such grafts are capable of preventing the functional maladaptation of striatal host neurons after dopamine neuron depletion. The use of optogenetics in stem cell based grafting paradigms presents a general strategy to bridge the gap between transplantation, behavioral assays and histological analysis and should contribute towards establishing more mechanism-based approaches in regenerative medicine.

F-3014
PERTURBATION OF GLIOMA SELFRENEWAL VIA DISRUPTION OF POWER LAW IN CLONAL GROWTH BY PI3K INHIBITOR

Sugimori, Michiya
University of Toyama, Graduate School of Medicine and Pharmaceutical Sciences, Toyama, Japan

Glioma tumor growth can depend on a rare population of tumor initiating (TI) cell is not yet understood how TI cells contribute to tumor progression, and this is difficult to discern due to tumor heterogeneity. Here clonal analysis was used to define the growth of single glioma cells leading to a mathematical law, a power-law, describing TI cell self-renewal. Using this model we identified the PI3K inhibitor LY294002 as a potent inhibitor of the self-renewal of the heterogeneous population of TI cells. This study serves as a proof of principle that clonal analysis with power-law modeling is a valuable read-out phenomenon to identify drugs that target TI self-renewal, in combat glioma growth.

F-3015
PTP1B ACTS AS A NOVEL EFFECTOR OF THE ACTIVIN PATHWAY TO CHOOSE MESENDODERMAL OR NEURAL FATE IN EMBRYONIC STEM CELLS

Matulka, Kamil[1], Lin, Hsuan-Hwai[2], Hříbková, Hana[3], Uwanogho, Dafe[3], Dvořák, Petr[3], **Sun, Yuh-Man**[3]
[1]Masaryk University Faculty of Medicine, Brno, Czech Republic, [2]Department of Internal Medicine, National Defense Medical Center, Taipei, Taiwan, [3]Biology, Masaryk University, Brno, Czech Republic

During embryogenesis, the Activin/Nodal pathway promotes mesendodermal lineage and inhibits neural fate. The molecular mechanisms underlying this role of the Activin/Nodal pathway are not clear. In this study, we report a novel role for protein tyrosine phosphatase 1B (PTP1B) in Activin-mediated early fate decisions during ESC differentiation and show that PTP1B acts as a novel effector of the Activin pathway to determine mesendodermal or neural fate. We found that the Activin/ALK4 pathway recruits PTP1B through the binding of PTP1B to ALK4 and releases PTP1B from the endoplasmic reticulum by the cleavage of PTP1B by ALK4. Sequentially, PTP1B constitutes a non-canonical pathway of Activin signaling to suppress p-ERK1/2 signaling, which leads to the inhibition of neural specification and a promotion of the mesendodermal phenotype.

F-3016
IMPAIRED NEURONAL MATURATION ON SECKEL SYNDROME IS CAUSED BY LOSS OF SELF-ORGANIZATION AND CENTROSOME INTEGRITY DURING EARLY NEURONAL DEVELOPMENT

Suzuki, Naoya[1], Samata, Bumpei[1], Habu, Toshiyuki[2], Watanabe, Akira[1], Nakahata, Tatsutoshi[1], Takahashi, Jun[1], Saito, Megumu[1]
[1]Center for iPS Cell Research and Application, Kyoto, Japan, [2]Radiation System Biology, Radiation Biology Center, Kyoto, Japan

Seckel syndrome (SS) is a rare autosomal recessive disorder, characterized by intrauterine and postnatal growth delay, mental retardation and severe microcephaly. Due to lack of the appropriate in vitro model, roles of SS-related genes during neuronal development in human remain to be elucidated. To address this issue, here we established induced pluripotent stem cell (iPSC) lines from two SS patients. One patient (ATR-SS) inherits a homozygous mutation causing aberrant splicing in Ataxia Telangiectasia Mutated and Rad3-related (ATR) gene which maintains genome integrity at aberrant replicative sites during S phase. In another patient, no mutation was identified among the coding region of known SS-related genes (other-SS). Both SS-iPSCs showed normal ES cell-like morphology, the expression of pluripotent marker genes and the formation of three germ line layers in vivo. Karyotypes were normal in all examined iPSC clones. Interestingly, the ATR pathway was not defected in ATR-SS-iPSCs because downstream CHK1 phosphorylation was not impaired. However, in ATR-SS-iPSC derived neural progenitors, CHK1 phosphorylation was significantly impaired, indicating that functional deficit of ATR was restored during pluripotent state. Next, we differentiated SS-iPSC lines into neuronal lineages by forming self-organizing neurospheres (NSs). On day 36, control iPSC lines-derived neurospheres (cNSs) were composed almost entirely of mature neurons. On the other hand, in both SS-iPSC lines-derived neurospheres (SS-NSs), most of cell failed to commit or maturate into neurons. Transplantation of iPSC-derived neuronal progenitors into the brain of immunodeficient NOG mice revealed the defective maturation of ATR- and other-SS-derived neuronal progenitors into mature neurons in situ. To evaluate whether the aberrant neural differentiation takes place in the earlier phase, we examined day 12 NSs which were composed by immature neural progenitors. Intriguingly, although both SS-NSs showed the round shape similar to cNSs, concentric layered structure usually observed in cNSs was completely abrogated in both ATR- and other-SS-NSs. Interestingly the underlying cell biological appearance in SS-neuronal progenitors seemed distinct, because d12 ATR-SS-NSs showed G1 cell cycle arrest but are not apoptotic, while d12 other-SS-NSs showed no cell cycle arrest but are proapoptotic. Abnormalities in centrosome amplification and mitotic spindle formation were significantly increased in both SS-iPSCs-derived neural progenitors. In conclusion, our results demonstrate that, in SS patients, impaired self-organization and dysregulated mitotic events underlie as common pathophysiological mechanisms that lead to abnormal layer structure in vivo. This study highlights the unprecedented potential of SS-iPSCs as a tool for dissecting the pathogenesis of SS and the biological function of SS-related genes during brain development.

F-3017

A CELL-BASED NEURONAL MODEL DERIVED FROM MUCOPOLYSACHARIDOSIS TYPE I PATIENT FIBROBLAST CELLS FOR LEAD COMPOUND DISCOVERY

Swaroop, Manju[1], McKew, John C.[1], Zheng, Wei[2]
[1]*Therapeutics of Rare and Neglected Disease, National Center for Advancing Translational Sciences, National Institute of Health, Bethesda, MD, USA,* [2]*NIH/NCATS/DPI, Rockville, MD, USA*

Mucopolysacharidosis type 1 (MPS I), also known as Hurler syndrome, is an autosomal recessive disease caused by mutations in the gene encoding alpha-L-iduronidase (IDUA), a lysosomal hydrolase responsible for degrading glycosaminoglycans (GAGs), heperan sulfate and dermatan sulfate[1]. Deficiency of IDUA function results in defective degradation, lysosomal accumulation of heperan sulfate and dermatan sulfate, and progressive dysfunction of many tissues including the central nervous system (CNS). Although enzyme replacement therapy (ERT) can relieve the peripheral symptoms, it cannot penetrate the brain and has no effect on neuronal symptoms. Thus, a small, CNS permeable drug is needed for treatment of Hurler syndrome. In addition, a combination treatment of ERT and a small molecule drug, targeting distinct pathways, may provide a more efficacious approach for treatment of LSDs and reduce side effects by allowing lower doses of each therapeutic. We have generated MPS I iPS cells from patient fibroblasts and differentiated them to neuronal stem cells displaying disease phenotype. We have further developed and optimized a phenotypic Lysotracker staining assay using these cells. These MPS I neuronal stem cells display enlarged lysosomes due to accumulation of storage materials. Using this cell-based phenotypic disease model, we have carried out a drug-repurposing screen of 5000 known drugs and tool compounds. We have found a set of active compounds that reduce the enlarged lysosomes in the MPS I cells. Further studies with these active compounds may reveal the mechanism of action leading to identification of new drug targets. We hope that this approach will lead us to rapid drug development for new therapeutics to treat MPS 1.

F-3018

OXIDATIVE STRESS-INDUCED NEURAL STEM CELL ACTIVATION IN THE ADULT MOUSE MIDBRAIN

Sze, Christie C., Shi, Han, Edens, Brittany, Ma, Yong-Chao
Northwestern University, Chicago, IL, USA

Dopaminergic neurons, located in the ventral midbrain, play an important role in controlling movement and a range of behavioral systems including emotion and reward. Degeneration of substantia nigra dopaminergic neurons leads to Parkinson's disease, the second most common neurodegenerative disorder. Although extensive effort has been spent on differentiating dopaminergic neurons from various stem cell resources for cell replacement therapy, little is known if endogenous neural stem cells exist in the adult midbrain or if dormant neural stem cells can be activated under specific conditions. Previous studies have demonstrated activation of stem cells to proliferate for a reparative role upon tissue injury in the adult heart and lung, although this has yet to be tested in the adult midbrain. Existing evidence points to presence of progenitor cells with neurogenic potential in the adult mouse ventral midbrain, though current research has yet to illustrate whether these neural stem cells could be activated in response to oxidative stress-induced damage in the midbrain. Oxidative stress is widely considered a key contributor to motor neuron degeneration in Parkinson's disease. Therefore, we employed a toxin-induced Parkinsonian mouse model to test if oxidative stress-mediated damage of ventral midbrain could lead to endogenous neural stem cell activation and dopaminergic neuron neurogenesis. Surprisingly, we found robust generation of dopaminergic neurons in ventral midbrain in response to oxidative stress-induced injury. Using BrdU labeling, we found significantly more BrdU-incorporated neurons in the ventral midbrain of oxidative stress-inducing toxin MPTP-treated mice in comparison to that of untreated mice. Via confocal microscopy, we observed co-localized expression of BrdU together with neuronal nuclear marker NeuN and dopaminergic neuronal marker FoxA2, but not of BrdU with glial marker Glial Fibrillary Acidic Protein (GFAP). These data suggest that the BrdU+ cells are newborn dopaminergic neurons and not glia. We also found that these BrdU+ cells are not induced to partake in DNA-damage repair using the TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) assay. We are currently employing lineage-tracing approach to isolate these adult neural stem cells in the mouse midbrain. We plan to study gene expression programs and epigenetic mechanisms regulating stem cell differentiation into dopaminergic neurons in response to oxidative stress-induced injury. Together, our studies suggest that adult neural stem cells exist in the ventral midbrain and can be activated to give rise to dopaminergic neurons in response to oxidative stress-induced damage. These early findings provide implications for treating Parkinson's disease and other related neurological disorders through activating endogenous adult dopaminergic neural stem cells.

F-3019

TWO NEUROGENIC FACTORS CEND1 AND NEUROGENIN 2 DRIVE REPROGRAMMING OF MOUSE ASTROCYTES AND EMBRYONIC FIBROBLASTS TOWARDS MULTIPOTENCY AND NEUROGENESIS

Aravantinou-Fatorou, Katerina[1], Matsas, Rebecca[1], Berninger, Benedikt[2], **Thomaidou, Dimitra**[3]
[1]*Cellular and Molecular Neurobiology, Hellenic Pasteur Institute, Athens, Greece,* [2]*Adult Neurogenesis and Cellular Reprogramming, Mainz, Germany,* [3]*Hellenic Pasteur Institute, Athens, Greece*

Recent studies demonstrate that astroglia isolated from non-neurogenic brain regions has the potential to be reprogrammed into functional neurons through forced expression of factors known to instruct neurogenesis. Based on our previous studies on the potential of the neurogenic gene Cend1 in directing neural stem/precursor cells (NSCs) to exit the cell cycle and acquire a neuronal phenotype, in parallel with evidence demonstrating activation of Cend1 expression by genes of the neurogenin family, we explored the combined effect of Cend1 and Neurogenin-2 (Ngn-2) on reprogramming postnatal mouse cortical astrocytes. To this end, forced expression of either Cend1, Ngn-2 or both, resulted in an important increase of cells with elongated morphology, that had lost GFAP expression, while strongly expressed the radial glial marker GLT-1. Further characterization revealed that a subpopulation of these cells trans-differentiated towards the neuronal lineage, as they were exhibiting differentiated neuronal morphology and stably expressed βIII-tubulin and the neuronal subtype-specific markers, GABA, Glutamate and TH. Cell tracking analysis following long time live cell imaging for 1-week period indicated that, while Cend1-transduced astrocytes pass from several divisions prior to differentiating to GABAergic neurons, the majority of Ngn2+ astrocytes directly trans-differentiate to TH+ and Glutamate+ neurons without passing from a proliferative stage. Surprisingly, only in double-transduced cultures, a subpopulation of Cend1+/Ngn2+ astrocytes formed highly proliferative Glast+/Nestin+ spheres ("astrospheres") attached to the culture dish. When "astrospheres" were isolated and cultured under NSC conditions, they grew as neurospheres that, in the absence of growth factors, differentiated into neurons, astrocytes and oligodendrocytes, an observation implying that they exhibit neural stem cell-like properties. In order to investigate whether

Cend1 and Ngn2 have a broader neurogenic potential and are capable of trans-differentiating more distant in lineage cell types, we forced-expressed the two molecules in mouse embryonic fibroblasts (MEFs). Ectopic expression of Cend1, Ngn2 or both resulted in MEFs reprogramming, initially towards neural progenitor cells and, at later stages, towards neurons. Depending on which factor or combination of them was expressed the 3-dimensional structure, as well as survival, proliferation and differentiation properties of trans-differentiated cells varied, however in all cases, by 20 days following viral transduction, differentiated subtype-specific neurons forming synapses appeared in significantly high numbers above 50-60% of the total cell population. The latter finding demonstrates that common reprogramming mechanisms exist, instructing neuronal trans-differentiation of different cell types. It also highlights the existence of a group of common factors that inactivates the differentiated cell program and activates genes associated with NSCs' proliferation and differentiation state.

F-3020
A HUMAN NEURAL STEM CELL CYTOTOXICITY MODEL FOR ASSESSING NEUROTOXICANTS IN A QUANTITATIVE HIGH-THROUGHPUT SCREENING ASSAY

Tong, Zhi-Bin, Huang, Ruili, Shinn, Paul, Gerhold, David L.
Division of Preclinical Innovation, National Center for Advancing Translational Sciences (NCATS), National Institutes of Health (NIH), Rockville, MD, USA

We used human neural stem cells (hNSC) to develop a cytotoxicity model for assessing chemical sensitivities and to elucidate toxic mechanisms of action. Neural stem cells in the adult nervous system are self-renewing, pluripotent and capable of differentiating into multiple lineages such as neurons, astrocytes and oligodendrocyes. To assess potential cytotoxicity of drugs and other environmental chemicals to neural stem cells, we developed an *in vitro* assay using human neural stem cells (hNSC) derived from H9 human embryonic stem cells (hESC). By titrating the levels of 33 neurotoxic compounds, we compared cytotoxic responses of hNSC cells to the much-studied human neuroblastoma cell line SH-SY5Y cells. Cytotoxicity was assessed using the sensitive CellTiter-Glo® assay for ATP content. The studies were adapted, from 384 well plates (10000 cells/30 µl/well), to 1536-well plates (2000 cells/5 µl/well) for quantitative high-throughput screening (qHTS). For this qHTS format, 32 compounds were assessed with 11 dosages from 1.7 nM to 100 µM, in triplicate, in a single plate. Dose-response data were fitted to the Hill model in an automated process to determine AC_{50} and derive quality-of-fit metrics. The drug treatment time for 36-48 hours appeared to be both necessary and sufficient to manifest cytotoxicities. While we noted that hNSC and SH-SY5Y cells showed similar sensitivities to 14-of-33 chemical compounds, they demonstrated significant differences in response to 19-of-33 tested compounds. For example, SH-SY5Y cells were much more sensitive to rotenone and 6-hydroxydopamine (6-OHDA), whereas hNSC cells were markedly more sensitive to malachite green oxalate and cytosine arabinoside (Ara-C). In addition, the hNSC cells cultured in suspension like 'neurospheres' were much more sensitive to some compounds than hNSC cells in adherent culture. For example, AC_{50} values obtained from the suspension and adherent cultures of the hNSC cells treated with chorambucil for 36 hours were 2.15 µM and 42.86 µM, respectively. In summary, hNSC provides a qHTS model to screen drugs and environmental compounds for their cytotoxicities in a large scale. The hNSC model is also suitable to evaluate cytotoxicity quantitatively as a basis for further studies on drug mode-of-action by using multiple gene expression technology or high-content imaging analyses . (This research was supported by the Intramural Research Program of the NCATS/NIH and National Toxicology Program.)

F-3021
DIVERGENT ROUTES FOR DERIVATION OF ALTERNATIVE GLIAL CELL FATE FROM BONE MARROW STROMAL CELLS OF ADULT RATS FOR REMYELINATION

Tsui, Yat-Ping[1], Chan, Ying-Shing[2], Shum, Daisy Kwok-Yan[1]
[1]Biochemistry, The University of Hong Kong, Hong Kong, China, [2]Physiology, The University of Hong Kong, Hong Kong, China

Loss of myelin due either to traumatic injuries or congenital abnormalities impacts severely on neuronal function. Focusing on cell replacement based remyelination therapy for both CNS and PNS, we attempted to direct differentiation of bone marrow stromal cells (BMSCs, adult rats) along the oligodendrocyte and Schwann cell lineage in vitro. BMSCs acquired phenotypes of neural progenitors in non-adherent culture conditions. To foster differentiation along the oligodendroglial lineage, BMSC-derived neural progenitors (BM-NPs) were then maintained in adherent culture supplemented with 3 glial inducing factors: β-heregulin, PDGF-AA and bFGF. Oligodendrocyte precursors positive for markers - NG2, Olig2, PDGFRa and Sox10, were detected within 2 weeks. To derive Schwann cells, BM-NPs were co-cultured with purified dorsal root ganglia (DRG) neurons and simultaneously treated with the 3 glial inducing factors. Schwann cells positive for markers - p75 and S100b, were detected within 2 weeks. DRG neurons prevented the neural progenitors from committing oligodendroglial fate and promoted their differentiation along the Schwann cell lineage. To test for myelination capability, BMSC-derived OPCs (BM-OPCs) and Schwann cells (BM-Schs) were co-culture with purified DRG neurons for 2 weeks, respectively. BM-OPCs matured into oligodendrocytes and extended myelin basic protein (MBP)-positive processes along multiple axons, indicating myelination. BM-Schs lined themselves along axons and were positive for MBP, also indicating myelin formation. Our findings indicate BMSCs as a possible source of both OPCs for and Schwann cells for remyelination therapy.

F-3022
STIMULATING ENDOGENOUS STEM CELLS MAY BE A MECHANISM OF NEUROREHABILITATION BY CONSTRAINT-INDUCED MOVEMENT THERAPY IN HEMIPLEGIC CEREBRAL PALSY

Umebayashi, Daisuke[1], Mahmud, Neemat[2], Coles, Brenda[1], Sachewsky, Nadia[2], Yammine, Samantha[1], Morshead, Cindi M.[2], van der Kooy, Derek J.[1]
[1]Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada, [2]Department of Surgery, University of Toronto, Toronto, ON, Canada

Cerebral Palsy (CP) is the most common cause of movement disability in pediatrics, and hemiplegic CP is one of the most common subtypes. The most effective neurorehabilitation for this type of CP is constraint-induced movement therapy (CIMT): forced use of the disabled limb through inhibition of the unaffected limb. Although many clinical studies report CIMT improved locomotor function and upper limb function in children with hemiplegic CP, the mechanisms underlying these benefits are not clearly understood. We are testing the hypothesis that new endogenous neural precursors are generated by CIMT. Although there is a literature studying CIMT in adult animal models of stroke, there is little information on CIMT in infant animal models because it is difficult to inhibit limb movements with casts in growing animals. We developed a CIMT model by injecting onabotulinustoxin A (botox) into the 3 muscles of one early postnatal mouse forelimb

instead of using casting or taping procedures. These injections transiently immobilize the injected forelimb and force use of the other forelimb. The forelimb immobilized by botox recovered completely a few days after the injections, and this time course was controlled by adjusting the number of botox injections. We applied this CIMT model to postnatal day 8 hypoxia/unilateral ischaemia (HI) injured mice and measured the numbers of endogenous neural stem cells by using the in vitro clonal neurosphere formation assay from each hemisphere of the forebrain. The clonal neurosphere assay revealed that CIMT ipsilateral to the HI injury increased the numbers of neural stem cells bilaterally compared to HI alone. Interestingly, CIMT without HI also increased forebrain neural stem cells bilaterally, and this effect appeared earlier in time than that of CIMT with HI. Even CIMT contralateral to the HI injury (immobilization of disabled limb and forcing use of the good limb) increased the numbers of neural stem cells (especially on the uninjured side of the forebrain), although contralateral CIMT has adverse effects in adult rodents. Additionally, HI injury itself decreased the number of forebrain stem cells bilaterally compared to uninjured control mice of the same early postnatal age, unlike the increases seen after adult HI injury. Our results indicate there may be at least 2 different time-dependent types of beneficial effects produced by CIMT - in the uninjured forebrain and in the injured forebrain. These results also suggest the mechanisms of CIMT in infant animals are different from those of adult ones.

F-3023
EPIGENOMIC CONTROL OF THE EARLY NEURAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

Valensisi, Cristina[1], Maia, Malonzo[2], Pasumarthy, Kalyan Kumar[3], Lund, Riikka Johanna[4], Lähdesmäki, Harri[2], Hawkins, David[5]
[1]Medical Genetics, University of Washington, Seattle, WA, USA, [2]Department of Information and Computer Science, Aalto University, Espoo, Finland, [3]Turku Centre for Biotechnology, Turku University, Turku, Finland, [4]Turku Centre for Biotechnology, University of Turku and Abo Akademi University, Turku, Finland, [5]Genome Sciences and Medical Genetics, University of Washington, Seattle, WA, USA

Neural differentiation of human embryonic stem cells (hESCs) recapitulates in vitro neuroectodermal specification. The earliest signature of neuroectodermal specification is the formation of rosette-like structures1, referred to as neural rosettes (NRs), a transient cell population that closely recapitulates gene expression and morphological timeframe of neural tube formation in human embryos2. Therefore, NRs represent a valuable system to investigate how crucial decisions of lineage differentiation are orchestrated. NRs likely represent a neural stem cell population. NRs further differentiate in to neural progenitor cells (NPCs), a multipotent and self-renewing cell population that gives rise to all neurons and glial cells during CNS development3. The highly dynamic status of the epigenome during neural differentiation, comprising changes in DNA methylation, histone modifications and ncRNAs represents a developmentally unique balance between self-renewal of multipotent progenitors and fate commitment during lineage specification. However its regulation remains largely unknown, especially regarding the early stages4. We aim to determine how the epigenome controls this early stage of neuroetodermal specification and how different epigenetic regulators interplay in this scenario. Toward this goal, we employed a chemically defined culture condition to neuralize hESCs (H9) and isolate NRs, which were plated to give rise to NPCs. Immunostaining of NRs and NPCs have shown that our system is highly efficient in generating Nestin-PAX6-SOX2 positive NRs as well as Nestin-SOX1 positive NR-derived NPCs. Whole transcriptome profiling by strand-specific RNA-seq showed about 250 protein-coding transcripts and over 400 long non-coding (lnc) transcripts expressed exclusively in NRs. Almost 200 protein-coding and about 350 lnc transcripts were found expressed exclusively in NPCs. A significant number of these transcripts are associated with neurodevelopment and CNS functions, such as HOXA and HOXB cluster antisense RNAs and GBX2. Among the transcripts that resulted expressed in both cell types, differential expression (DE) analysis showed over four thousands protein-coding transcripts are differentially regulated between NRs and NPCs. We also determined if unique transcriptomes correlate with unique signature in DNA methylation. We mapped genome-scale, single-nucleotide resolution DNA methylation profiles in NRs and early NPCs. Our results showed over a thousand differentially methylated CpG islands (CGIs) and three times more CpGs shore regions comparing NRs versus NPCs. Correlation of methylation and expression data focused attention to transcripts regulated, at least in part, by changes in DNA methylation. Ongoing analysis is focused on differentially methylated regions (DMRs) outside of promoters and CGIs. We are currently investigating enhancer landscapes in NRs using histone tail modifications in order to shed more light on how the epigenome defines early neural progenitors and distinguishes regulatory differences between NRs and NPCs.

F-3024
ACTIVATED T CELLS HAVE DIVERGENT EFFECTS ON AUTOLOGOUS HUMAN NEURAL STEM CELLS AND OLIGODENDROCYTE PRECURSOR CELLS

Wang, Tongguang, Choi, Elliot, Medynets, Marie, Nath, Avindra
Translational Neuroscience Center, National Institute of Neurological Disorders and Stroke/National Institutes of Health, Bethesda, MD, USA

T cell infiltration and activation in the central nervous system is an important component in many neuroinflammatory diseases such as multiple sclerosis. The possible detrimental effect of activated T cells on neurons and neurogenesis has drawn intense attention. However, studying the mechanism of T cell mediated effects on neurogenesis and neuro-glial cell function in humans has been challenging due to the difficulty in obtaining autologous neural stem cells and T cells from affected individuals. To address these issues we have developed an *in vitro* neuroinflammatory model using cocultured autologous T cells and neural stem cells (NSC) directly derived from adult peripheral CD34+ cells. Directly derived NSC were generated by transfecting purified CD34+ cells from adult peripheral blood with Sendai virus containing transcription factors Sox2, Oct3/4, C-myc and Klf4, followed by culturing in NSC selective media. Immunostaining with neural cell markers indicated that the derived NSC were nestin and SOX-2 positive and OCT4 negative and capable of differentiating to functional neurons and glial cells by induction using corresponding differentiation media. When coculturing with activated autologous T cells, the derived NSC cultures showed deceased proliferation and neuronal differentiation, which was similar to primary cultured neural progenitors. Furthermore, using this model we found that contrary to the effect on neural progenitor cells, activated T cells enhanced O4+ oligodendrocyte progenitors proliferation through the release of VEGF. These results indicate that activated T cells have different effects on specific neural progenitor cells and the in vitro neuroinflammatory model using neural stem cells derived from peripheral CD34+ cells and activated autologous T cells could be useful in delineating the pathophysiology of neuroinflammatory diseases and for potential drug development targeting T cell-mediated effect on neurogenesis.

F-3025

CHARACTERIZATION OF DCX-EXPRESSING CELL POPULATIONS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Wang, Yanling, Martinez, Refugio, Grimley, Joshua, Levi, Boaz, Ku, Sherman, Menon, Vilas, Nelson, Angel, Lee, Chengkyu, Mulholland, Heather, Shapovalova, Nadiya, Krostag, Anne-Rachel, Thomsen, Elliot, Ye, Chaoyang, Thompson, Carol, Kaykas, Ajamete, Ramanathan, Sharad, Phillips, John

Allen Institute for Brain Science, Seattle, WA, USA

Recently developed methods to model human corticogenesis using human ES cells (hESC) enable the detailed cellular, molecular and functional studies of human cortical development. We have developed a monolayer-based, highly efficient and reproducible cortical projection neuron differentiation method that recapitulates the time course of corticogenesis *in vitro*. To enrich and isolate the cortical neuronal populations, we generated an H1 hESC line stably expressing Citrine fused to DCX, a microtubule-associated protein essential for the migration of immature neurons, using site-specific nucleases (TALENs) and homologous recombination donors. Using our differentiation method, we show that the cellular expression of DCX-citrine is dynamic over the course of cortical differentiation; and DCX-citrine reporter faithfully recapitulates expression of the DCX endogenous protein. Moreover, we show that DCX-citrine is expressed in diverse subtypes of cortical projection neurons including both early-born lower and late-born upper layer neurons. Using Transwell and embryoid bodies (EB)-based migration assays, we show that the DCX-citrine + cells are migratory; and the migratory properties are enhanced upon the cortex-enriched environment cues. Fluorescence active cell sorting and replating experiments enable us to enrich the early born and later born cortical projection neurons for further molecular analysis. Finally, the DCX-citrine + cells are functional active, undergo synapogensis and form excitatory circuits *in vitro*. Overall our data demonstrates that H1 DCX-citrine reporter line provides a useful tool to understand the cortical development and neuronal specification.

F-3026

CHROMOSOMAL STABILITY OF DIRECT REPROGRAMMING INTO NEURAL CELLS COMPARED TO THAT OF PLURIPOTENT AND ADULT STEM CELLS IN HUMAN AND MOUSE

Weissbein, Uri[1], Ben-David, Uri[2], Benvenisty, Nissim[3]

[1]Genetics, Hebrew University of Jerusalem, Jerusalem, Israel, [2]Hebrew University Stem Cell Unit, Jerusalem, Israel, [3]Hebrew University, Jerusalem, Israel

Differentiation of mature cells such as neural cells can be achieved from either pluripotent stem cells or adult stem cells, but also by direct cellular reprogramming through trans-differentiation mediated by tissue master regulators. We have previously shown that human and mouse pluripotent stem cells acquire large chromosomal aberrations during their derivation and upon prolong culturing. Similarly, we have shown that human adult stem cells can acquire chromosomal abnormalities in culture. The chromosomal aberrations are non-random and are lineage specific. Here, we have compared the chromosomal aberrations in neural cells differentiated from pluripotent and adult stem cells to that observed in direct conversion of mature cells into neural cells. In our analysis, we have examined the chromosomal integrity of over 550 samples of neural cells, by analyzing their gene expression profile, using e-karyotyping methodology. Our analysis included 84 studies where mouse or human cells were differentiated into either neural stem cells or neurons. In neural cells derived from human pluripotent or adult stem cells, chromosomal aberrations were documented in 8% and 6% of the samples, respectively. Neural cells from mouse pluripotent or adult stem cells showed even higher incidence of chromosomal aberrations, resulting in 28% and 37% of aberrant samples, respectively. Importantly, chromosomal aberrations were documented in about half of the studies with human or mouse pluripotent stem cells; and in about third of the studies with adult stem cells. Interestingly, analyzing neural cells derived by direct reprogramming showed much lower incidence of aberrations, where only one out of 9 studies in human, and one out of 7 studies in mouse showed any chromosomal aberrations. Among the chromosomal aberrations detected in the neural cells differentiated from pluripotent cells were trisomies 8 and 11 in mouse, and 1, 12 and 17 in human. Those aberrations are known to be selected during derivation and culture adaptation of pluripotent cells. However, we also detected distinct recurrent aberrations positively selected during differentiation of neural cells, either from pluripotent or adult stem cells. These included chromosome 19 in both mouse and human cells. Amazingly, aberrations detected in the direct reprogramming samples also included mouse chromosome 11 and human chromosome 17, the signature chromosomal aberrations in pluripotent cells. Careful analysis of the direct reprogramming methodologies in these cases revealed that in addition to brain specific transcription factors, pluripotent genes were also utilized, suggesting that the cells dedifferentiated into a pluripotent stage prior to their final neural differentiation. Overall, our analysis revealed surprising high levels of genomic instability in neuronal stem cells and in the neurons differentiated from them. This instability was very pronounces in cells derived from pluripotent stem cells, but also common in adult neural stem cells and neurons derived from them. Neural cells differentiated by direct reprogramming are rather chromosomally stable, with the exception of those reprogrammed with pluripotent factors. Our analysis sheds light on the mechanism of direct reprogramming, in addition to pointing to the genetic stability and thus to the potential value of trans-differentiated cells in future regenerative medicine.

F-3027

NEURAL STEM CELL TRANSPLANTATION PROMOTES NEUROBEHAVIOURAL, NEUROPHYSIOLOGICAL AND NEUROANATOMICAL IMPROVEMENT IN A BILATERAL CONTUSION/COMPRESSION MODEL OF CERVICAL SPINAL CORD INJURY

Wilcox, Jared[1], Satkunendrarajah, Kajana[2], Zuccato, Jeffrey[1], Lip, Alyssa[1], Nassiri, Farshad[1], Derrick, Tam[1], Fehlings, Michael G.[3]

[1]Institute of Medical Science, University of Toronto, Toronto, ON, Canada, [2] University Health Network, Brampton, ON, Canada, [3]Toronto Western Hospital, Toronto, ON, Canada

Cervical spinal cord injury (SCI) presents complex personal and societal issues with few clinical interventions. Although the majority of injuries involve the cervical cord, few studies of cell transplantation have used clinically relevant models of cervical SCI, limiting translation. Given this knowledge gap, we examined the effects of neural precursor cell (NPC) transplants in a rodent model of cervical contusion-compression injury. Rats were given a validated bilateral contusion-compression injury at C6, and randomized at 14 days to receive: i) adult brain-derived (a) NPCs, ii) embryonic stem cell-derived (ES-)NPCs, iii) vehicle control, or iv) sham operation. Treated groups received growth factors and minocycline for 7 days post-transplant, and given either Cyclosporine A or FK506 until sacrifice. Neurobehavioural testing was conducted for up to 16 weeks post-injury including hindlimb locomotion and gait, forelimb strength and dexterity, allodynia and pain, electrophysiologic conduction. Long-term survival of cell grafts was observed with cells localized rostrocaudally surrounding the lesion, throughout grey matter

and motor tracts. Grafts exhibited myelin deposition, nodal structure normalization, and apposition to synaptic connectivity. White and grey matter volumes were significantly improved as early as 2 weeks post-transplant in aNPC and ES-NPC-treated animals, with tissue preservation and concomitant reduction in GFAP+/CSPG+ glial scar volumes sustained to endpoint. Forelimb function and grip strength improved nearly two-fold following NPC engraftment compared to vehicle controls, and cervical neurophysiology also improved as measured by in vivo electrophysiology and axonal tract tracing. Fine motor control improvements were also observed by CatWalk gait analysis following NPC transplantation, including swing speed, duty cycle, and print width, length and max area at up to 16 weeks post-injury. Treatment-related allodynia, hyperalgesia and pain responses were not observed. These data indicate transplantation of NPCs in the bilaterally injured cervical spinal cord results in significantly improved spinal tissue and forelimb function, warranting further work with preclinical cervical models to enable translation to the clinic.

F-3028
AN RNA-SEQ TRANSCRIPTOME AND SPLICING DATABASE OF DIVERSE CELL CLASSES AND PROGENITORS OF THE CEREBRAL CORTEX

Wu, Jiaqian[1], Zhang, Ye[2], Chen, Kenian[1], Sloan, Steven[2], Bennett, Mariko[2], Scholze, Anja[2], O'Keeffe, Sean[3], Phatnani, Hemali[3], Guarnieri, Paolo[3], Caneda, Christine[2], Ruderisch, Nadine[4], Deng, Shuyun[1], Liddelow, Shane[2], Zhang, Chaolin[3], Daneman, Richard[4], Maniatis, Tom[3], Barres, Ben[2]

[1]Neurosurgery, Center for Stem Cell and Regenerative Medicine, The University of Texas Medical School at Houston, Houston, TX, USA, [2]Stanford University, Stanford, CA, USA, [3]Columbia University, New York, NY, USA, [4]University of California San Francisco, San Francisco, CA, USA

Brain is comprised of diverse classes of cells which differ in their developmental processes, metabolism, signaling, and function. This complicates the task of characterizing gene expression and limits the utility of data obtained from tissue homogenates. In order to address this problem, we used a combination of immunopanning and fluorescence assisted cell sorting (FACS) to purify eight classes of cells (astrocytes, neurons, oligodendrocyte precursor cells, newly formed oligodendrocytes, myelinating oligodendrocytes, microglia, endothelial cells and pericytes) from mouse brain. Subsequently, we employed next generation RNA sequencing (RNA-Seq) technology and a highly sensitive alternative splicing algorithm to characterize the transcriptomes of these purified populations of cells. Bioinformatic analyses identified new cell type-enriched transcripts and alternative splicing isoforms as novel markers for cell identification and tools for genetic manipulation. Previously undetected cell type specific transcription factors, secreted ligands and membrane receptors, ion channels, cell adhesion molecules, and enzymes were found. For example, our data provides clues as to how astrocytes, unlike neurons, can dynamically regulate glycolytic flux and lactate generation. A number of these cell type enriched genes are validated by Fluidigm and in situ hybridization as novel markers. Weighted gene co-expression network analysis (WGCNA) and gene set enrichment analysis revealed gene modules that are related to cell identity. We deposited the complete dataset in an interactive web browser and database. This study provides an extremely valuable resource for the research community and will help to advance our understanding of the molecular basis of the brain functions, diseases and regeneration.

F-3029
MICROCEPHALY ASSOCIATED PROTEIN WDR62 INTERACTS WITH MEKK3 TO REGULATE JNK SIGNALING AND NEURAL PROGENITOR CELL MAINTENANCE IN THE DEVELOPING NEOCORTEX

Xu, Zhiheng

Institute of Genetics and Developmental Biology, CAS, Beijing, China

Human autosomal recessive primary microcephaly (MCPH) is a neural developmental disorder hallmarked by significantly reduced brain size and variable intellectual disability. Mutation of WD40-repeat protein 62 (WDR62) is the second major cause of MCPH and we have reported recently that WDR62 regulates the maintenance of neural progenitor cells (NPCs) during cortical development through JNK1 (Xu et al., Cell Reports 2014). However, the detailed biological function of WDR62 and the underlying mechanism by which WDR62 regulates JNK signaling are still not very clear. Here, we generate Wdr62 deficient mice which exhibit reduced brain size. We demonstrate that WDR62 controls neurogenesis through a novel protein complex including WDR62, MEKK3, MKKs and JNK1. MEKK3, WDR62 and JNK1 depletion or deficiency phenocopy each other in defects including premature differentiation of NPCs. WDR62 and MEKK3 regulates each other's stability and JNK activity synergistically. In addition, we find that the stability of WDR62 is negatively regulated by an E3 ligase through the proteasomal pathway. Our findings demonstrate that WDR62 is required for the maintenance of NPC via MEKK3 and JNK1 and disclose the molecular mechanisms underlying MCPH pathogenesis.

F-3030
MYELIN INHIBITS THE FORMATION OF PRIMITIVE NEURAL STEM CELL-DERIVED NEUROSPHERES FROM THE ADULT SPINAL CORD

Xu, Wenjun, Sachewsky, Nadia, Morshead, Cindi M.

University of Toronto, Toronto, ON, Canada

Definitive neural stem cells (NSCs) in the adult forebrain are thought to comprise a subpopulation of GFAP-positive (GFAP+) subependymal (SE) cells, which proliferate and give rise to neuroblasts that migrate to the olfactory bulb and differentiate into mature interneurons. Most recently we have demonstrated that NSCs in the adult brain are derivatives of a rare population of LIF-receptor/Oct4+ primitive NSCs (pNSCs) that are found during early development and persist in the adult brain. In vitro, LIFR+/ Oct4+ AdpNSCs and GFAP+ NSCs give rise to clonally derived, multipotent colonies of stem and progenitor cells. Herein we demonstrate that similar to the brain, the embryonic and adult spinal cord contain both definitive and primitive NSC populations. Using a number of transgenic mouse models we have shown that definitive NSCs in the spinal cord express GFAP. GFAP-thymidine kinase (TK) transgenic mice that permit the selective ablation of GFAP+ cells in the presence of gancilovir (GCV) reveal the complete loss of NSC derived colony formation (i.e. neurosphere formation) following exposure to GCV in vitro or in vivo (intraventricular infusion for 5 days). GCV exposure had no effect on the numbers of neurospheres derived from littermate controls. In regard to the LIF responsive AdpNSCs, we were unable to isolate these cells from the adult spinal cord but successfully isolated small numbers of LIF responsive NSCs from the embryonic and early postnatal spinal cord. We hypothesized that (1) this was due to the rare nature of the cells (approximately 10 per adult brain) and/or that (2) inhibitory factors were present in the adult spinal cord that inhibited pNSC colony formation. We hypothesized that myelin may be inhibitory to sphere formation. We confirmed this hypothesis by demonstrating that

pNSCs could be isolated from both adult and embryonic shiverer mice, which lacks myelin basic protein (MBP). To further investigate the effect of myelin, we performed co-culture experiments using wildtype YFP+ and adult shiverer spinal cord tissue. We found a reduction in the number of shiverer-/- derived neurospheres in the presence of mature myelin or conditioned media from control YFP+ mice. Moreover, as predicted, MBP alone inhibited neurosphere formation in a dose depended fashion. Finally, we found that the size of the neural stem cell pools (GFAP+ and LIF responsive) in the spinal cord was increased significantly in a minimal injury model. We propose that the enhanced proliferation observed following injury may be regulated by the amount of MBP in the injured cord. We conclude the adult spinal cord contains two distinct neural stem cell populations identical to what is found in the adult brain.

F-3031

DISSECTION OF GENE REGULATORY NETWORK INVOLVED IN DIRECT REPROGRAMMING OF MOUSE FIBROBLAST INTO NEURAL STEM CELL

Yaqubi, Moein[1], Mohammadnia, Abdulshakour[2], Fallahi, Hossein[3], Massumi, Mohammad[4]

[1]Nanobiomaterial and Tissue Engineering, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran, [2]Nanobiomaterial and Tissue Engineering, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran, [3]Department of Biology, Razi University, Kermanshah, Iran, [4]National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

Introduction: Direct reprogramming of fibroblast into induced neural stem (iNS) cells is a straightforward way to generate neural cells in which the generation of pluripotent cells has been bypassed. This aim has been achieved by overexpression of key transcription factors (TFs) involved in neural development. This bioinformatics study is aimed to predict some new TFs involved in neuronal direct reprogramming by scrutinizing the high throughput gene expression profile of iNS cells. Method: Microarray data were obtained from GEO database (NCBI) using GSE31598 accession number. Robust Multiple Averaging (RMA) and fold change algorithms were used for normalization and detection of differentially expressed (DE) genes, respectively. Fold changes at 3 times set as threshold for differential gene expression analyses and fold changes 2 set as threshold for TFs regulate DE genes. DAVID 6.7 was used for functional clustering of DE genes and Enrichment scores more than 1.3 assumed as significant. Network construction and visualization were conducted using Cytoscape 3.0.2. MCODE plug-in was used to find high score modules in protein-protein interaction network. Results: Data analysis from direct conversion of mouse fibroblast to iNS cell showed 2167 DE genes. From those, 1020 genes were up-regulated, whereas 1147 genes were down-regulated. Based on ChIP enrichment analysis 46 TFs were detected as regulators of 1832 out of 2167 DE genes. Based on gene regulatory network ontology, the nervous system development related term was identified with 236 DE genes and SUZ12, MTF2, POU5F1, NANOG, EZH2, TCF3, SOX2, JARID2, SMARCA4, and MYC as their main regulators. TFs includes CEBPB, STAT4, SUZ12, RAD21, TCF3, GATA3, TFCP2L1, WT1, OLIG2, EZH2, JARID2, NANOG, SALL4, SOX2, NR0B1, MYB, ZIC3, and POU5F1 are present in 5 top active modules during conversion of mouse fibroblast to iNS cells. Mitogen-activate protein kinase (MAPK) cascade was assigned 72 DE genes. TFs including SUZ12, NANOG, TCF3, POU5F1, and MTF2 (ordered by number of regulatory interactions) are main regulators of MAPK cascade. Collectively, this study shed light on the direct reprogramming of mouse fibroblast to iNS cell path and introduces new TFs, which could be applied as a map of road to check the process of neural direct reprogramming.

F-3032

IRRADIATION OF THE JUVENILE BRAIN PROVOKED A SHIFT FROM LONG-TERM POTENTIATION TO LONG-TERM DEPRESSION

Zanni, Giulia[1], Xie, Cui Cui[2], Riebe, Ilse[3], Zhu, Changlian[2], Hanse, Eric[3], Blomgren, Klas[1]

[1]Department of Women's and Children's Health, Karolinska Institute, Stockholm, Sweden, [2]Center for Brain Repair and Rehabilitation, Göteborg University, Göteborg, Sweden, [3]Department of Neuroscience and Physiology, Göteborg University, Göteborg, Sweden

Radiotherapy is common in the treatment of brain tumors in children, but often causes deleterious, late-appearing sequelae, including cognitive decline. This is thought to be caused, at least partly, by suppression of hippocampal neurogenesis. However, the changes in neuronal network properties in the dentate gyrus (DG) following irradiation are still poorly understood. We characterized the long-lasting effects of irradiation on the electrophysiological properties of the DG in adult rats after 8 Gy whole brain irradiation on postnatal day 11. Assessment of basal excitatory transmission in the medial perforant pathway (MPP) by examining the volley/EPSP ratio showed an increase of the synaptic efficacy per axon in irradiated animals compared to sham. The paired-pulse ratio at the MPP-granule cell synapses was lower in irradiated animals compared to sham, pointing to a higher release probability of neurotransmitters. Surprisingly, the induction of long-term synaptic plasticity in the DG by applying four trains of high-frequency stimulation provoked a shift from long-term potentiation to long-term depression in irradiated animals compared to sham. These data suggest that irradiation of the juvenile brain caused permanent changes in synaptic plasticity that would seem consistent with impairment of declarative learning. Although the mechanisms of these synaptic alterations need to be elucidated, these findings provide a better understanding of the effects of irradiation in the developing brain and the cognitive deficits observed in young patients who have been subjected to cranial radiotherapy.

F-3033

ASTROCYTES ACTIVATED IN TRAUMATIC BRAIN INJURIES PROMOTE ALS-LIKE DEGENERATION OF HUMAN MOTOR NEURONS

Zhou, Qiao[1], Tripathi, Pratibha[2], Sandoe, Jackson[3], De Boer, Sophie[2], Gupta, Shailesh[2], Rubin, Lee[4], Eggan, Kevin[2]

[1]Harvard University, Cambridge, MA, USA, [2]Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA, USA, [3]Harvard Univeristy, Cambridge, MA, USA, [4]Harvard University Department of Stem Cell and Regenerative Biology, Cambridge, MA, USA

Traumatic brain injury (TBI) has been proposed as an environmental factor that may contribute to the development of a range of neurodegenerative conditions including Amyotrophic Lateral Sclerosis (ALS). Astrocyte is an important cellular mediator of ALS pathogenesis. We established a coculture system of TBI-activated astrocytes and human motor neurons (hMN). Our studies showed that injury-activated wild-type astrocytes can strongly promote motoneuron degeneration with characteristic ALS features including branching and survival defects and formation of $TDP43^{+}ubiquitin^{+}$ inclusions. We used two different brain injury models including stab injury and controlled cortical impact (CCI). Adult mouse astrocytes were harvested after injury and cultured with defined serum-free medium. Genetic lineage tracing (GFAP-CreER::CAG-dtomato) confirmed that the cultured astrocytes derived from pre-existing astrocytes, and not progenitor cells. Gene profiling showed that these astrocytes have strongly elevated expression of extracelluar

matrices, pro-inflammatory factors, reactive genes, as well as factors thought to promote neuronal health and synaptogenesis such as BDNF, thrombospondins and glypican6. hMNs were derived from differentiation of human embryonic stem cells (hES) carrying an Hb9-GFP reporter and highly purified by fluorescent activated cell sorting (FACS). In addition to wild-type hMNs, we used an isogenic SOD^{A4V} hES line to produce mutant hMNs. hMNs cocultured with control non-reactive astrocytes (derived from neonatal rodent brain with standard method) exhibit robust branching and synaptogenesis. In contrast, coculturing with injury-derived astrocytes strongly diminished hMN branching and synaptogenesis. Strikingly, in 2-month cocultures with injury-activated astrocytes, a small but significant number of wild-type hMNs developed $TDP43^{+}$ and $ubiquitin^{+}$ inclusions (5.5±1.7% and 3.9±0.7% of total hMNs, respectively) that are characteristic of sporadic ALS cases. Our studies further showed that hMNs carrying the SOD^{A4V} mutation have reduced survival and greatly increased ubiquitin and SOD1 inclusions (12.9±1.7% and 18.8±3.1% of total hMNs, respectively) when cocultured with activated astrocytes. In contrast, wild-type hMNs cocultured with non-reactive astrocytes developed no inclusions. SOD^{A4V} mutant astrocytes cocultured with non-reactive astrocytes developed only a small number of ubiquitin and SOD1 inclusions (1.9±1.2% and 1.1±0.5%, respectively). To our knowledge, robust induction of proteinaceous inclusions in human models of ALS has not been reported before. These data collective indicate that injury-activated wild-type astrocytes are sufficient to induce ALS-like motoneuron degeneration. Genetic mutations synergize with the reactive state of the astrocytes and accelerate disease. This study further emphasizes the role of astrocyte as a key cellular mediator of ALS and established an in vitro model for investigating mechanisms of neurodegeneration in traumatic brain injuries.

F-3034
CELL REPLACEMENT THERAPY WITH BDNF-OVEREXPRESSING NEURAL STEM CELLS IN MOUSE MODELS OF CHOREA HUNTINGTON

Zimmermann, Tina, *Remmers, Floortje, Leschik, Julia, Lutz, Beat*
University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany

Huntington's Disease (HD) is an inherited neurodegenerative disease with fatal motoric failures and is characterized by the loss of striatal medium spiny neurons. Neuronal cell death has been linked to impaired expression and axonal transport of the neurotrophin BDNF (brain derived neurotrophic factor). Several studies aimed to add BDNF exogenously to the striatum, which was either virus-mediated or with BDNF overexpressing cell lines. In fact, neuroprotection via BDNF has been demonstrated in HD mouse models, but failed to show long-term motor behaviour improvement. HD still lacks effective therapy, but embryonic stem cell derived neural transplantation may provide an effective treatment. Recently, a mouse embryonic stem (ES) cell line overexpressing BDNF has been produced in our lab, which shows enhanced neuronal and specifically GABAergic differentiation. In the present study, the two therapeutic approaches (i.e., exogenous supply of BDNF and cell replacement therapy with ES cells) were combined. Pre-differentiated ES cells were used for grafting to assure in vivo differentiation into medium spiny neurons. Cells were sorted via magnetic cell sorting (MACS) to remove the tumorigenic potential of early progenitors, and grafted immediately into the striatum of quinolinic-acid lesioned mice. Mice transplanted with BDNF-overexpressing cells are expected to show a long-term recovery in several motor tests. Neuronal differentiation, the number of GABAergic neurons, integration into the host tissue and migration of transplanted cells are analyzed via immunhistology. In addition, BDNF has been shown to potentiate neuronal differentiation and maturation of neuroblasts derived from the subventricular zone (SVZ). Therefore, the effect of transplanted cells overexpressing BDNF on neurogenesis in the SVZ and the striatum is examined.

F-3035
LIPOPOLYSACCHARIDE-STIMULATED CORTICAL MICROGLIA ELABORATE SOLUBLE FACTORS WHICH INHIBIT IN VITRO NEUROGENESIS OF ADULT NEURAL STEM/PROGENITOR CELLS

Zusso, Morena, Mercanti, Giulia
Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy

Adult neurogenesis is the process of generating new neurons from neural stem/progenitor cells (NPCs) occurring during adult life within specialized areas of the central nervous system (CNS) (i.e., the subventricular zone of the lateral ventricle and the subgranular zone of the dentate gyrus of the hippocampus). NPCs may provide an endogenous or transplantable source of newly generated cells, with potential therapeutic utility in numerous CNS pathological conditions, particularly neurodegenerative diseases. A prerequisite for this is an appropriate sequence of NPC proliferation, differentiation, survival, and progressive maturation into fully functional and integrated neurons. Since most CNS disorders are associated with inflammatory processes, it is important to understand NPC development under inflammatory conditions. In the CNS, the inflammatory process is driven by local infiltration of immune cells as well as resident microglial cell activation. The latter respond rapidly to pathological changes in the brain by producing and releasing various pro- and anti-inflammatory cytokines, chemokines, neurotransmitters, and reactive oxygen and nitrogen species, in turn modulating the different steps of adult neurogenesis. In the present study we used lipopolysaccharide (LPS)-activated rat cortical microglia as an in vitro model of neuroinflammation to analyze the effects of their conditioned medium (CM) on proliferation and differentiation of NPCs obtained from the subventricular zone of adult mice. Microglia LPS-CM, containing increased concentrations of pro-inflammatory cytokines [e.g., interleukin (IL)-1β, IL-6 and tumor necrosis factor-α], significantly decreased proliferation and neuronal differentiation of NPCs, without affecting glial differentiation. Interestingly, microglia LPS-CM induced an abnormal NPC-derived cellular phenotype characterized by the presence of "hydrid" cells sharing the morphological and antigenic profile of both neurons and astrocytes, suggesting dysregulation of NPC fate switching. Within this context, microglia LPS-CM down-regulated transcription of the Pax6 gene, a transcription factor regulating the neural fate specification process, confirming the negative effect of activated microglia on neuronal differentiation. Conceivably, NPCs could be reprogrammed to switch their expected developmental fate by extracellular signals, such as microglia-released cytokines. Understanding the molecular mechanism(s) underlying cell fate switching of adult NPCs will be important in designing pharmacological strategies which promote stem cell therapies targeting neurodegenerative diseases with a strong inflammatory component.

F-3036

ANALYSIS OF NIEMANN PICK TYPE C1 HIPSC REVEALS A NEW CHOLESTEROL TRAFFICKING PATHWAY IN HUMAN NEURONS

Ordonez, Paulina[1], Goldstein, Lawrence S.B.[2]
[1]*Pediatrics, University of California San Diego, San Diego, CA, USA,* [2]*University of California San Diego, La Jolla, CA, USA*

Successful development of effective therapeutic interventions for neurodegenerative diseases will require a deeper understanding of mechanisms of disease initiation and progression. Niemann-Pick type C1 (NPC1) is a progressive and incurable pediatric dementia caused by mutations of the lysosomal cholesterol transporter NPC1. Although rare, NPC1 imposes an emotional and economic burden in patients, families, and society that is disproportionate to its relative infrequency. We used reprogramming technology to develop sets of NPC1 and control hIPSC lines, and we systematically generated patient-specific pure neuronal cultures using a standard differentiation protocol. We found that NPC1 neurons have disrupted mitochondrial turnover by autophagy that leads to mitochondrial depolarization and increased production of reactive oxygen species, all of which are likely to contribute to the neuronal failure observed in NPC1. Our data also raise the important and new possibility that NPC1 neurons initially survive cholesterol accumulation because they activate autophagy. We have evidence that in NPC1 mutant neurons, autophagy may function as a backup pathway that releases and distributes trapped cholesterol, albeit at lower efficiency, but sufficient to protect neuronal viability until birth and perhaps for a few additional years. Further mechanistic studies lead us to identify a potential new transporter that mediates autophagy-dependent cholesterol efflux from the late endosomal compartment. Therefore, we have characterized a novel cholesterol trafficking pathway in hIPSC-derived neurons. Furthermore, our work suggests a different drug development approach for NPC1 that focuses on rescuing the mitochondrial turnover phenotype without reducing autophagy, which may be necessary for normal viability as the backup system responsible for distributing accumulated cholesterol.

F-3037

ABVENTRICULAR STEM CELLS IN THE DEVELOPING HUMAN VENTRAL FOREBRAIN

Nicholas, Cory[1], Wang, Xiaoqun[2], Harwell, Corey[3], Chen, Jiadong[1], Rubenstein, John[4], Alvarez-Buylla, Arturo[5], Kriegstein, Arnold R.[1]
[1]*Department of Neurology, University of California San Francisco, San Francisco, CA, USA,* [2]*Institute of Biophysics, Chinese Academy of Sciences, Beijing, China,* [3]*Department of Neurobiology, Harvard University, Boston, MA, USA,* [4]*Department of Psychiatry, University of California San Francisco, San Francisco, CA, USA,* [5]*Department 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), an important subpallial birthplace of 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 as it 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 oRG and bipolar non-translocating daughter cells. Furthermore, we detected many oRG daughters that expressed ASCL1 and DLX2, indicative of differentiation toward a GABAergic 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. Thus, human MGE progenitors include a non-epithelial radial glia-like stem cell type with unique orientation and behavior that 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.

EYE OR RETINAL CELLS

F-3038

HUMAN OCULAR EPITHELIAL CELLS ENDOGENOUSLY EXPRESSED SOX2 AND OCT4 YIELD HIGH EFFICIENCY OF PLURIPOTENCY REPROGRAMMING

Qizhou, Lian
University of Hong Kong, Hong Kong, China

A variety of pluripotency reprogramming frequencies from different somatic cells has been observed, indicating that the cell origin is a critical factor. Identifying the cell types from which induced pluripotent stem cells (iPSCs) can be efficiently derived, and defining their advantages or disadvantages, is therefore important. We discovered that conjunctival tissues derived-ocular epithelial cells (OECs) endogenously express reprogramming factors OCT4 and SOX2. Reprogramming OECs yielded very high efficiency of iPSCs generation. Furthermore, OECs-generated iPSCs display excellent potential for ocular epithelial cell-type differentiation. We observed that OECs-generated iPSCs have high PAX6 expression, an essential element for ocular development. The findings in present study not only uncover OECs may serve as a novel somatic cell source for high efficiency of iPSCs generation, but also demonstrate OECs-generated iPSCs may be advantageous for ocular epithelial differentiation and cell replacement therapy. Keywords: ocular epithelial cells, endogenous OCT4 and SOX2 expression, reprogramming, pluripotency, PAX6, differentiation.

F-3039

THE FUNCTIONAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF TRANSPLANTED HUMAN PLURIPOTENT STEM CELL-DERIVED RETINAL PIGMENTED EPITHELIUM IN SUBRETINAL SPACE OF RCS RATS

Satarian, Leila[1], Kiani, Sahar[1], Daftarian, Narsis[2], Rezaei Kanavi, Mozhgan[3], Ahmadieh, Hamid[2], Baharvand, Hossein[1]
[1]*Department of Stem Cells and Developmental Biology at the Cell Science Research Center, Royan, Tehran, Iran,* [2]*Ophthalmic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran,* [3]*Ocular Tissue Engineering Research Center, Shahid Baheshti University of Medical Sciences, Tehran, Iran*

Retinal pigment epithelial cells (RPE) play critical roles in the eye. The oxidative damage and immunological insult to RPE cause severe visual impairment in elderly persons that known as age related macular degenaeration. Current therapies show only limited efficacy, although

recently RPE cells have been obtained from in vitro differentiation of human pleuripotent stem cells (hPSC) and this has created a hope for treating blinding diseases by replacement of pathological RPE with healthy tissue. Current protocols for RPE generation are long, low efficient with heterogeneous differentiated cells, therefore here we used an efficient protocol that involves the serial addition of noggin, basic fibroblast growth factor (bFGF), retinoic acid and sonic hedgehog (Shh) for the differentiation these cells to RPE in a serum- and feeder- free adherent condition. For completely cell characterization, immunocytoflourecence and Flow cytometry assessment showed that around 95% the differentiated cells expressed RPE65 and Bestrophine at 60th days after differentiation. Ultrastructure and electrophysiological properties of generated cells was the same native RPE and they had latex bead phagocytosis ability. For evaluation of changes in the visual behavior of RCS rats during two months, we transplanted 125000 cells/5µl in subretinal space of them and the results showed improvement of retinal function by multifocal electroretinography that emphasized by outer nuclear layer preservation in transplanted eyes. This short-term, simple and efficient protocol offer an opportunity to cell replacement therapies to replenish RPE in age related macular degeneration and related RPE diseases.

F-3040
IDENTIFICATION OF HUMAN TRABECULAR MESHWORK SPECIFIC DIFFERENTIATION MARKERS

Sathiyanathan, Padmapriya, Stanton, Lawrence W.
Genome Institute of Singapore, Singapore

Glaucoma as the second leading cause of blindness is a world health problem. The common subtype, primary open-angle glaucoma (POAG) is associated with increased resistance to aqueous humor outflow in the trabecular meshwork (TM). Decline in TM cells and malfunctioning existing ones are believed to contribute to the resistance. Regenerative medicine to restore functionality to the diseased tissue is widely being investigated. Previously we reported the isolation and characterization of progenitors from the TM as TM-derived mesenchymal stem cells (TM-MSC). The potential for TM-MSC to replace lost/ dysfunctional TM cells is being investigated. The lack of TM-specific markers, however, has challenged such efforts of regenerative medicine for POAG. The purpose of this study is to identify markers specific to the TM that can also be used to track TM lineage differentiation. To this end, gene expression profiling by microarray was performed on the TM tissue and TM-MSC to find TM differentiation markers. An additional comparison with the expression profiles of the corneal and scleral tissues identified markers enriched in the TM. 13 genes were found to be TM-specific with respect to cornea and sclera. Immunofluorescence of the markers shows their specificity to the TM in the anterior segment of the eye. These genes also have differential expression between the TM cells and TM-MSC as analyzed by qRT-PCR. Preliminary differentiation results also indicate their relevance as TM differentiation markers.This study reports the identification of a panel of genes that (i) ascertains TM cell type and also (ii) applicable as TM differentiation markers. They have the possibility to advance the search for a cell-based therapy for POAG.

F-3041
LIMBAL REGENERATION IS FACILITATED BY PEDF PEPTIDES

Tsao, Yeou-Ping
Mackay Memorial Hospital, Taipei, Taiwan

Damage of cornea limbus deprives cornea with source of stem cell and eventually leads to cornea epithelial failure. In this study we investigated the potential PEDF peptide to regenerate damaged limbus. Limbus damage is induced by half circumference lamellar excision to remove limbus epithelium and a layer of stroma. Eyes treated with PEDF peptide healed with limbus-like epithelium, in the absence of fibrovascular tissue and conjunctival epithelium invasion. Such regenerated limbus remained stable for more than 6 months and contained NP63 and ABCG2 containing cells. Cells harvested from regenerated limbus form colonies in culture, and express ABCG2, bmi-1, integrin alpha6 and K15. The symmetric healing pattern of cornea epithelial wound suggested that regenerated limbus supplied cells for cornea wound healing. Extensive limbal excision expending 270 degree in which PEDF peptide completely regenerated the limbus and sustained cornea epithelium after repeated wounding. These observations suggest that PEDF peptide may facilitate the regeneration of a functional limbus.

F-3042
RETINAL PIGMENTED EPITHELIAL CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS EXHIBIT CYTOKINE PROFILES SIMILAR TO OTHER IN VITRO HUMAN RPE MODELS

Yanagida, Aya, Knight, Kaitlen J., Chao, Jennifer R.
Ophthalmology, University of Washington, Seattle, WA, USA

Purpose: Age-related macular degeneration is the leading cause of blindness in elderly populations in the developing countries, and currently there are no effective long-term treatments available. In order to better understand AMD disease pathology, there has been significant interest in studying patient-specific iPSC-derived retinal pigmented epithelium (RPE) cells, which are known to play a central role in AMD. To confirm that iPSC-derived RPE cells recapitulate the cell morphology and function of native RPE, we compared them to cultures often used to study AMD, including human fetal RPE, ARPE 19 cells and human embryonic stem cell (hESC) derived RPE. Methods: iPSC- and hESC-derived RPE cells, human fetal RPE (gestational age 18-20 weeks), and ARPE-19 cells were cultured on transwell filter membranes in order to establish cell polarity and monolayers that replicate native RPE. After 4 and 8 weeks, cell cultures were assessed by light, confocal, and transmission electron microscopy (TEM). They were also assessed by transepithelial resistance (TER) measurements in order to detect the formation of tight junctional complexes. Secreted proteins in media (both apical and basal) were analyzed by multiplex protein analysis after 4 and 8 weeks in culture. Results: All cell types expressed RPE markers (CRALBP, ZO-1, and Otx2) by 4 weeks in culture. iPSC-RPE, hESC-RPE, and human fetal RPE, but not ARPE-19 cells, developed tight junctional complexes and apical microvilli, as determined by TEM. In addition, all cell lines except ARPE-19 cells exhibited TER measurements similar to that of native RPE by 8 weeks, indicating the establishment of tight junctions. In a profile of 44 secreted proteins in apical and basal media, iPSC-RPE cells were most similar to hESC-RPE and least similar to ARPE-19 cells. iPSC-RPE were similar to native RPE in a polarized (basal > apical) secretion of VEGF-A and apolipoprotein E, two important factors in AMD disease development. Conclusions: Human iPSC-derived RPE cells have similar structure and cytokine profiles compared to native RPE and are the most similar to ES cell derived RPE cells in our study. Our findings provide support for the use of patient-specific iPSC-derived RPE in studying the pathology of retinal degenerative diseases, including AMD.

F-3043

DIFFERENTIATION OF RETINAL GANGLION CELLS AND PHOTORECEPTOR PRECURSORS FROM MOUSE IPS CELLS CARRYING A MATH5/ATOH7 LINEAGE REPORTER

Yang, Xian-Jie[1], Xie, Bin-Bin[2], Zhang, Xiangmei[1], Hashimoto, Takao[1], Tien, Amy[1], Chen, Andrew[1], Ge, Jian[2]

[1]Ophthalmology, Jules Stein Eye Institute, University of California Los Angeles, Los Angeles, CA, USA, [2]State Key Laboratory of Ophthalmology, Zhong-Shan Ophthalmic Center, Sun Yat-Sen University, Guangzhou, China

The neural retina constitutes a critical part of the visual system, which provides the majority of sensory inputs in humans. Various retinal degenerative diseases can result in the permanent loss of retinal neurons, especially the light-sensing photoreceptor cells and retinal ganglion cells (RGCs), which are the projection neurons connecting the retina with the higher visual centers in the brain. In optic nerve neuropathy and glaucoma, RGCs undergo cell death leading to decline of the vision. The repair of RGC damage is particularly challenging, as both RGC specification and the subsequent axonal growth and projection require complex and precise regulation. To begin addressing the serious roadblocks in RGC production and repair using stem cell technology, we have established mouse iPS cells that are genetically marked for a transcription factor, Math5/Atoh7, which is critical for RGC fate specification. Math5-Cre knock-in mice were crossed with Rosa.YFP cre reporter mice to derive Math5-Cre(KI);Rosa.YFP MEFs. Lentiviruses expressing the Yamanaka factors under TetO control were then used to induce the MEFs into Math5-Cre; Rosa.YFP iPS cells that express pluripotent markers and are capable of generating teratomas in SCID mice. Embryoid bodies of Math5-Cre.Rosa.YFP iPSCs were formed under anterior neural induction conditions, and further differentiated under retinal neurogenic conditions. Immunocytochemistry detected neurons co-expressing various RGC markers and YFP with extensive neurites and/or axons, indicating that these neurons were derived from Math5 lineage. Consistent with previous in vivo cell lineage studies, Math5 YFP-expressing cells also gave rise to a subset of photoreceptor precursors. Furthermore, FACS analyses showed that inhibition of Notch signaling in the Math5 iPSC cultures enhanced YFP reporter positive cells, and increased the Brn3a+YFP+ cells from 8.1$\pm$0.8 to 12.5$\pm$0.9 %, and Crx+YFP+ population from 7.3$\pm$1.1 to 16.4$\pm$1.9 %. Together, these results indicate that the Math5 reporter iPS cells can be used to demarcate a sub-lineage derived from retinal progenitor cells, and to study the development and survival of RGCs and photoreceptors.

F-3044

HUMAN INDUCED RETINAL NEURONS GENERATED FROM PATIENTS' SCALP CELLS

Zhu, Jianhong[1], Zhang, Helen L.[2]

[1]National Key Lab for Medical Neurobiology, Fudan University Huanshan Hospital, Shanghai, China, [2]Department of Genetics, Harvard Medical School, Boston, MA, USA

Optic nerve injury neuritis and glaucoma are among the leading causes of incurable vision loss across the world. What is worse, neither pharmacological nor surgical interventions are significantly effective in reversing or halting the progress. Retinal ganglion cells (RGCs) are retinal projection neurons, their long axons form the optic nerve and collectively transmit visual information from the retina to cerebral cortex. Direct reprogramming of human fibroblasts into functional retinal neurons in vitro by defined factors provides an invaluable resource for regenerative medicine. However, clinical applications must consider the risk of immune rejection, thus patient-specific induced neuronal cells may serve as an ideal source for autologous cell replacement. In this study, we report a robust process for functional retinal neuronal cells from the patients' scalp fibroblasts by gene delivery of Mash1,Math5 and RAX. These induced retinal neurons are similar to human ganglion neuronal cells in morphology, surface antigens, gene expression. We performed patch-clamp recordings to evaluate the electrophysiological phenotype of the scalp-derived ganglion neurons after 30 days of initial induction. Whole-cell patch-clamp recordings confirmed that the induced ganglion neuronal cells exhibited properties of functional mature neurons. About 66% (n=10 out of 15 cells recorded) of the induced retinal cells fired mature action potentials in response to depolarizing current injection, and expressed the voltage-gated inward Na+ and outward K+ currents. After implantation into mouse eye, the scalp-derived induced ganglion neuronal cells were able to develop the voltage-gated ion channels, generated spontaneous action potentials. Our findings might provide a source of patient-specific retinal ganglion neurons for cell therapy.

MUSCLE CELLS

F-3049

MUSCLE DERIVED STEM CELL CHARACTERIZATION

Bardag-Gorce, Fawzia, Oliva, Joan, Tiger, Kumar, Wood, Andrew, Niihara, Yutaka

LA Biomed at Harbor UCLA Medical Center, Torrance, CA, USA

There is an increasing interest in the therapeutic potential of muscle derived stem cells and simultaneously an increasing challenge to isolate and identify a homogenous muscle stem cell population with great regenerative capacity. Based on the characteristic that muscle derived stem cells adhere weakly in culture condition to collagen-coated flasks, a small biopsy of rabbit skeletal muscle was performed to isolate and culture muscle derived stem cells using the preplating techniques. Every 24 hours for 6 days, the non-adherent cells were collected and reseeded for further isolation and culture. Muscle cell markers and adhesion molecules levels were analyzed and the results showed that the cells collected from day one and day two of seeding stained markedly for Vimentin suggesting that the attached cultured cells were mainly fibroblast cells that adhered faster to the culture ware surface. The cells from days three and day four of seeding stained markedly with the early myogenic marker MyoD and the satellite cell adhesion molecule markers CD34 and Vcam-1. Moreover Pax-7 transcription factor that is essential for early myogenic differentiation of satellite cells stained positive in cells from day three and day four of seeding. However cells from days five and day six of seeding stained negative for CD34 and Vcam-1 as well as for Pax-7 and stained positive for Sox-2 which suggested their pluripotency. These findings indicating that based on their weak adhesion characteristics, cells from day five and day six of seeding might be the MDSCs, a promising population of cells for muscle regeneration in response to injury and disease.

F-3050

CHARACTERIZATION OF IPSC-DERIVED MYOGENIC PROGENITORS ISOLATED FROM MOUSE MODELS OF DUCHENNE MUSCULAR DYSTROPHY

Bengtsson, Niclas E.[1], Hall, John K.[1], Lieber, André M.[2], Chamberlain, Jeffrey S.[1]

[1]Neurology, University of Washington, Seattle, WA, USA, [2]University of Washington, Seattle, WA, USA

Duchenne muscular dystrophy (DMD) affects approximately 1 in 5000 children and is characterized by progressive muscle wasting, weakness

and premature death. DMD is caused by mutations in the gene encoding dystrophin, which plays an essential role in maintaining muscle integrity by providing a structural link between the cytoskeleton and the extracellular matrix. In the absence of dystrophin, affected muscles experience repeated bouts of injury, inflammation and necrosis, resulting in reduced regenerative capacity and the replacement of muscle mass with fibrotic and adipose tissue. Both gene and cell therapies are candidate approaches to replace the defective dystrophin gene. While gene therapy has the potential to halt disease progression in targeted muscles, cell therapies could both introduce replacement genes and generate new muscle or muscle stem cells to affected tissues. Cell therapies for DMD have thus far shown moderate success due to limited ex vivo expansion potential of progenitor cells, poor survival and migration of cells following transplantation, inefficient myogenic conversion of non-myogenic cells as well as host rejection of donor cells. Most of these limitations could potentially be addressed using gene corrected induced pluripotent stem (iPS) cells derived from biopsies of affected patients. We have established and characterized several iPSC lines from control, dystrophic (mdx) and transgenic-mdx mice. Selected iPS cells expressed markers associated with pluripotency, exhibited normal karyotypes and were able to form all three germ layers during in vivo teratoma assays. To generate myogenic progenitors from iPS cells, we have investigated the use of an inducible form of the myogenic regulatory factor MyoD (MyoD-ER[T]). Upon activation of MyoD-ER[T], iPSCs are readily converted into myogenic progenitor cells that can efficiently differentiate into myocytes and fuse into myotubes in vitro. iPS cells derived from dystrophic mice and which carry different versions of dystrophin expression cassettes can engraft into mdx muscles in vivo and generate dystrophin-positive myofibers. We are currently comparing the efficiency of various myogenic cell populations resulting from MyoD-ER[T] induction, to generate functional myogenic engraftment in vivo following transplantation into mdx mouse hosts. Together, these approaches show potential for ex vivo gene therapy of DMD using dystrophin-corrected autologous cell transplantation.

F-3051
SATELLITE CELL FUNCTION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE: A ROLE FOR CD34?

Blanchet, Marie-Renee, Pagé, Mélissa, Langlois, Anick, Porlier, Alexandra, Debigare, Richard
Centre de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec, Quebec City, QC, Canada

Rationale: chronic obstructive pulmonary disease (COPD) is highly associated to cigarette smoking. This disease is characterized by chronic inflammation and irreversible airway obstruction. Beyond the severe respiratory symptoms occurring in COPD patients, the skeletal muscle atrophy which develops in these patients significantly reduces their quality of life, and increases the need for hospital stay. Recently, studies revealed that satellite cell (SC) function in COPD patients is altered, reducing the muscle's capacity to regenerate following damage and possibly contributing to muscle atrophy in this disease. SC function is tightly regulated, and some recent work revealed an important role for CD34 in SC trafficking and activation in the context of toxin-induced muscle damage. However, the importance of CD34 in muscle regeneration and SC function in the context of COPD has never been studied. Hypothesis: we postulate that CD34 plays an important role in SC activation and muscle regeneration in response to hypoxic and inflammatory stimuli (such as those found in COPD). Altered CD34 expression in COPD patients could lead to the skeletal muscle wasting observed in these patients. Methods: The effect of hypoxic (10% O2) and inflammatory (TNF-alpha) conditions on the differentiation capacity of SC cells was studied in vitro. SC differentiation was verified using CD34 (undifferentiated) and myosin heavy chain (MHC) (differentiated) expression over a 7-day differentiation assay. To assess the possible alteration of CD34 expression in SC from COPD patients, CD34 mRNA expression was verified in fresh SC cultures or biopsies obtained from healthy subjects and COPD patients (quadriceps). Results: Compared to cells exposed to normal levels of O2, there was an increase in CD34 expression on cells exposed to 10% hypoxic conditions after 7 days of differentiation, while MHC expression was completely abrogated following exposure to hypoxic conditions. When cells were exposed to TNF, CD34 expression was also increased compared to control conditions, while MHC expression did not vary. Also, a decrease in CD34 mRNA expression was observed in SC from COPD patients, when compared to healthy SC cultures. Conclusion and perspectives: This suggests that in normal subjects, hypoxic and inflammatory conditions found in COPD trigger CD34 expression and retain SC in an undifferentiated state. This may promote muscle repair of damage induced by these conditions. However, SC isolated from COPD patients demonstrate a reduction in CD34 expression, which could lead to alterations in muscle regeneration and muscle atrophy in this disease. The mechanisms by which COPD patients loose the capacity to induce CD34 expression is yet to be elucidated, but could lead to interesting avenues in the prevention or treatment of muscle atrophy in COPD.

F-3052
IN VITRO MODELING OF FACIOSCAPULOHUMERAL MUSCULAR DYSTROPHY

Block, Gregory Joel[1], Narayanan, Divya[1], Petek, Lisa[1], Rickard, Amanda[1], Miller, Daniel G.[2]
[1]Pediatrics, University of Washington, Seattle, WA, USA, [2]University of Washington, Seattle, WA, USA

Facioscapulohumeral Muscular Dystrophy (FSHD) is one of the most common progressive myopathies and is characterized by complete wasting of select skeletal muscles throughout the body. The disease is thought to be caused by inappropriate expression of a transcription factor called Double Homeobox Protein 4 (DUX4); however, DUX4 has proven very difficult to detect and measure, leading to considerable doubt that it is involved in disease pathogenesis. Using muscle precursor cells derived from patients with FSHD we show a dramatic FSHD-specific apoptotic phenotype in terminally differentiated myotubes that is entirely dependent on activation of DUX4. Measuring DUX4 levels and concomitant toxicity has allowed us to evaluate genes and pathways that regulate DUX4 expression in myogenesis, thus exposing potential targets to prevent disease progression.

F-3053
MOLECULAR REGULATION OF SATELLITE STEM CELL FATE DETERMINATION

Chang, Natasha C.[1], Rudnicki, Michael A.[2]
[1]Sprott Centre for Stem Cell Research, Ottawa Hospital Research Institute, Ottawa, ON, Canada, [2]Ottawa Hospital Research Institute, Ottawa, ON, Canada

Satellite cells, the adult stem cell of skeletal muscle, represent a heterogeneous population of stem cells and committed myogenic precursor cells. Satellite stem cells constitute approximately one tenth of the satellite cell population and are essential for skeletal muscle regeneration and maintenance of the satellite cell pool. The ability of satellite stem cells to undergo self-renewal is established via both

symmetric and asymmetric cell divisions, the latter of which generates both a satellite stem cell and a committed myogenic daughter cell. The intrinsic molecular mechanisms that underlie these cellular fate decisions, however, have remained elusive. The paired box transcription factor Pax7 is required for specification of satellite cells and controls their entry into the myogenic program. Myogenic regulatory factor Myf5 transcription is directly targeted by Pax7 and its expression indicates myogenic commitment of satellite cells. During asymmetric satellite stem cell division, Pax7 transcriptional activity is regulated through methylation by the arginine methyltransferase Carm1. Here we identify the mitogen activated protein kinase p38-gamma as a regulatory kinase of Carm1. Specific depletion of p38-gamma in satellite cells revealed a preference for asymmetric cell division and inhibition of symmetric satellite stem cell divisions. Moreover, in the absence of p38-gamma we observed enhanced Carm1/Pax7 associations. We conclude that in contrast to Carm1, which is necessary for asymmetric satellite stem cell division, p38-gamma is required for satellite stem cell self-renewal via symmetric expansion. Insight into the molecular pathways that stimulate satellite stem cell self-renewal is ultimately beneficial for the advancement of therapeutic strategies to treat muscle degeneration.

F-3054
MECHANISMS OF ALTERNATIVE POLYADENYLATION IN MUSCLE STEM CELLS

de Morrée, Antoine, Gan, Qiang, Biressi, Stefano, Rando, Thomas A.
Stanford University School of Medicine, Stanford, CA, USA

Stem cell regulation depends on tight control of key regulatory factors. Pax3 is a key regulator in developmental myogenesis and expressed in adult quiescent muscle stem cells. Its expression is tightly regulated at multiple levels including post transcriptionally by miR206. Two miR206 target sites in the 3'UTR of Pax3 allow for degradation of the transcript in quiescent and activating muscle stem cells in lower hind limb muscles. Surprisingly, a novel mechanism of post tanscriptional regulation allows muscle stem cells in diaphragm to protect Pax3 transcripts against miR206. In these cells, the Pax3 transcripts have shorter 3'UTRs that exclude the miR206 target sites, making them resistant. As a result these cells express higher levels of Pax3. We explored the mechanisms that enable diaphragm muscle stem cells to express Pax3 transcripts with shorter 3'UTRs. Pax3 has four sequential PolyAdenylation Sites (PASs) in its 3'UTR allowing the cell to cleave and polyadenylate nascent pre-mRNA transcripts at different points along the gene. We identified conserved elements flanking the PASs that regulate the cell's ability to choose a particular PAS, including binding sites for U1 small nucleolar RNA (snRNA). Changing the levels of U1 snRNA in cells affects the 3'UTR length of reporter genes bearing the Pax3 3'UTR and endogenous transcripts. Our results hint at a novel mechanism for post transcriptional control of gene expression in muscle stem cells.

F-3055
GINGIVAL MUCOSA - NEW SOURCE FOR MYOBLAST DERIVATION

Eremin, Ilya I.[1], Zorin, Vadim[2], Kopnin, Pavel B.[3], Zorina, Alla[2], Pulin, Andrey A.[1]
[1]*Burnasyan Federal Medical Biophysical Center, Moscow, Russian Federation,* [2]*Human Stem Cells Institute, Moscow, Russian Federation,* [3]*Institute of Carcinogenesis, Russian Blokhin Cancer Research Center, Moscow, Russian Federation*

Myoblasts, muscle tissue's cells, are able to divide in vitro and migrate with subsequent fusion and formation of muscle tubes. Myoblasts could be successfully used in regenerative medicine for treatment of patients with different muscle diseases. It was shown earlier that myoblasts transplantation leads to improvement of muscle tissue's contraction in patients with Duchenne muscular dystrophy. As result duration and quality of life of that patients were increased. The major source of myoblasts is skeletal muscle tissue. However, this source and method of biopsy are not optimal. Also biopsy leads to damage of muscle tissue and development of temporary disability of patient with relatively long rehabilitation period. In addition, myocytes derived from skeletal muscles are characterized in vitro by low proliferative potential especially in elderly patients and patients with muscular dystrophies. Due to this fact autologous cells transplantation in such category of patients is ineffective. Our team revealed presence of myoblasts in gingival mucosa specimens and developed new method of myoblasts obtainment from it. This source of cells is easily accessible and biopsy of mucosa is not highly invasive. Given method involves several steps: biopsy of gingival mucosa specimen 2-3 mm2 from retromolar area; obtainment of primary culture of myoblasts; immune markers analysis to confirm myogenic features of cells; cryopreservation and storage of cells (myoblasts viability after thawing is 92 ± 2%). Our research showed that culture of gingival mucosa derived myoblasts has great proliferation activity in comparison to skeletal muscle derived myoblasts. It could play critical role in clinical application of this type of cells. It was shown that gingival mucosa cells in vitro pass following steps: adhesion to the plastic; proliferation of myoblasts progenitors; formation of myoblasts, their fusion and assembling of multinuclear myotubes; formation of myofibril. Immunocytochemical analysis confirmed myogenic features of cells. Culture of cells expressed myoD1, SK-myosin, SK-actin.

F-3056
IL-1-BETA INDUCED MATRIX METALLOPROTEINASE-13 IS ACTIVATED BY A DISINTEGRIN AND METALLOPROTEASE 28 REGULATED PROLIFERATION OF HUMAN SKELETAL MUSCLE STEM CELL DERIVED OSTEOBLAST-LIKE CELLS

Hiyama, Taiki[1], Ozeki, Nobuaki[1], Yamaguchi, Hideyuki[1], Hase, Naoko[1], Kinoshita, Katsue[1], Mogi, Makio[2], Nakamura, Hiroshi[1], Nakata, Kazuhiko[1]
[1]*Endodontics, Aichi Gakuin University, Nagoya, Japan,* [2]*Medicinal Biochemistry, Aichi Gakuin University, Nagoya, Japan*

We reported previously that matrix metalloproteinase (MMP)-13 accelerates bone remodeling in oral periradicular lesions, and indicated a potentially unique role for MMP-13 in wound healing and regeneration of alveolar bone. The ADAM (a disintegrin and metalloproteinase) family is a set of multifunctional cell surface and secreted glycoproteins, of which ADAM-28 has been localized in bone and bone-like tissues. In this study, we show that interleukin (IL)-1β induces the expression of MMP-13 and ADAM-28 in homogeneous α7 integrin-positive human skeletal muscle stem cell (α7+hSMSC)-derived osteoblast-like (α7+hSMSC-OB) cells, and promotes proliferation while inhibiting apoptosis in these cells. At higher concentrations, however, IL-1β failed to induce the expression of these genes and caused an increase in apoptosis. We further employed ADAM-28 small interfering RNA (siRNA) to investigate whether IL-1β-induced MMP-13 expression is linked to this IL-1β-mediated changes in cell proliferation and apoptosis. Silencing ADAM-28 expression potently suppressed IL-1β-induced MMP-13 expression and activity, decreased cell proliferation and increased apoptosis in α7+hSMSC-OB cells. In contrast, MMP-13 siRNA had no effect on ADAM-28 expression, suggesting ADAM-28 regulates MMP-13. Exogenous MMP-13 induced α7+hSMSC-OB cell proliferation and could rescue ADAM-28 siRNA-induced apoptosis, and we found that proMMP-13 is partially cleaved into its active form

by ADAM-28 in vitro. Overall, our results suggest that IL-1β–induced MMP-13 expression and changes in cell proliferation and apoptosis in α7+hSMSC-OB cells are regulated by ADAM-28.

CARDIAC CELLS

F-3058
ROLE OF CARDIAC BIOPSY DERIVED CONDITIONED MEDIA IN MODULATING BONE MARROW DERIVED MESENCHYMAL STEM CELLS TOWARDS CARDIOMYOCYTES LIKE CELLS

Kakkar, Anupama[1], **Mohanty, Sujata**[1], B Nandy, Sushmita[2], Bhargava, Balram[3], Airan, Balram[4]

[1]Stem Cell Facility, All India Institute of Medical Sciences, New Delhi, India, [2]Department of Biomedical Sciences, Texas Tech University, El Paso, TX, USA, [3]Department of Cardiology, All India Institute of Medical Sciences, New Delhi, India, [4]Department of CTVS, All India Institute of Medical Sciences, New Delhi, India

Human mesenchymal stem cells (hMSC) are multipotent stem cells and can be isolated from different sources. They have great regenerative potential and have been used in various clinical trials because of their immune privilege, paracrine properties and absence of ethical concern. It has been shown that bone marrow-derived Mesenchymal stem cells (BM-MSCs) can differentiate into cardiomyocytes in vitro. Several methods have been used to induce the differentiation of stem cells into cardiomyocyte-like cells, which include the co-culture with neonatal rat cardiomyocytes, treatment with cardiac tissue extracts, treatment of MSCs with chemicals such as 5-azacytidine, oxytocin, DMSO etc. In case of MSC, only co-culture method gives beating cardiomyocytes. However, the differentiation efficiency using chemical inducers is very low. The use of conditioned medium (CM) from human cardiac explants (HCEs) as a cardiomyogenic inducer has yet not been looked into extensively. We initiated this study to explore the use of HCE for the differentiation of MSCs into cardiomyocytes like cells. After differentiation into cardiomyocytes like cells, cells were evaluated for cardiac related genes like Myosin Light Chain-2v (Mlc-2v), Connexin 43 and cardiac Troponin I (cTnI). The present study aimed at cardiomyogenic differentiation of hBM-MSC using HCE conditioned media and its evaluation in terms of expression of cardiac related genes. This study was initiated after IEC (Institute Ethics Committee) and Stem cell Ethics Committee clearance and samples were collected after proper informed consent from patients. The cardiac biopsy was collected from patients aged 2-10 year undergoing treatment for tetralogy of Fallot at Department of CTVS, AIIMS, New Delhi. It was processed within 4 hours of collection of sample. After antibiotic wash, tissue was chopped in pieces of size 2X2cm2. Explants were cultured in HG-DMEM/F12 in a ratio of 3:1 supplemented with 10% FBS. Their conditioned media was collected every 4th day. MSC were isolated from BM on the basis of plastic adherence property and 3rd passage cells were used for differentiation after their characterization by surface markers (CD105, CD29, CD90, CD73 and HLA I and II, CD34/45) studies using Flow cytometry. At 50-60% confluency cells were induced with 100% of pooled HCE conditioned media for 21 days with regular media change in between (Group A). BM-MSCs treated with 5-Azacytidine (6uM) for 30 days was taken as reference (Group B). After differentiation cells were characterized for cardiac specific markers Myosin Light Chain-2v (Mlc-2v), Connexin 43 and cardiac Troponin I (cTnI) by RT-PCR and Mlc-2v and cTnI by Immunofluorescence and Flow cytometry (n=3). qPCR was done for cardiac specific marker Mlc-2v. These hBM-MSCs were positive for CD105, CD90, CD29, CD73, HLA I and negative for HLAII and CD34/45. After differentiation cells in Group A and Group B were found to be positive for Mlc-2v, CA, Cx4, cTnI by RT-PCR and by Flow Cytometry showed the expression of cardiac related markers cTnI and Mlc-2v which was less as compared to cells treated with 5-Aza (reference). qPCR for one gene Mlc-2v which showed expression of 16 fold in Group B and 9.5 fold in Group A as compared to untreated BM-MSCs. These Initial studies reveal that HCE might provide a source of soluble factors which triggers the differentiation of MSCs into cardiomyocyte-like cells. Further analysis of secretome of cardiac explants is going on and will add to the pool of existing knowledge.

F-3059
TOBACCO CIGARETTES AND E-CIGARETTES DELAY CARDIAC MATURATION IN VITRO AND IN VIVO

Palpant, Nathan[1], Hofsteen, Peter[1], Reinecke, Hans[1], Pabon, Lil[1], Murry, Charles[2]

[1]University of Washington, Seattle, WA, USA, [2] University of Washington - Center for Cardiovascular Biology, Seattle, WA, USA

Maternal smoking is a risk factor for low birth weight and other developmental delays. We sought to determine the impact of extracts derived from e-cigarettes (e-cig) compared to standard tobacco cigarettes (3R4F) on heart development in vitro and in vivo. Human embryonic stem cells were used as a model for in vitro cardiac development and were differentiated using monolayer cardiac differentiation. Cells were exposed to purified nicotine or extracts from e-cig or tobacco cigarettes containing 1.7, 3.4, 6.8, and 13.7 μM nicotine, or vehicle control throughout differentiation. On day 14 control cells had an intrinsic beating rate of 39.5 ± 7.2 beats per minute (bpm), cardiomyocyte cell yield of 2.5x106 ± 4.3x105 cells, and cardiomyocyte purity of 83.6% ± 1.1% cTnT+ cells by flow cytometry. Cells exposed to purified nicotine or e-cig extracts showed no effect on intrinsic beating rate, cardiomyocyte yield, or cardiomyocyte purity under all concentrations tested. In contrast, there was no survival of cells exposed to 13.7 μM nicotine from tobacco cigarettes. Furthermore, cells exposed to 6.8 μM tobacco nicotine showed markedly reduced intrinsic beating rate (4.5 ± 0.9 bpm), but no effect on cardiomyocyte yield or purity. Cardiomyocytes exposed to 1.7, 3.4, and 6.8 μM tobacco nicotine showed significantly increased levels of α-smooth muscle actin, consistent with cardiomyocyte immaturity. To determine if these data correlate in vivo, zebrafish embryos were raised in 3.4, 6.8, and 13.7 μM e-cig, tobacco extract or control from 0-72 hours post fertilization. Tobacco-exposed fish showed significant decrease in heart rate, survival, general development and increased incidence of heart malformation in a dose dependent manner. In contrast, e-cig exposed fish closely resembled the time-matched controls. To understand molecular mechanisms underlying the impact of cigarette smoke exposure on heart development we carried out molecular profiling using quantitative PCR and immunohistochemistry at various stages of differentiation including mesoderm, cardiac progenitor, and definitive fetal cardiomyocyte. These data showed marked up-regulation of mesendoderm markers GSC and Nodal as well as decreased Wnt/β-catenin ligand expression in tobacco smoke treated samples compared to control and e-cigarette treated samples. At day 5, cardiac progenitor cells had decreased expression of cardiac transcription factors GATA4 and Nkx2.5 in both cigarette treated groups compared to control. In fetal stage cardiomyocytes, both e-cigarette and tobacco cigarette treated samples showed reduced expression of a wide range of transcription factors, calcium handling proteins, and myofilament proteins compared to control samples. These data suggest that e-cigarettes and tobacco cigarettes have a marked impact on early heart development by delaying the progression through stages of maturation. Furthermore, tobacco cigarettes showed a much more profound impact on global development compared to e-cigarettes on the basis of cytotoxicity

cardiac developmental defects in vitro and in vivo.

F-3060
CELL ORIGIN OF POSTNATAL CARDIOMYOGENESIS

Liu, Yuan-Hung[1], Lai, Ling-Ping[2], Huang, Shih-Yun[1], Lin, Yi-Shuan[1], Lin, Jiunn-Lee[2]

[1]Section of Cardiology, Cardiovascular Center, Far Eastern Memorial Hospital, New Taipei City, Taiwan, [2]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan

Recent studies report that postnatal mammalian hearts undergo cardiomyocyte refreshment; however, evidence is lacking for the cell origin of the cells involved in postnatal cardiomyogenesis. We first confirmed that myocardial infarction triggers the expression of embryonic cardiogenesis genes. We demonstrated that Nkx2.5 cardiac lineage-specific progenitor cells exist in the postnatal Nkx2.5 enhancer-eGFP transgenic mice and would differentiate into striated cardiomyocytes in vitro. We created coronary artery ligation on the Nkx2.5 enh-eGFP transgenic mice. The number of Nkx2.5 enh-eGFP cells was increasing following MI. We further confirmed that cardiac progenitor cells contributed directly to postnatal cardiomyogenesis by lineage tracing using inducible Cre system. The Nkx2.5 cardiac progenitor cells expressed markers of cardiac precursors, but not markers of mature cardiomyocytes, hematopoietic cells or fibroblasts. Transcriptomic analysis of activated Nkx2.5 enh-eGFP cells showed heart development genes up-regulated remarkably and significantly. To trace the developmental origin of the activated Nkx2.5 enh-eGFP cells, we created different lineage-Cre/Nkx2.5 enh-eGFP/ROSA26 reporter triple transgenic mice. Post-MI Nkx2.5 enh-eGFP+ cells originated from the embryonic epicardial cells, not from the pre-existing cardiomyocytes, endothelial cells, cardiac neural crest cells. Together, this study confirmed that cardiac-lineage progenitor cells contribute to postnatal mammalian cardiomyogenesis. The discovery of a cardiomyogenic cell population in the postnatal heart enables future cell therapy for cardiac regeneration.

F-3061
CELL SHEET TRANSPLANTATION TO RAT INFARCT HEART USING GENOMIC INTEGRATION-FREE HUMAN IPS CELLS GENERATED WITH EPISOMAL PLASMID VECTORS: A STRATEGY FOR TUMOR-FREE CARDIAC CELL THERAPY

Masumoto, Hidetoshi[1], Ikuno, Takeshi[2], Okita, Keisuke[3], Marui, Akira[4], Minakata, Kenji[4], Ikeda, Tadashi[4], Sakata, Ryuzo[4], Yamashita, Jun[1]

[1]Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan, [2]Center for iPS Cell Research and Application, Kyoto University, kyoto, Japan, [3]Dept. of Reprogramming Science, CiRA, Kyoto University, Kyoto, Japan, [4]Department of Cardiovascular Surgery, Kyoto University, Kyoto, Japan

Backgrounds: The potential for tumor formation is a major safety roadblock to clinical application of human induced pluripotent stem cells (hiPSCs) for cardiac regeneration. To date, there are few reports focused on potential of tumor formation after hiPSC-derived cell transplantation to animal heart, and the observation periods are insufficient to eliminate the possibility. Genomic integration-free hiPSCs are expected to bring lower tumor formation. Here we examined whether transplantation of integration-free hiPSC-derived cardiac cell sheets to infarct hearts brings functional benefits without tumor formation under long-term observation. Methods and Results: We used an integration-free hiPSC line generated with episomal plasmid vectors. We employed a monolayer culture-based hiPSC differentiation protocol to induce cardiomyocytes (CMs) together with vascular cells (endothelial cells [ECs]/ vascular mural cells [MCs]). During the serum-free differentiation culture, percentages of undifferentiated cell marker TRA-1-60+ cells among cultured cells were significantly reduced (59.6±2.5 vs 4.4±2.3 %, days 0 vs 16, $P<0.001$, n=4). We collected cells on day 16 and transferred them onto temperature-responsive culture dishes (UpCell; CellSeed, Tokyo, Japan). After 4 days of culture, we successfully collected self-pulsating cardiac cell sheets with 5.7×105±1.0 (n=5) of cells containing cTnT+-CMs (59.4±8.3%), VE-cadherin+-ECs (14.7±10.7%), PDGFRβ+-MCs (18.8±18.7%) and TRA-1-60+ undifferentiated cells (0.2±0.3%). A regular and synchronized calcium transient was observed throughout the sheet along with spontaneous beating. We performed transplantation of three-layered cell sheets to an athymic rat infarct heart followed by monthly echocardiogram to assess cardiac function and tumor formation. We found a significant and sustained improvement of systolic function of left ventricle after transplantation (fractional shortening: 23.2±4.5 vs 32.0±6.5%, $p<0.001$, n=12) (pre-treatment vs 5 months after transplantation). No tumor formation was observed during observation (6.9±1.5 months, 9 months at longest, n=12). Conclusion: Transplantation of integration-free hiPSC-derived cardiac cell sheets ameliorates cardiac dysfunction after myocardial infarction without tumor formation. Integration-free hiPSCs would provide safe and efficient strategies in cell transplantation.

F-3062
THE DEVELOPMENT OF THREE-DIMENSIONAL HUMAN VASCULARIZED BIOENGINEERED CARDIAC TISSUE FOR TRANSPLANTATION BY THE INTEGRATION OF CELL SHEET TECHNOLOGY AND SCALABLE IPS CELL CULTURE SYSTEM

Matsuura, Katsuhisa[1], Shimizu, Tatsuya[1], Komae, Hyoe[2], Yamato, Masayuki[3], Okano, Teruo[4]

[1]Institute of Advanced Biomedical Engineering and Science (TWIns), Tokyo Women's Medical University, Tokyo, Japan, [2]Department of Cardiac Surgery, Tokyo University, Tokyo, Japan, [3]Tokyo Women's Medical University, Tokyo, Japan, [4]Tokyo Women's Medical University Institute of Biomedical Engineering, Tokyo, Japan

Human iPS cell (hiPSC) research and cell sheet technology might enable us to fabricate human cardiac tissues. However the usability of bioengineered cardiac tissue for transplantation remains unclear. We previously reported that the 100mL bioreactor-based cultivation of hiPSC produced over 100 millions of cardiomyocytes and fibroblasts in a single run, and cardiac cell sheets that were fabricated using cells after the enzyme dissociation were mainly composed of cardiomyocytes and fibroblasts. The risk of tumor formation by the contamination of remaining iPS cells in cardiac cell sheets hinders the potentiality of bioengineered cardiac tissue for transplant. Recently we have developed the novel culture methods to eliminate remaining hiPSC in the process of cell sheet fabrication using the methionine free medium. Consistent with the findings that methionine free culture condition prohibited the survival and growth of hiPSC in monoculture, the expression of Nanog and Oct3/4 were significantly downregulated after the cultivation of cardiac cell sheets in that condition. It should be noted that cardiomyocytes showed the spontaneous beating even in the methionine free condition and the gene expression of several cardiac contractile proteins and extracellular matrix proteins were also not different regardless of methionine existence. Next we transplanted triple layered human cardiac cell sheets onto the subcutaneous tissue of nude rats. The cardiac grafts showed the spontaneous beating independent of the heart beats from the host animals even 6 months after the transplantation. The electrical mapping shows that the measured electric conduction flows in a non-random pattern, indicating that

there was no arrhythmia Further the electrical mapping shows that the measured electric conduction flows in a non-random pattern, indicating that there was no arrhythmia. Many of cardiomyocytes in the grafts expressed Ki67 and phospho-histone H3 until 2 weeks and around 200 μm dense cardiac tissues were fabricated at 6 months. Transplanted cardiac tissue was vascularized from host tissues and the electron microscopic analysis revealed that cardiomyocytes showed the matured sarcomeric structure and contained abundant mitochondria and sarcoplasmic reticulum, suggesting that cardiomyocyte maturation might be promoted in the host tissue. Finally we examined whether fabricated cardiac tissue grafts could be transplanted like a free flap. When cardiac grafts 2 weeks after the transplantation on the fat tissue of the inguinal portion were resected with the femoral artery and vein and re-transplanted ectopically to the neck portion of another rat by anastomosing the graft vessels with the host's jugular artery and vein, cardiac tissue were engrafted and showed the spontaneous beating. Herein bioengineered human cardiac tissue using hiPSC-derived cells migh be useful for transplantation and could be used to support cardiac contraction by transplanting onto the heart surface or even around vessels to assist circulation.

F-3063
INHIBITION OF EGF RECEPTOR DETERMINES FATE SWITCH BETWEEN CARDIAC AND NEURONAL DURING DIFFERENTIATION OF PLURIPOTENT STEM CELLS

Mehta, Ashish, Ramachandra, Chrishan J.A., Sequiera, Glen L., Sudibyo, Yuliansa, Manasi, Manasi, Shim, Winston
National Heart Centre Singapore, Singapore

Differentiation of human pluripotent stem cells (hPSCs) towards mesendodermal lineages is hampered due to the spontaneous ability of PSC's commitment towards neuroectodermal lineage. The epithelial growth factor (EGF)/epithelial growth factor receptor (EGFR) signalling cascade is a key regulator of cell proliferation, survival and differentiation. EGFR functions either as a homo- or as a hetero-dimer by combining with HER2, HER3 and HER4. While EGFR signalling is important for neural development, HER2 signalling is vital for cardiac development. Aim: We hypothesize that selective EGF-R inhibition is required for priming cardiac differentiation in hPSCs by fate switching between cardiomesodermal and neuroectodermal commitment. Methods: An EGF-R selective inhibitor AG1478 was introduced during directed embryoid body (EB)-based cardiac differentiation across 3 cell lines (hESC, normal and diseased hiPSC). Real-time PCR, western blot, immunostaining and flow cytometry was performed to ascertain the effects of EGF-R during cardiac commitment in hPSCs. Results: AG1478 treatment during cardiac differentiation resulted in an up-regulation of cardiac transcription factors; Nkx2.5 (50-fold), Gata4 (80-fold) and Mef2c (80-folds) in day 8 EBs. AG treatment also resulted in increased ErbB2 and ErbB4 protein levels with a significant reduction in EGFR expression. These protein changes in EGF family members translated into enhanced NKx2.5 and cTnT protein levels along with complete elimination of Pax6 protein. Concomitantly, cardiac subtype structural markers; MLC2v (53-folds), MLC2a (21-folds) and HCN2 (9-folds) also showed an increase in mRNA levels suggestive of an overall increase in cardiac subtypes. Immunostaining revealed that AG1478 treatment replaced Sox2+ neural precursor cell (NPCs) populations with cTnT+ cardiomyocyte (CMs) populations. AG1478 treated EBs showed earlier contractility and an overall improved contractile efficiency with enhanced cTnT expression levels as verified by flow cytometry (Treated vs. non-treated; cTnT+ cells 65.3% vs. 32.5%). Conclusions: We demonstrated that inhibition of EGF-R with AG1478 significantly enhanced cardiac commitment in hPSCs with concomitant restriction on neuronal fate by modulating β-catenin signalling. These results indicate that EGF-R is a crucial checkpoint for determining fate switching in hPSCs.

F-3064
CARDIOMYOGENIC DIFFERENTIATION WITH ELECTRO MECHANICAL STIMULI OF HUMAN ADIPOSE DERIVED MULTIPOTENT STEM CELLS ASCS ON POLYURETHANE BASED SCAFFOLDS.

Minonzio, Greta[1], Boffito, Monica[2], Corazza, Mattia[1], Mariotta, Luca[1], Mattu, Clara[2], Sartori, Susanna[2], Gola, Mauro[1], Ciardelli, Gianluca[2], Soldati, Gianni[1]
[1]Swiss Stem Cell Foundation, Gentilino, Switzerland, [2]Politecnico di Torino, Department of Mechanical and Aerospace Engineering, Torino, Italy

Introduction: The goal of this project is the differentiation of adult human adipose tissue-derived multipotent stem cells (ACSs) into cardiomyocytes for cardiac tissue engineering/regenerative medicine. For this, three innovative approaches that mimic the physiological environment of the heart have been investigated: first the use of human growth factors in a defined serum-free culture medium, second the use of a biomimetic scaffold and third the application of electrical and mechanical stimuli. We previously developed a protocol for the extraction and culturing of ASCs that fulfills the strict European regulations concerning the Advanced Therapy Medicinal Products. ASCs were obtained from liposuction aspirates, cultured and expanded using a protocol developed in our laboratory. In the presented work, ASCs were seeded on polyurethane (PUR)-based scaffolds and subjected to a mechanical stress in presence of a new cardiogenic serum-free culture medium. Differentiation has been evaluated with cardiogenic markers. Materials and Results: Scaffolds with the required structural properties were successfully produced via thermally induced phase separation. The PUR used in this study was synthesized starting from poly(ε-caprolactone) diol, 1,4-budandiisocyanate and L-lysine ethyl ester. The scaffolds were characterized by scanning electron microscopy (SEM), tensile tests, contact angle and fluid uptake tests. Durability tests were conducted to evaluate scaffold capability to withstand cyclic mechanical stress (0.4±0.2N, 1Hz). ASCs-seeded scaffolds were cultured in a stress-controlled bioreactor, in the presence of a cardiogenic serum-free culture medium. Cell viability and morphology were evaluated through Alamar Blue assay and SEM imaging. ASCs cardiogenic differentiation was studied through real-time polymerase chain reaction with standard cybergreen technique. This specific serum-free medium was able to induce cardiogenic differentiation of ASCs in normal 2D cell culture conditions. When seeded on the scaffold, the ASCs presented a high vitality also after 15 days and seemed to homogenously colonize the 3D support. When the use of the scaffold and a mechanical stimulation were combined, a further two fold increase in mRNA expression of cardiac specific genes (i.e. Nkx2.5, Mef-2C, HAND2 and MHC) compared to the same induction carried out in conventional 2D was observed. Conclusions: In conclusion our results show that the engineering strategy described here appears to be very promising for the development of protocols for the treatment of myocardial infarction. In the near future this approach is further developed by applying an electrical field to the scaffold together with the mechanical stress to further increase the maturation of ASCs-derived cardiomyocytes and we hope to test soon the performance of our in vitro generated autologous cardiomyocytes scaffolds in a human clinical trial.

F-3065

A DROSOPHILA MODEL OF LAMIN-ASSOCIATED CARDIOMYOPATHY REVEALS ALTERED REDOX HOMEOSTASIS AND SHORTENED LIFE SPAN

Nikravesh, Mastaneh[1], Kalvakuri, Sreehari[1], Ponce, Jessica M.[2], Thiemann, Dylan A.[2], Young, Grant H.[2], Wallrath, Lori[2], Bodmer, Rolf[1], Melkani, Girish[3]

[1]Development, Aging and Regeneration Program, Sanford-Burnham Institute for Medical Research, La Jolla, CA, USA, [2]Biochemistry, University of Iowa, College of Medicine, Iowa City, IA, USA, [3]Molecular Biology, SDSU Heart Institute, San Diego State University, San Diego, CA, USA

Laminopathies are a group of genetic disorders caused by mutations in the *LMNA* gene encoding A-type lamins, intermediate filaments that line the inside of the nuclear envelope. Patients with laminopathies exhibit a spectrum of phenotypes including cardiac and skeletal muscle dysfunction, dysplasia, diabetes and progeria. Among these, dilated cardiomyopathy (DCM) is a major cause of death, yet the underlying mechanisms of pathology remain unknown. We have developed a Drosophila model to functionally dissect the roles of lamins in the heart. We investigated laminopathy-associated cardiac dysfunction by expressing Drosophila lamin (LamC), possessing mutations analogous to those that cause human disease, in the heart. Heart-specific expression of wild type LamC caused no observable phenotypes. In contrast, heart-specific expression of mutant LamC caused conduction defects accompanied by either restricted or dilated cardiomyopathy, depending on the specific mutation. Expression of mutant LamC in the heart also caused nuclear envelope deformation, cytoplasmic aggregation of lamins, age-dependent altered redox homeostasis, and a shortened life span. Currently, we are investigating candidate genes and pathways that are able to modulate these pathological phenotypes, which will provide potential avenues for therapeutic intervention.

F-3066

ISOLATION AND CHARACTERIZATION OF HUMAN CARDIAC PROGENITOR LINEAGES BASED ON ISL1 EXPRESSION

Palomares, Karina[1], Vidal, Jason G.[2], Martin, Jody C.[2], Emre, Nil[2], Evans, Sylvia[1], Chen, Ju[1]

[1]University of California, San Diego, La Jolla, CA, USA, [2]BD Biosciences, La Jolla, CA, USA

Several studies have provided evidence for multipotent progenitor cell populations that have the potential to differentiate into all of the major cell types of the heart, including cardiomyocytes, smooth muscle cells, and endothelial cells. Some of these cardiac progenitor populations can be identified by expression of the LIM homeodomain transcription factor Islet1 (Isl1). Comprehensive studies on Isl1+ progenitor populations have been hindered by the lack of a methodology to purify Isl1+ cells. Here we describe a powerful in vitro system to isolate and characterize Isl1+ cardiac progenitor cells derived from human embryonic stem cells (hESCs). First, we examined Isl1 expression during a directed cardiomyocyte differentiation that temporally modulates Wnt signaling. Analysis at multiple time points during differentiation revealed a putative cardiac progenitor population, marked by Isl1 expression and absence of Troponin I. In order to isolate and characterize this Isl1+ population, we performed a large unbiased screen of 242 antibodies that recognize cell surface epitopes while also analyzing for intracellular expression of Isl1 by flow cytometry. A comparative analysis of Isl1+ versus Isl1- expressing cells has identified a potential cell-surface signature to isolate Isl1+ progenitor population from the differentiation cultures. This novel cell surface signature is being utilized to purify Isl1+ cells for additional functional characterization. Generation and isolation of multipotent Isl1+ cardiac progenitors from hESCs and subsequent directed differentiation into the major cardiac cell types represents an attractive strategy for use in regenerative medicine, disease modeling, and drug screening.

F-3067

NILOTINIB IMPROVES CARDIAC FUNCTION FOLLOWING ISCHEMIC INJURY BY INHIBITING THE PROLIFERATION AND FIBROGENIC DIFFERENTIATION OF PDGFRA+ MESENCHYMAL PROGENITORS

Paylor, Ben, Lemos, Dario, Low, Marcela, Rossi, Fabio M.V.

University of British Columbia, Vancouver, BC, Canada

Classical dogma in the heart states that in situations of injury, "cardiac fibroblasts" differentiate into mature "myofibroblasts", the principal cell population involved in deposition of collagen. A lack of robust in vivo markers for both of these populations, as well as thorough understanding of heterogeneity within the loose definitions used to identify them has hampered efforts to develop of therapies targeting cardiac fibrosis. Recent data from our lab has demonstrated that multiple tissues, including the heart, harbour a tissue-resident population of mesenchymal progenitor cells (MPCs) that are involved in the development of fibrosis. We hypothesize that pharmacological modulation of cardiac-resident mesenchymal progenitor cell (Lin-:PDGFRα+:Sca1+) proliferation and differentiation following cardiac injury represents a potential therapeutic strategy to improve cardiac function We isolated RNA from freshly sorted cardiac Sca1+ PDGFRa+ (S+P+) and Sca1- PDGFRa+ (P+) and found them to contain the predominant expression of ECM-associated genes (Col1a1, CTGF, TGFB1) when compared to other cardiac cell populations (S-P-, CD31+ and CD45+). Utilizing two models of cardiac injury (isoproterenol and LAD ligation) we observed significant expansion of S+P+ cells at D3 after injury followed by expansion of P+ cells at D7 and D14 after injury. We also observed that proliferation of both populations (assessed by EdU incorporation) co-localized with collagen deposition and CD68+ macrophage infiltration in the myocardium. EdU treatment directly before damage was used to pulse chase the fate of D3 proliferating S+P+ cells and demonstrated they were the predominant source of D7 and D14 P+ cells, indicating these arose by differentiation of S+P+ cells into P+. This was further confirmed by performing LAD ligation on collagen-GFP mice and the observation that at D3, GFP+ cells were solely S+P+ while at later time points (D14, D28) GFP+ cells were predominantly P+. LAD ligations were performed on parabiotic WT:collagen-GFP pairs and no GFP+ cells were found in fibrotic regions of the WT pair, indicating little or no contribution of circulating cells to collagen deposition in the infarcted myocardium. In a high throughput screen, we identified nilotinib, a receptor tyrosine kinase inhibitor, to have significant anti-proliferative effects on S+P+ cells in vitro, and further demonstrated nilotinib attenuated TGFB-1 induced increases in GFP expression in cultured S+P+ cells from collagen-GFP mice. In vivo treatment with nilotinib (20mg/kg/day) led to increased S+P+ and decreased P+ populations at D7 after isoproterenol-induced cardiac injury and reduced fibrosis in the myocardium in these mice. 28-day treatment with nilotinib after LAD-ligation led to preserved cardiac function (ejection fraction 29% in treated vs 19% in untreated) highlighting this pharmacological strategy as a potential therapy to treat cardiac fibrosis. We conclude that nilotinib has anti-fibrotic effects following cardiac injury through its effect on PDGFRa+ mesenchymal progenitor proliferation and differentiation.

F-3069

IMPLANTATION OF A LAMININ-511-CONJUGATED COLLAGEN SHEET REPAIRS THE DAMAGED MYOCARDIUM IN RAT BY ENHANCING STEM CELL HOMING

Sougawa, Nagako[1], Miyagawa, Shigeru[1], Fukushima, Satsuki[1], Saito, Atsuhiro[1], Harada, Akima[1], Ishikawa, Tsuyoshi[1], Sato- Nishiuchi, Ryoko[2], Sekiguchi, Kiyotoshi[2], Sawa, Yoshiki[1]

[1]Cardiovascular Surgery, Osaka University Graduate School of Medicine, Suita, Japan, [2]Laboratory of Extracellular Matrix Biochemistry, Institute for Protein Research, Osaka University, Suita, Japan

Background: Recently it has become increasingly evident that the extracellular matrix (ECM), particularly basement membrane components such as laminins (LMs), plays important roles for differentiation and self-renewal of stem cells. Therapies targeting the ECM may develop a new strategy for the treatment of heart failure. In this study, we focused on LMs, which are the main component of basement membrane, and hypothesized that these ECM may affect regeneration processes by modulating stem cell activity *in vitro* and *in vivo*. Methods and Results: Immunostaining showed that LM511 and LM221 were the main ECM components of epicardium where stem cells were abundant. *In vitro*, we examined the activities to LM511 and LM221 of MSCs and c-kit positive cardiac stem cells which express some integrins. MSCs and c-kit positive cells showed strong adhesive and proliferate activities on LM511, on the other hand they showed weak adhesive and proliferate activities on LM221 (MSCs, adhesion: 47.5-, 2.0-fold to the cells on no coating, respectively, proliferation at day5: 5.7-, 3.7- fold to at day 0, respectively; $P<0.05$, c-kit positive cells, adhesion: 30.3-, 0.5-fold to the cells on no coating, respectively, proliferation at day5: 9.0-, 3.0- fold to at day 0, respectively; $P<0.05$,). To examine the therapeutic effect of the LMs *in vivo,* we produced recombinant LMs fused with the collagen binding domain (CBD) of fibronectin to immobilize them on collagen sheets and transplanted sheets with or without CBD-LM511 and CBD-LM221 in rat acute myocardial infarction model. Four week later, the number of both PDGFR and CD90 positive cells significantly higher in CBD-LM511 group compared with CBD-LM221 group and the control (CBD-LM511: 531±322 cells/mm^2, CBD-LM221: 240±126 cells/mm^2, control : 160±97 cells/mm^2 ; $p<0.05$). Furthermore the gene expression of *Sca-1* and *c-kit* tended to be higher in CBD-LM511 group than the control and CBD-LM221 group (Sca-1, CBD-LM511: 1.38-, CBD-LM221: 0.59-fold to the control, c-kit, CBD-LM511 group: 1.86-, CBD-LM221: 1.65-fold to the control). Histological examination moreover revealed that the number of ILB4 (endothelial cell marker) positive cells were significantly higher in CBD-LM511 group compared with CBD-LM221 group (CBD-LM511: 1289±172 cells/mm^2, CBD-LM221: 925±142 cells/mm^2, control: 768±141 cells/mm^2; $p<0.05$), and some of ILB4 positive cells expressed both PDGFR and CD90, suggesting that CBD-LM511 enhance the recruitment and attachment of stem cells in the implanted site. Infarct size was smaller in the CBD-LM511 group than in the control (CBD-LM511: 24.84±6.54%, CBD-LM221: 31.0±6.3%, control: 36.18±7.3%). In addition, real time PCR analysis revealed that the gene expressions of angiogenesis factors were higher in CBD-LM511 group than the control. Conclusion: Stem cells accumulate into the injured site by the transplantation of CBD-LM511 conjugating collagen sheet and may contribute to repairs the damaged myocardium in rat.

F-3070

EARLY CARDIAC CONNEXIN ACTS AS A CALCIUM HEMICHANNEL THAT REGULATES HEART MORPHOGENESIS VIA NFATC4 ACTIVATION

Sultana, Naznin[1], Nag, Kakon[2], Akira, Kato[1], Kawakami, Atsushi[1], Kawakami, Atsushi[1], Kutsuzawa, Koichi[1], Akaike, Toshihiro[1], Hirose, Shigehisa[1], Nakamura, Nobuhiro[1]

[1]Department of Biological Sciences, Tokyo Institute of Technology, Yokohama, Japan, [2]Tokyo Institute of Technology, Yokohama, Japan

Recently we have revealed that a novel connexin, early cardiac connexin (ECx) in zebrafish, regulates heart morphogenesis by establishing nkx2.5 expression; however, the substrate for ECx was unknown. In this study through extensive physicochemical screening we have discovered that ECx is a high affinity functional hemichannel for Ca+2 that helps survival of ftk and ecx-morphant larva by recovering relevant heart defects, including nkx2.5-reappearance, and has suggested cooperativity between Ca2+ and nkx2.5 expression. Radioactive 45Ca2+ and 22Na+ further confirmed the specificity and sub-nanomolar affinity of Ca2+ in HeLa cells and isolated early heart-tube from ecx promoter-driven cardiospecific GFP-expressing transgenic fish or morphant fish from same clutch. Finally, using human iPS cells in cardiomyocyte generation protocols we revealed that Ca+2 influx can mediate nkx2.5 expression via nuclear translocation of NFATc4 without any change in transcription of GATA4, NFATc4, TBX5, and MEF2c. Collectively we concluded that ECx is a high affinity Ca2+ channel that uptakes Ca2+ into the early cardiogenic cells to establish cardiogenic program via NFATc4 activation leading to nkx2.5 expression. Ca+2 has been known important for heart function but our study revealed that proper homeostasis of Ca+2 in developing cardiomyocytes is indeed critical for heart maturation including parallel organization of myofibril and trabaculation of heart muscle by establishing proper gene expression.

F-3071

MULTI-STAGE GLOBAL DNA METHYLATION AND TRANSCRIPTIONAL PROFILING OF THE PLURIPOTENT-TO-CARDIOMYOCYTE DIFFERENTIATION PROGRAM

Tompkins, Joshua[1], Chen, Chang-Yi Vincent[2], Jung, Marc[1], Lin, Ziguang (Bob)[2], Ye, Jinjing[2], Godatha, Swetha[1], Hsu, David[2], Couture, Larry[3], Riggs, Arthur[1]

[1]Diabetes and Metabolic Disease Research, City of Hope/Beckman Research Institute, Duarte, CA, USA, [2]Center for Biomedicine and Genetics, City of Hope/Beckman Research Institute, Duarte, CA, USA, [3]CATD, Beckman Research Institute, City of Hope, Duarte, CA, USA

Among potential regenerative medicine targets, myocardial infarction represents the foremost cause of death among industrialized nations. As a result, researchers have invested substantial resources into techniques for cardiac cell derivation and transplant strategies for the purposes of heart regeneration. With future clinical utility in mind, it is widely understood that the scalable expansion of pluripotent cells and their derivatives, including cardiomyocytes, must be carried out under fully defined, GMP compliant culture and differentiation systems. If is further understood that proper cellular programming is essential to product cardiomyocyte function and ultimately therapeutic efficacy and safety. However, genome-wide epigenetic maps describing pluripotent-to-cardiomyocyte differentiation are generally absent and those few that do exist remain insufficient in coverage and/or precision. In this work we describe the global DNA methylation and transcriptional program that defines 4 key time points on the journey hESCs take from pluripotency to beating cardiomyocytes. Working from a GMP generated hESC suspension bank we have derived pure cardiomyocytes using a fully defined small molecule based differentiation strategy. To

eliminate substantial heterogeneity expected and observed during hESC differentiation we carried out flow cytometry based isolation of mesoderm and cardiac progenitor populations by targeting established cell surface markers. RNA-seq results clearly establish that we have minimized the otherwise confounding transcriptional signatures present in uncommitted pluripotent cells and those differentiating towards alternative lineages. Therefore, we have used pure hESC, mesoderm, cardiac progenitor, and cardiomyocyte populations to define both the methylome and global transcriptional program of human cardiomyocyte differentiation. Our results identify Wnt, hedgehog, and TGF-beta signaling factors specifically involved in cardiomyocyte differentiation, and furthermore, identify miRNAs, lncRNAs, and alternative splicing events that accompany differentiation. In addition to describing global correlations between DNA methylation and transcription, we lastly reveal not only specific DNA methylation changes that occur pair-wise between hESCs and cardiomyocytes (ex: at MYOCD, MYH6 genes), but progressive and dynamic changes through differentiation uniquely testable by investigating mesodermal and progenitor cells (ex: at HOXA and B1, TRIM55 genes). Collectively, our results highlight the importance of isolating pure cell populations when conducting genome-wide association studies through cellular differentiation and provide a detailed DNA methylation and transcriptional map for pluripotent-derived cardiomyocytes under fully defined culture and differentiation conditions.

F-3072
CARDIAC-DERIVED EXTRACELLULAR MATRIX MATURES HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTE METABOLISM

Tran, David D.[1], Datta, Rupsa[1], Moya, Monica L.[1], Wang, Jean J.[2], Christman, Karen[3], Conklin, Bruce R.[4], Gratton, Enrico[1], George, Steven C.[1]

[1]University of California, Irvine, CA, USA, [2]University of California, San Diego, CA, USA, [3]University of California, San Diego, La Jolla, CA, USA, [4]J. David Gladstone Institutes, San Francisco, CA, USA

Introduction: Heart disease is the leading cause of death in the western world. Human induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) represent a new source of human cardiomyocytes with potential for new therapeutics to treat heart disease. Development of human iPS-CMs as a viable research and therapeutic tool has been accelerated by recent reports describing efficient differentiation in defined conditions. While our ability to create human iPS-CMs has significantly improved, the resulting cells are typically immature in structure and function. Past research has shown improvement in cardiomyocyte phenotype when features of the in vivo environment can be included in the culture conditions. Decellularized cardiac extracellular matrix (cECM) retains a protein and macromolecular composition (e.g., collagen, elastin and glycosaminoglycans) similar to the native heart, potentially possessing chemical and mechanical cues able to drive cardiomyocyte maturation. In this work, we use non-destructive fluorescent lifetime image microscopy (FLIM) phasor analysis to characterize the effect of extracellular matrix composition on the human iPS-CM metabolic state. Methods: Human induced pluripotent stem (iPS) cells (WTc-11 and WTb) were differentiated into cardiomyocytes following a small molecule Wnt-modulating differentiation protocol. Briefly, human iPS cells were cultured as a feeder-independent monolayer on matrigel and then sequentially exposed to CHIR99021 and IWP2 to first stimulate and then inhibit canonical Wnt signaling, respectively. Cardiomyocyte spheroids were formed by passaging differentiated cells into AggreWell plates. Spheroids were embedded within extracellular matrix (ECM) mixtures comprised of fibrin and porcine cECM. Two-photon microscopy captured FLIM images of the cardiomyocytes excited at 740 nm. The most abundant autofluorescent cellular cofactor at 740 nm excitation is nicotinamide adenine dinucleotide (NADH) in its free and protein-bound form. Phasor analysis, a mathematical transformation of FLIM described previously, allowed quantification of intracellular levels of free and bound NADH, indices of glycolysis and oxidative phosphorylation, respectively. Results and Discussion: After 8 days of culture, FLIM phasor analysis demonstrate a significant change in human iPS-CM metabolism when encapsulated in different ECM compositions. Human iPS-CM cultured within a pure fibrin matrix contained a high ratio of free NADH to bound NADH, indicative of a predominantly glycolytic metabolic state. In contrast, a pure cECM matrix significantly increased the cardiomyocyte intracellular of bound NADH to free NADH from 20.5% to 99.5% ± 7.3 indicative of a cardiomyocyte metabolic shift from glycolysis to oxidative phosphorylation. A major advantage of using non-destructive methods to assay phenotypic changes is the ability to do so temporally. Increased concentration of fibrin in the ECM increased the rate at which human iPS-CM shifted from oxidative phosphorylation to glycolysis over the initial five days. Conclusions: Through phasor analysis of NADH FLIM images, we have demonstrated that cECM promotes maturation of human iPS-derived cardiomyocyte metabolism towards primarily oxidative phosphorylation for efficient ATP generation. cECM has the potential to impact *in vitro* cardiac tissue models by replacing currently used scaffold materials with an ECM that matures cardiomyocyte metabolic phenotype.

F-3073
COMPARISON OF HUMAN FOETAL IN VIVO AND EMBRYONIC STEM CELL IN VITRO DERIVED CARDIOMYOCYTES

Vestergaard, Maj Linea, Anderson-Jenkins, Zöe, Mamsen, Linn Salto, Andersen, Claus Yding

Laboratory of Reproductive Biology, University Hospital of Copenhagen, Copenhagen, Denmark

Development of human cardiomyocytes from undifferentiated human embryonic stem cells (hESC) and from induced pluripotent cells has been demonstrated in several studies. However, the efficacy of deriving cardiomyocytes is highly dependent of the experimental set-up and reflects that precise robust differentiation protocols has not yet been established. The variability between caridomyocytes derived from different hESC lines has only to a limited extent been evaluated. Further, hESC-derived cardiomyocytes appears to resemble foetal cardiomyocytes, but no studies have thoroughly compared hESC-derived cardiomyocytes to human *in vivo* derived foetal cardiomyocytes in order to evaluate their similarity and to verify the cell model. The aim of the present study is to identify critical parameters in the differentiation protocol and to characterize and compare cardiomyocytes derived from different hESC lines. Further we want to compare these results with data from human foetal cardiomyocytes obtained during early development of heart formation. Using established techniques our laboratory has derived 25 unique hESC lines. Cardiomyocytes are derived from undifferentiated hESC by separate embryoid body differentiation in 96 well dishes. Media with high serum concentration together with reduced oxygen tension at particular stages is used to promote mesoderm and cardiac differentiation. Cardiomyocyte derivation is visually monitored by identifying beating areas of cell aggregates and further verified by gene expression analysis. Human foetal heart samples have been collected from tissue obtained from healthy pregnant women undergoing legal termination of pregnancy and who have given their informed consent.

Heart samples have been collected from foetal week 5 to 11 post conception and are subsequently divided in atrium and ventricular tissues. We derive cardiomyocytes from hESC in less than two weeks with efficiencies between 20-60% based on the visual identification of beating cell aggregates. The first moving cells are observed after 9 days of differentiation and reach a maximum after approximately 30 days of differentiation. Stable gene expression of the 6 consensus hESC transcription factors (*DNMT3B, GABRB3, GDF3, NANOG, POU5F1, TDGF1*) is observed in all the hESC lines used prior differentiation. During differentiation the expression of all hESC transcription factors decreases and become replaced by a peak in *DKK1* expression indicating a population of cardiomyocyte progenitor cells around 9 days into the differentiation process. Subsequently, the gene expression of cardiac transcription factors increase (*GATA4, NKX2.5, TBX5*) and become replaced by the expression of functional cardiac genes as *CTNT* and *ACTN2* after 12-16 days; at the same time as most new beating cells are observed. Results from different hESC lines will be presented at the conference. Human foetal heart samples have been collected and are being prepared for molecular analysis. Comparison of results from hESC derived and *in vivo* derived cardiomycytes will be presented at the conference.

F-3074
MONITERING OF THE CARDIAC DEVELOPMENTAL PROCESS USING A NKX2-5^{EGFP}/MLC2V^{MCHERRY} HUMAN EMBRYONIC STEM CELL LINE

Yamauchi, Kaori[1], Nakatsuji, Norio[2], Suemori, Hirofumi[1]
[1]*Department of Embryonic Stem Cell Research, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan,* [2]*Institute for Intergrated Cell-Material Sciences, Kyoto University, Kyoto, Japan*

Human pluripotent stem cell (hPSC)-derived cardiomyocytes are expected to be a valuable cell source for transplantation therapies. Although many methods to obtain a large amount of the cardiomyocytes from the hPSCs have been developed, effective protocols for selective induction of the specific cardiac cell types such as ventricular, atrial, and pacemaker cells are yet to be established. Use of a mixed population of various cardiac cells generated during the differentiation process results in hampering of proper functional analysis and evaluation of the cell properties. The information of human cardiac development would be useful to figure out how the mixed cardiomyocytes were differentiated. However, the studies of the human cardiac development are highly limited because of lack of the *in vitro* human model systems. To address the issue, we have previously established the hESC-derived model system that recapitulates the early cardiac developmental process. In the study, the generated cardiomyocytes captured the similar properties with ventricular, atrial, and pacemaker cells by an electrophysiological analysis. However, the mixture of these cardiomyocytes was observed. Here, we established *NKX2-5^{eGFP}/MLC2v^{mCherry}* hESCs, which enable labeling, tracing and purifying the ventricular-specific cells from the cardiac differentiated cells. *NKX2-5* is well known as a marker to detect cardiac progenitor cells. *MLC2v* is a good marker to identify the differentiation of the ventricular cells. We targeted mCherry to the *MLC2v* locus of the *NKX2-5^{eGFP}* hESCs by using a CRISPR/Cas system. The targeting efficiency was 10~40% in our study, suggesting that the system is very effective for genome engineering in the hPSCs. The *NKX2-5^{eGFP}/MLC2v^{mCherry}* hESCs showed the same ability with the parental cell line in the cardiac differentiation *in vitro*. As expected, the mCherry expression following the GFP expression was observed under the culture condition we previously developed. These results demonstrated that the *NKX2-5^{eGFP}/MLC2v^{mCherry}* hESCs allow monitoring the development process of the cardiac tissue cells from early to late stages. In the clinical applications, obtaining the homogeneous cardiomyocyte population (e.g. ventricular cells) would be ideal, because the contamination of pacemaker cells into the transplanted cells could cause irregular beating of the heart. The purification and the selective induction of the specific cell types would be one of best ways to resolve the problem. Our study will help to establish the methods for safe and effective cell transplantation.

MESENCHYMAL CELL LINEAGE ANALYSIS

F-3076
UNDERSTANDING THE ROLE OF THE OSTEOGENIC NICHE IN DIRECTING CELL LINEAGE AND METABOLISM

Corradetti, Bruna[1], Taraballi, Francesca[1], Powell, Sebastian[1], Weiner, Bradley K.[2], Tasciotti, Ennio[1]
[1]*Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX, USA,* [2]*Division of Spinal Surgery, Department of Orthopaedic Surgery, The Houston Methodist Hospital, Houston, TX, USA*

All regenerative processes in living tissues draw on reservoirs of stem cells, which boast the unique skill of generating committed phenotypes able to progress along maturation. Among these, mesenchymal stem cells (MSCs) are multipotent progenitors showing promising potential for regenerative medicine applications. MSCs are intricately connected with their niche and receive from it constant chemical, structural and mechanical inputs, which drive their commitment and specification. MSCs have been isolated from several adult and fetal tissues and the different properties of each tissue make them prone to differentially respond to the stimuli provided by their tissue of origin. In the present study we aim to understand the nature and role of MSCs derived from the rat compact bone (CB-MSCs) as opposed to cells classically harvested from bone marrow (BM-MSCs). Despite both tissues arise from the same mesoderm-derived population during embryonic development, we hypothesized that the chemical, structural and mechanical signals provided to the MSC by the osteogenic niche could affect their proliferation and differentiation potential as well as the way they react in an inflammatory environment. CB-MSCs displayed a slower proliferation rate compared to BM-MSCs with a 50% increase in the doubling time mean value. Flow cytometric analysis showed that cells isolated from the compact bone represent a very homogeneous population, in which 99% of cells were positive for mesenchymal markers (CD29, CD90, and CD44) and negative for the hematopoietic marker CD34. Gene expression analysis confirmed the expression of these and other markers (CD105, CD73) up to passage 10. Moreover, when appropriately induced CB-MSCs showed the capability to differentiate toward the chondogenic, adipogenic and chondrogenic lineages. However, their osteogenic potential was found greater than BM-MSCs. Osteogenic assays qualitatively and quantitatively confirmed the more committed osteogenic nature of CB-MSCs showing an increase in the mineral deposition and in the expression of osteogenic lineage markers such as osteocalcin and osteopontin. To assess their contribution in de novo bone formation, CB- and BM-MSCs were seeded in a three-dimensional macroporous collagen scaffolds and implanted subcutaneously for 4 weeks. Both populations generated vascularized bone tissue, however, histology and osteopontin immunostaining proved that only CB-MSCs were able to form mature osteoid. In the process of evaluating their immunosuppressive potential, we found out that CB-MSCs respond to the pro-inflammatory cytokine TNF-α (50 ng/ml) significantly increasing the expression of PGES, COX-2 and iNOS, which is accompanied with a marked production of prostaglandin E2 and nitric oxide. While BM-MSCs secrete these

molecules only in response to pro-inflammatory stimuli, CB-MSCs displayed surprisingly high levels even in basal conditions. We believe that these molecules could be preferentially used by CB-MSCs to communicate in the context of the compact bone. This study suggests that the physiologic niche in which the CB-MSCs reside might have a role in directing their fate toward a osteogenic lineage and provides supporting information about the effect that the environment has on the their response to extrinsic signals.

F-3077
CD146 EXPRESSION AS A BIOMARKER FOR HMSC POTENCY IN NORMAL CELLS EXPANDED WITH THE QUANTUM SYSTEM

Jones, Mark E., Startz, Thomas, Frank, Nathan, Vang, Boah, Peters, Rebecca, Hill, Domicinda, Kilian, Rachel, Nankervis, Brian, Nguyen, Kim
Innovation and Development, Terumo BCT, Inc., Lakewood, CO, USA

In the course of biopharmaceutical development, the potency of human mesenchymal stromal cells (hMSCs) is frequently evaluated by differentiation (adipogenesis, chondrogenesis or osteogenesis) for use in regenerative medicine. Such hMSC differentiation protocols generally require two to three weeks to complete following the induction of cell populations that are characterized by heterogeneity. CD146 (MCAM) is a cell surface biomarker on hMSCs that plays a role in cell adhesion as a member of the immunoglobulin super family. Prior work by Russell *et al* indicates that CD146 exhibits a correlation between biomarker expression and cultured MSC potency. More recent studies by Espagnolle *et al* suggest that MSCs with high CD146 expression also correlate to a vascular smooth muscle cell lineage in normal cells. At Terumo BCT, we have begun to measure the CD146 surface biomarker expression by flow cytometry as an early indicator of hMSC differentiation potency at the time of harvest from the Quantum Cell Expansion System. In addition, we followed the CD146 expression profile in differentiated bone marrow derived hMSCs. BM-hMSCs from three normal donor aspirates were cultured in a hollow fiber membrane bioreactor. At harvest and after differentiation, hMSCs were stained with a conjugated PE/Cy7 anti-human CD146 tandem fluorochrome, fixed, and analyzed for the presence of the biomarker. The Median Fluorescence Intensity (MFI) was subsequently used to quantify the expression of CD146+ on hMSCs as well as on differentiated cells and compared to unstained cells using a BD Canto-II flow cytometer with Diva 6.1.3 software. The average CD146+ MFI of uninduced, stained hMSCs in the three donor cell lineages was 2,329 versus the average MFI of unstained, uninduced hMSCs which was 22. By comparison, the average CD146+ MFI of induced adipocytes was 405 versus the unstained, induced adipocytes MFI of 120. And the average CD146+ MFI of induced chondrocytes was 854 versus the unstained, induced chondrocytes MFI of 202. Furthermore, images of hMSC adipogenesis and chondrogenesis were acquired for confirmation purposes using QCapture Pro 6.0 software. These data suggest that CD146 biomarker expression can be used to quantify the potency of hMSC differentiation prior to induction. The results also reflect the progression of hMSCs from multi-potency, through lineage commitment, and on to differentiation in terms of a cell adhesion biomarker.

F-3078
HYPERCHOLESTEROLEMIA IMPAIRS ADIPOSE DERIVED MESENCHYMAL STEM CELLS FUNCTION IN APOE KNOCKOUT MICE

Porto, Marcella Leite[1], Rodrigues, Bianca[1], Campagnaro, Bianca[2], Amigo, Igor[1], Menezes, Thiago[1], Vasquez, Elisardo[1], Meyrelles, Silvana[1]
[1]UFES, Vitoria, Brazil, [2]Universidade de Vila Velha, Vila Velha, Brazil

Background: Mesenchymal stem cells derived from adipose tissue (ADSC) are multipotent stem cells, they are easily harvested and exhibit promise in a variety of regenerative applications. The purpose of this study was to evaluate the hypercholesterolemic condition impact on ADSC, relating to oxidative stress, DNA damage, inflammation markers and apoptosis. Methods: Male mice (n= 8) were divided into C57 and apoE-/- (2-month-old) groups. ADSCs were isolated from adipose tissue of mice, the cells were cultered until passage six, when the experiment started. Cells were harvested by trypsinisation and stained with antibodies against Sca-1, CD105, CD29, CD44, or matched isotype control. Oxidative stress was assessed by dihydroethidium (DHE) and 2,7-dichlorofluorescein (DCF) by flow cytometer. Nitric oxide (NO) was also measured by diaminofluorescein (DAF) fluorescence. DNA damage was analysed by propidium iodide (PI) and inflammation markers, such as interleukin-6 (IL-6) and monocyte chemoattractant protein-1(MCP-1), were detected using cytometry bead array assay on supernatant of cultured cells. For apoptosis analysis, ADSC were resuspended in Binding Buffer and incubated Annexin V-FITC and (PI). A FACSCanto II cytometer was used for the flow cytometric analysis. Data were acquired and analyzed using BD FACSDiva, FCAP Array and FCS Express 4 softwares. Lipid profile was also analyzed. Data are mean±SEM. Statistical analysis was performed using Student´s t test. Results: Hypercholesteromia increased hydrogen peroxide levels, DNA damage, inflammation markers and apoptosis. Superoxide anions stained with DHE showed no significant difference, however DCF showed an increase in apoE-/- mice compared with C57 (600 ±35 vs. 875 ± 35 MFI, $p<0,05$). Hypercholesterolemic mice showed lower level of NO, but not significant different. DNA damage was augmented in apoE-/- compared with control mice (1,2 ± 0,4 vs. 9,7 ± 0,9%, $p<0,05$). Inflammation markers showed higher levels in hypercholesterolemic mice (IL-6: 260 ± 8,8 vs. 310 ± 7,5 pg/ml; MCP-1: 300 ± 35 vs. 770 ± 16 pg/mL, $p<0,05$). Apoptosis was also increased with hypercholesterolemia (2,4 ± 0,08 vs. 18 ± 0,33%, $p<0,05$). As expected, total plasma cholesterol level was markedly increased in apoE-/- (81 ± 18 vs. 222 ± 44 mg/dL, $p<0,05$) Conclusion: The present study demonstrated that hypercholesterolemia affect ADSC functionality. Possibly increased cholesterol levels may influence inflammation markers, promoting an imbalance of reactive oxygen species, causing DNA damage and apoptosis. These results provide novel rationales for the optimal use of ADSC in therapies.

F-3079
IN VIVO GENETIC LINEAGE TRACING OF ADULT DERMAL STEM CELLS DURING HAIR FOLLICLE REGENERATION

Rahmani, Waleed, Hagner, Andrew, Abbasi, Sepideh, Biernaskie, Jeff
University of Calgary, Calgary, AB, Canada

The hair follicle mesenchyme (dermal sheath and dermal papilla) contains cells that when isolated *in vitro*, exhibit self-renewal and multipotency. Moreover, transplantation studies have shown that these skin-derived precursor cells (SKPs) can induce hair follicle growth and may play a role in dermal maintenance. However, the primary source of these precursors and lineage relationship between mesenchymal cells within the hair follicle are poorly understood. Moreover, how inductive dermal papilla (DP) cell numbers are maintained over

consecutive regenerative cycles is unknown. Our preliminary work and by others, have suggested that proliferation within the hair follicle mesenchyme largely occurs in the dermal sheath and not in dermal papilla. Here we asked whether αSMA+ cells within the dermal sheath (DS) represent a progenitor population that migrate into the DP during the course of hair morphogenesis. To address this, we generated two inducible Cre-lox transgenic mice, αSMA-CreER^{T2}:ROSA26^{eYFP} and αSMA-CreER^{T2}:ROSA26^{confetti}, to perform *in vivo* genetic lineage tracing experiments. Tamoxifen treatment during early anagen (hair follicle growth phase) exclusively labeled DS cells and not the DP. We then documented the fate of these cells over multiple hair follicle cycles (1-30 days and 7 months post treatment). Our results identify a small population of multipotent self-renewing adult mesenchymal stem cells that envelop the telogen DP, are activated at the onset of anagen to regenerate the DS and are retained at the end of each hair follicle cycle for at least 8 months. More importantly, these cells also contributed new cells to the DP. This work provides definitive evidence for the existence and location of a mesenchymal stem cell within the adult hair follicle and provides new insights into the lineage relationships within the mesenchymal compartment of the hair follicle. Moreover, since abnormal DP function is the primary contributor of androgenetic alopecia, this work has direct implications toward our understanding of pathological mechanisms that underlie hair loss disorders.

F-3080
ANALYSIS OF MESENCHYMAL STROMAL CELL FATE IN TISSUE REGENERATION

Scott, Wilder[1], Schreiner, Petra[1], Choi, Heejung[1], Kim, Hailey[1], Rossi, Fabio M.V.[2], Underhill, Tully Michael[1]
[1]Biomedical Research Centre, University of British Columbia, Vancouver, BC, Canada, [2] University of British Columbia, Vancouver, BC, Canada

Mesenchymal stromal cells (MSCs) play fundamental roles in tissue regeneration and repair. In homeostasis, MSCs are mostly in a quiescent state and become "activated" in response to various signals. Activation is typically associated with MSC re-entry into the cell cycle where they produce an environment to support tissue regeneration. To better understand the role of MSCs in regeneration, we have used a notexin-induced skeletal muscle damage model in conjunction with two newly created mouse lines based on the gene, Expressed in mesenchymal cells (Emc). Analysis of a conditional knockout mouse line incorporating a nuclear LacZ reporter into the Emc locus along with EMC immunodetection showed that Emc/EMC expression is localized to interstitial and perivascular MSCs. To track the fate of Emc+ cells, a CreERT2 knock-in mouse was generated. When bred to a tdTomato reporter line, tdTomato expression is only observed following tamoxifen administration and the pattern of tdTomato parallels that observed with the Emc nLacZ knock-in allele. This mouse model was used to follow the fate of Emc+ cells following muscle injury. Shortly after notexin-induced damage, Emc+ cells re-enter the cell cycle and are disseminated throughout the damaged region. TdTomato+ processes can be found enveloping the regenerating myofibres as well as the vasculature, and appear to play a fundamental role in both stabilizing the damage region and supporting tissue regeneration. Following regeneration, Emc+ cells can be found throughout the regenerated muscle, however, no tdTomato+ myofibres were found, indicating that the Emc+ MSCs do not directly contribute to the myogenic lineage. These two mouse lines are currently being used to investigate the function of Emc and the role of Emc+ cells in tissue regeneration and repair. Operating grants from the Canadian Institutes of Health supported this research.

F-3081
SPECIFIC MICRORNA TARGETING APAF1 REGULATES APOPTOSIS OF HYPOXIC MESENCHYMAL STEM CELLS VIA INTRINSIC MITOCHONDRIA-MEDIATED APOPTOTIC PATHWAY

Seung, Minji[1], Ham, Onju[1], Lee, Se-Yeon[1], Lee, Chang Youn[2], Park, Jun-Hee[2], Lee, Jiyun[1], Seo, Hyang-Hee[1], Yun, Ina[1], Han, Sun M.[1], Choi, Eunhyun[1], Hwang, Ki Chul[3]
[1]Yonsei University College of Medicine, Brain Korea 21 Plus Project for Medical Science, Seoul, Republic of Korea , [2]Yonsei University, Department of Integrated Omics for Biomedical Sciences, Seoul, Republic of Korea , [3]Yonsei University College of Medicine, Severance Biomedical Science Institute, Seoul, Republic of Korea

Due to the genesis of harsh environmental conditions of infarcted hearts such as hypoxia, abatement of cell proliferation and augmentation of cell apoptosis occur and jeopardize the survival of mesenchymal stem cells (MSCs) after transplantation. Apoptotic protease activating factor 1 (Apaf-1), a protein involved in the assembly of the apoptosome, is known to initiate the cleavage of procaspase 9 in the intrinsic cell death pathway. To target this relevant protein, we used microRNAs (miRNAs) - post-transcriptional regulators that bind to complementary sequences in the 3'UTR of mRNAs, typically resulting in inhibition of mRNA translation. The present study established that miRNAs targeting Apaf-1 regulate apoptosis of MSCs after injection to the infarcted hearts. Using rat myocardial infarction (MI) models, we confirmed that there was significant increase in Apaf-1 levels in the infarct region compared to that of the control. *In vitro* results using bone marrow derived rat MSCs showed that when hypoxia-stimulated intrinsic apoptosis occurs, Apaf-1 expression levels amplified alongside other apoptotic signals such as Bax, cytochrome c, caspase-9, and caspase-3. Contrastingly, once MSCs were transfected with miRNA, Apaf-1 and apoptotic signals declined while survival percentages increased. In accordance to the *in vitro* results, decreased detection of TUNEL-stained cells indicated that miRNA-transfected MSCs (miR-MSC) showed higher survival efficacy. Infarct size, fibrosis area, and inflammation size also decreased in miR-MSC-injected MI hearts *in vivo*. Subsequently, we report that the downregulation of Apaf-1 expression levels via microRNAs will promote the magnitude of MSC survival after transplantation in the infarct region of myocardial infarction.

F-3082
ISOLATION AND CHARACTERIZATION OF MULTIPOTENT TROPHOBLAST CELLS DERIVED FROM HUMAN PLACENTA TISSUE

Shablii, Volodymyr[1], Kuchma, Maria[1], Kyryk, Vitaliy[2], Svitina, Hanna[1], Shabliy, Yulia[1], Skrypkina, Inessa[1], Lukash, Lubov[3], Lobintseva, Galina[1]
[1]Cryobank, Institute of Cell Therapy, Kiev, Ukraine, [2]Cryobank, State Institute of Genetics and Regenerative Medicine Academy of Medicine of Ukraine, Kiev, Ukraine, [3]Human Genetics, Institute of Molecular Biology and Genetics of National Academy of Science of Ukraine, Kiev, Ukraine

The aim of this study was to investigate the immunophenotype, the expression of some trophoblast and mesenchymal related genes, the heterogeneity of subpopulations, the multipotent capacity and the therapeutic potential of multipotent trophoblast cell (MTCs). Placental MTCs was obtained by classical culture methods, placental cells were immunophenotyped by flow cytometry and immunocytochemistry, gene expression was analyzed by RT-PCR. Proteins expression in the placental cells in situ was studied by immunohistochemistry and flow cytometry. For investigation of surviving, homing and integrating into damaged cardiac tissue of MTCs was used FVB

line of mice and isoproterenol induced model of cardiomyopathy. MTCs simultaneous expressed both mesenchymal (vimentin, CD90, CD73, CD105) and trophoblast markers such as pan-cytokeratin (pCK), cytokeratin 7 (CK7), chorionic ganadotropin (CG), tissue non-specific alkaline phosphatase (TNAP), epidermal growth factor receptor type 2 (HER2). RT-PCR analysis showed that MTCs expressed mRNA of transcriptional factors that regulated formation of trophoectoderm such as caudal type homeobox transcription factor 2 (CDX-2), eomesodermin (EOMES). MTCs also expressed other markers which commonly define embryonic stem cells POU5F1 and three primordial cell layers included NKX2-5, SPP1(osteopintin), PPAR-γ2 (peroxisome proliferator-activated receptor gamma 2), COL2A1(collagen type 2). Populations of colony-forming cells with immunophenotype Vim + CG + HER2 + CK7 - CD90 + (CK7-) and Vim + CG + HER2 + CK7 + CD90 + (CK7+) were present in the culture of placental MTCs. Both CK7+- and CK7-- progenitors expressed mRNA of EOMES, CDX-2 that is characteristics of trophoblast stem cells and had capacity to differentiate into adipogenic and osteogenic lineages. After 14 days of term placental explant culturing in tissue was found cells with immunophenotype like to both CK7+ and CK7- progenitor and some of these cells were positive for Ki-67 in contrast to freshly isolated term placenta that suggest about some progenitors cells acquiring MTCs immonophenotype during cultivation in vitro. Placental tissue in situ contains MTC-like populations with immunophenotype CD90 + CD73 + CD45 - CD34 - CD14 -- and CD90 - CD73 + CD45 - CD34 - CD14-. In stromal part of chorion villi we observed double positive cells for vimentin and CG but all of CG+-cells were co-localized with monocyte marker CD68 that suggested about CG + Vim + -cells represented the tissue specific macrophages distinct from MTCs. After intravenous administration of human MTCs into FVB-mice with isoproterenol-induced cardiomyopathy we found these cells in myocardium at 2nd and 28th. Imaging at 2nd showed that MTCs mainly located inside small vessels. On the other hand at 28th we found donor's cells in walls of blood vessels and outside of vessels in myocardium tissue. Placental MTCs simultaneously expressed mesenchymal and trophoblast markers (Vim, CK7, pCK, CG, HER2, TNAP) and transcription factors (EOMES, CDX-2, POU5F1, PPAR-γ2) and contained CK7+- and CK7-- progenitors. Both progenitors had capacity to differentiate into osteogenic and adipogenic lineages and arose from some unknown populations cells during cultivation in vitro. MTCs could to survive, homing and integrating into damaged cardiac tissue of mice in vivo.

F-3083
INVESTIGATION OF THE IN VITRO HETEROGENEITY AND ANGIOGENIC POTENTIAL OF MSC CULTURES

Shaw, Georgina, Dooley, Yvonne, Barry, Frank, Murphy, Mary
REMEDI, National University of Ireland, Galway, Ireland

Mesenchymal stromal cell (MSC) cultures are heterogenous in nature. MSCs are initially isolated based on their ability to adhere to tissue culture plastic and form CFU-F. They are also capable of withstanding trypinization and following trypsinization re-adhere to plastic and proliferate. In most laboratories there are no other selection processes resulting in heterogeneous cultures. In addition to containing cells with tri-lineage differentiation capability, within MSC cultures there are also progenitors with varying differentiation potential. In order to elucidate the complexity of these cultures, a panel of single cell clones was.established. by limiting dilution . These clones were utilised to identify progenitor-specific markers and therapeutically efficacious subpopulations. Tri-lineage differentiation assays were performed to illustrate the heterogeneity in panels of clones established from 2 donor MSC cultures. From this panel, 20 tri-potential clones (OCA) were identified. Additionally, 11 osteo-chondral progenitors (OC), 1 adipo-chondral progenitor (AC), 1 adipogenic progenitor (A) and 3 chondrogenic progenitor (C) clones were also found. Analysis of clones with a panel of antibodies as recommended for the characterisation of MSCs in the ISCT position statement was carried out. The results show that there was no correlation between the positive markers suggested and the ability of MSCs to differentiate to one or more lineages. The angiogenic potential of the clones was assessed using matrigel tubule assays. Assessment of angiogenesis with human umbilical vein epithelial cells (HUVEC)–MSC in co-culture or MSC conditioned medium and HUVEC in matrigel revealed large differences in the ability of clones to facilitate tubule formation. Staining with markers of perivascular and pericyte-like cells identified a phenotype of high 3G5 expression and low CD146 expression on osteo-chondral clones with greatest tubule stabilising properties in the co- culture assay. The conditioned medium from clones was analysed using a Human Angiogenesis Array kit (R and D Systems, Inc). The conditioned medium from 2 clones was significantly more tubulogenic than that of the parent and other clones. Furthermore, the secretome from these two clones displayed distinct angiogenic profiles. These clones also represent a specific subpopulation of osteochondral progenitors, which express high levels of CD146 and high 3G5. In summary, use of these markers may represent a novel strategy to pre-select MSC populations for therapeutic applications such as critical limb ischaemia, where optimal angiogenesis would be a desirable feature.

F-3084
ELASTICITY DEPENDENCE OF PROLIFERATION AND DIFFERENTIATION OF HUMAN AMNIOTIC STEM CELLS CULTURED ON OLIGOPEPTIDE IMMOBILIZED SURFACES

Wang, Pin Yu, Higuchi, Akon, Yu-Hsuan, Chou, Jia-yi, Lin
Department of Chemical Engineering and Material Sciences, National Central University, NO.300, Zhong Da Road, Zhongli City, Taoyuan County, Taiwan

Stem cells derived from human amniotic fluid (AFSCs) are pluripotent fetal cells capable of differentiating into multiple lineages, including representatives of the three embryonic germ layers. Therefore, AFSCs may become a more suitable source of stem cells in regenerative medicine and tissue engineering. However, stem cell characteristics, such as proper differentiation and maintenance of pluripotency, are regulated not only by the stem cells themselves but also by their microenvironment. Furthermore, physical characteristics of cell culture substrate influence the fate of stem cell differentiation. However, there have been no report from our database study that investigates the optimal elasticity to keep pluripotency of stem cells for a long time and studies the optimal combination of physical cue and biological cue on cell culture substrates. Here we report pluripotent maintenance and differentiation efficiency of AFSCs cultured on cell culture substrates immobilized extracellular matrix-derived oligopeptides, which have different elasticity. We prepared dishes coated with polyvinylalcohol-co-itaconic acid (PVA-IA) films having different elasticity by controlling the crosslinking time in crosslinking solution containing glutaraldehyde, and grafted with several ECM-derived cell-adhesion peptides though N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) chemistry in an aqueous solution. qRT-PCR measurements suggest that pluripotent genes, Nanog, Oct4 and Sox2, were kept on PVA-IA hydrogels grafted with oligopeptide derived from vitronectin (oligoVN) and fibronectin (oligoFN) having moderate elasticity around 1-8 MPa during two to five passages. However, Nanog, Oct4, and Sox2 expression of

AFSCs cultured on conventional tissue culture polystyrene dishes dramatically decreased with increasing passage numbers from two to five. We found that there is an optimal elasticity of cell culture matrix to keep pluripotency of AFSCs for their culture. Furthermore, early differentiation marker of osteoblasts (Runx2) were found on AFSCs cultured on stiffer PVA-IA hydrogels grafted with oligopeptides derived from collagen type I (oligoCOL I) in expansion medium without any induction (differentiation) components, suggesting stiffer culture substrates grafted with oligoCOL I guide AFSCs into osteoblast lineage, whereas early neural differentiation marker of nestin were more expressed on softer PVA-IA hydrogels grafted with oligoVN in the expansion medium, suggesting softer culture substrates grafted with oligoVN promote differentiation into neural cell lineages. It is suggested that physical cues such as stiffness of culture materials as well as biological cues of extracellular matrix components can guide and decide pluripotency and differentiation lineages of stem cells.

F-3085
MIR-155 NEGATIVELY REGULATES IMMUNE MODULATORY PROPERTIES OF MESENCHYMAL STEM CELLS

Wang, Ying, Xu, Chunliang, Xiaoyan, Han, Liangyu, Lin, Shi, Yufang
Institute of Health Sciences, SIBS, CAS, Shanghai, China

Mesenchymal stem cells (MSCs) widely exist in almost all tissues that can be easily isolated and expanded in vitro. Due to their immunosuppressive properties, MSCs hold the great promise in treating immune disorder diseases, such as autoimmune diseases. Their immunosuppression on T cells is mediated by nitric oxide that should be induced by IFNγ and TNFα or IL-1. With these inflammatory cytokines stimulation, vigorous enhancement of the expression of iNOS and chemokines are often observed in MSCs. We here investigated, mainly focusing on microRNAs, the molecular mechanisms underlying this strong effect of cytokines. We found that miR-155 is most significantly up-regulated in inflammatory cytokines stimulated MSCs. Using miR155 inhibitor and mimics, we demonstrated the negative regulation of miR-155 on the immunosuppression of MSCs. Furthermore, this up-regulated miR-155 inhibits the immunosuppressive capacity of MSCs by decreasing iNOS expression via targeting to TAK1-binding protein 2 (TAB2). Since inflammatory cytokines pretreated MSCs only show marginal inhibition on autoimmune hepatitis, we will further use miR-155 modified MSCs to detect whether the better therapeutic effects can be acquired. Thus, decreasing the level of miR-155 in MSCs may promote MSCs to do more beneficial to autoimmune diseases.

F-3086
ANGIOGENIC, CYTOPROTECTIVE, AND IMMUNOSUPPRESSIVE PROPERTIES OF HUMAN FETAL MEMBRANE-DERIVED MESENCHYMAL STEM CELLS

Yamahara, Kenichi[1], Harada, Kazuhiko[1], Ohshima, Makiko[1], Ishikane, Shin[1], Ohnishi, Shunsuke[1], Tsuda, Hidetoshi[1], Otani, Kentaro[1], Taguchi, Akihiko[2], Soma, Toshihiro[3], Ogawa, Hiroyasu[3], Yoshimatsu, Jun[1], Ikeda, Tomoaki[1]
[1]National Cerebral and Cardiovascular Center, Suita, Japan, [2]Institute of Biomedical Research and Innovation, Kobe, Japan, [3]Hyogo Medical College, Nishinomiya, Japan

Although mesenchymal stem cells (MSCs) can be obtained from the fetal membrane (FM), little information is available regarding biological differences in MSCs derived from different layers of the FM or their therapeutic potential. Isolated MSCs from both amnion and chorion layers of FM showed similar morphological appearance, multipotency, and cell-surface antigen expression. Conditioned media obtained from amnion- and chorion-derived MSCs inhibited cell death caused by serum starvation or hypoxia in endothelial cells and cardiomyocytes. Amnion and chorion MSCs secreted significant amounts of angiogenic factors including HGF, IGF-1, VEGF, and bFGF, although differences in the cellular expression profile of these soluble factors were observed. Transplantation of human amnion or chorion MSCs significantly increased blood flow and capillary density in a murine hindlimb ischemia model. In addition, compared to human chorion MSCs, human amnion MSCs markedly reduced T-lymphocyte proliferation with the enhanced secretion of PGE2, and improved the pathological situation of a mouse model of acute graft-versus-host disease. Our results highlight that human amnion- and chorion-derived MSCs, which showed differences in their soluble factor secretion and angiogenic/immuno-suppressive function, could be ideal cell sources for regenerative medicine.

F-3087
SUPPRESSION OF ENDOGENOUS MICRORNA181A TARGETING HEXOKINASE II ENHANCES SURVIVAL OF MESENCHYMAL STEM CELLS IMPLANTED INTO ISCHEMIA/REPERFUSION INJURED HEART

Yun, Ina[1], Ham, Onju[1], Lee, Se-Yeon[1], Lee, Chang Youn[2], Park, Jun-Hee[2], Lee, Jiyun[1], Seo, Hyang-Hee[1], Seung, Minji[1], Han, Sun M.[1], Choi, Eunhyun[1], Hwang, Ki Chul[3]
[1]Yonsei University College of Medicine, Brain Korea 21 Plus Project for Medical Science, Seoul, Republic of Korea, [2]Yonsei University, Department of Integrated Omics for Biomedical Sciences, Seoul, Republic of Korea, [3]Yonsei University College of Medicine, Severance Biomedical Science Institute, Seoul, Republic of Korea

Adult mesenchymal stem cells (MSCs) transplantation has emerged as a powerful tool to treat many cardiovascular diseases such as a myocardial ischemia/reperfusion (I/R) injury. However, generated reactive oxygen species (ROS) in the injured heart is an obstacle to survival of MSCs. For increasing the survival efficiency of transplanted MSCs, regulation of anti-apoptotic proteins is required. Hexokinase II (HKII) is a pro-survival protein that is mostly localized in mitochondria outer membrane and displaced into cytosol in the early phase of apoptosis. Previously, we observed that mitochondria-bound HKII decreased in infarcted zone following I/R injury. We therefore hypothesized that upregulation of HKII in MSCs would increase survival after I/R injury. MicroRNA (miR) transcriptionally downregulates gene expression in a variety of biological processes such as apoptosis. Our assay revealed that miR-181a targets HKII and acts as an apoptotic miR. We examined whether downregulation of miR-181a by treating with anti-miR-181a could control the ROS-induced apoptosis. Our data suggested that the expression of pro-apoptotic proteins were attenuated in anti-miR-181a transfected MSCs (anti-181a MSCs). *In vivo* experiments indicated that the survival of transplanted anti-181a MSCs was significantly higher than that of MSCs. Moreover, left ventricular functions were restored and cardiac fibrosis was recovered after transplantation of anti-181a MSCs into I/R injured heart. Collectively, our observations implicated that downregulation of HKII-targeting miR-181a in MSCs inhibits ROS-induced apoptosis following myocardial I/R injury via upregulating mitochondria-bound HKII levels.

MESENCHYMAL STEM CELL DIFFERENTIATION

F-3088
STEM CELL FUNCTIONAL MARKERS AS KEY QUALITY ATTRIBUTES OF LARGE SCALE EXPANSION

Murrell, Julie, Stankiewicz, Nikolai, Aysola, Manjula
EMD Millipore, Bedford, MA, USA

Human mesenchymal stem cells (hMSCs) are an attractive target for clinical studies due to their easy accessibility, the potential for in vitro expansion and their immunomodulatory properties. The characterization of stem cells and their specialized derivate cells is crucial for their use as therapeutic agents. Here we report the development of several assays for the simultaneous, specific and sensitive detection of a variety of stem cell lineage markers including intracellular proteins, cell surface structures and soluble factors. Three panels focusing on lineage specific markers in hMSCs (adipocytes, chondrocytes and osteoblasts) with a distinct temporal expression pattern will allow a faster and easier assessment of the differentiation state of bone marrow derived hMSCs. The assays can be used to replace the traditional assays with an earlier readout. Application data of assays for the monitoring of differentiation in hMSCs show a differential marker expression pattern typical of lineage specific differentiation. However, this temporal expression pattern is absent in samples from the bioreactor, confirming the usefulness of the bioreactor for expanding large number of high quality cells that maintain an undifferentiated cell state.

F-3090
EFFECTS OF HYDROXYAPATITE SCAFFOLD ON THE OSTEODIFFERENTIATION OF SUBCULTURED HUMAN MESENCHYMAL STEM CELLS

Rojas, Nina Rosario, de Guzman, Maria Krishna D.
Department of Chemistry, Ateneo de Manila University, Quezon City, Philippines

Prior to clinical use, mesenchymal stem cells (MSCs) have to undergo expansion *in vitro* to achieve a sufficient number of cells required for the intended treatment. However, studies have shown that several rounds of subculture tend to lower replicative capacity and multipotency. Nevertheless, cellular behaviour can still be regulated by interaction with the environment. In this study, hydroxyapatite (HA) scaffold was fabricated to determine its effect on the osteogenic potential of MSCs subjected to serial subculture. Hydroxyapatite was successfully extracted from bovine bone based on X-ray diffraction (XRD) and infrared (IR) spectroscopy. The scaffold was biocompatible, porous and supported cell growth and attachment. Subcultured human bone marrow-derived MSCs at passage 2 (P2), passage 4 (P4) and passage 6 (P6) were treated with osteogenic supplements (dexamethasone, β-glycerophosphate and ascorbic acid-2-phosphate) for 21 days, with and without the scaffold. During this period, calcium deposition, alkaline phosphatase (ALP) activity and osteocalcin content were determined to monitor the extent of differentiation. In general, treatment with the scaffold and osteogenic supplements resulted to higher values of ALP activity and calcium deposition in all passages tested. Even without osteogenic supplements, significant differentiation was still observed when the scaffold was present. On the other hand, only basal levels were detected for osteocalcin, a late marker for differentiation. As passage number increased from P2 to P6, cell proliferation decreased but calcium deposition increased. Even in cultures with neither osteogenic supplements nor HA, mineralization also increased with passage number, suggesting preference of the cells to the osteoblast lineage.

F-3091
AN ANIMAL COMPONENT-FREE CHONDROGENIC STIMULATORY MEDIUM FOR THE EFFICIENT DIFFERENTIATION OF HUMAN MESENCHYMAL PROGENITOR CELLS

Sampaio, Arthur V.[1], Duronio, Chris[1], Thomas, Terry E.[1], Eaves, Allen C.[2], Louis, Sharon A.[1]
[1]STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]STEMCELL Technologies Inc. and Terry Fox Laboratories, Vancouver, BC, Canada

Human bone marrow (BM)-derived mesenchymal progenitor cells (MPCs), expanded in serum-containing or serum-free media, are routinely used as a source of cells for chondrogenic differentiation *in vitro*. However, efficient differentiation of MPCs has been challenging, in part due to the inconsistency of sera used in home-made and commercial media. To avoid the use of serum, we developed a defined Animal Component-Free (ACF) Chondrogenic Medium (ACF-Ch) that supports consistent differentiation of culture expanded MPCs. Human mononuclear cells were isolated from fresh BM (n=5) and expanded in StemPro SFM, MesenCult™ (serum-containing), MesenCult™-XF (Xeno-free), and MesenCult™-ACF. Passage 1 cultures at 80% confluence were dissociated, harvested separately and counted. From each suspension, 5 x 10^5 cells were transferred to 15 mL conical tubes, pelleted at 300x *g* for 5 minutes and the supernatant discarded. Next, 0.5 mL of either ACF-Ch Medium or 3 competitor chondrogenic media (Ch1, Ch2, and Ch3) was added to the tubes. Cells were pelleted again and maintained for 21 days in each differentiation medium as 3D pellets at 37°C with 5% CO_2 in tubes with loosened caps. Complete media changes were performed every 3 days and the cell pellets were either stained with Alcian Blue (AB) and Nuclear Fast Red (FR) at day 21, or processed at day 9 and 21 for qPCR analysis of the chrondrogenic transcripts *Acan*, *Col2a*, *Sox9, Col10a,* and *Mmp13*. MPCs expanded in MesenCult™, MesenCult™-XF , or MesenCult™-ACF and differentiated in our new ACF-Ch formed dense cartilage as demonstrated by positive AB staining and contained an abundance of evenly distributed isogenous groups with minimal hypertrophic areas as observed by FR staining (n=5). In contrast, MPCs differentiated in Ch1, Ch2, and Ch3 consistently showed weak, uneven or no AB staining, demonstrating poor or no cartilage formation. Chondrogenic transcripts from MPCs differentiated in either ACF-Ch, Ch1 (analyzed at day 9) or Ch2 (analyzed at day 21) were normalized to *Tbp* expression (internal control). The normalized transcript expression for MesenCult™ and MesenCult™-ACF expanded MPCs that were differentiated for 9 days in ACF-Ch were: 13.86 and 18.17 (*Acan)*; 1.86 and 25.58 (*Col2a)*; 8.42 and 29.15 (*Sox9)*; 23.36 and 20.83 (*Col10a)*; and 46.55 and 6.31 (*Mmp13)*; respectively, whereas transcript expression for MPCs differentiated in Ch1 were: 0.04 and 0.10 (*Acan);* 0.00 and 0.00 (*Col2a);* 0.07 and 1.62 (*Sox9);* 0.00 and 0.55 (*Col10a);* and 2.01 and 4.79 (*Mmp13)* respectively. For MesenCult™, MesenCult™-ACF, or StemPro expanded MPCs, the normalized chondrogenic transcript for MPCs differentiated for 21 days in ACF-Ch were: 5.87, 23.43, and 13.96 (*Acan)*; 10.56, 65.19, and 76.94 (*Col2a)*; 5.16, 17.49, and 19.36 (*Sox9)*; 78.78, 314.39, and 518.40 (*Col10a)*; and 18.47, 0.86, and 12.02 (*Mmp13)* respectively; whereas transcript expression for MPCs differentiated in Ch2 medium were 0.05, 0.38, and 0.04 (*Acan)*; 0.42, 0.61, and 4.05 (*Col2a)*; 1.04, 1.70, and 1.17 (*Sox9)*; 9.13, 17.61, and 14.94 (*Col10a)*; and 738.43, 46.74, and 43.19 (*Mmp13)* respectively. Together, these results demonstrated that ACF-Ch consistently induces early and stronger expression of key chondrogenic transcripts in expanded MPCs compared to all competitor chondrogenic media. In addition, the ability to expand MPCs in MesenCult-ACF, and differentiate in ACF-Ch in a complete

animal component-free culture system, may benefit translational research for cartilage tissue engineering.

F-3092

THE RELATIONSHIP BETWEEN REACTIVE OXYGEN SPECIES AND STEMNESS IN MURINE BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

Sato, Yukio[1], Okano, Hideyuki[1], Matsuzaki, Yumi[2]
[1]Keio University, School of Medicine, Tokyo, Japan, [2]Institute of Medical Science, Tokyo Medical University, Tokyo, Japan

Mesenchymal stem cells (MSCs) are an attractive candidate for regenerative medicine because they are easily obtained, but do not retain their proliferative and multilineage differentiative capabilities after prolonged in vitro expansion. Recently, it has been reported that the hypoxic condition in cultures prolonged their proliferative capability. However, the mechanism of MSCs proliferation and differentiation is still unclear. In this study, we attempted to determine whether the reactive oxygen species (ROS) are involved in regulation of stemness in murine bone marrow-derived MSCs (BM-MSCs). To evaluate the oxygen condition of MSCs in murine BM, we isolated BM-MSCs from adult C57B/L6 wild-type mice after intraperitoneal injection of pimonidazole, a marker of hypoxia. BM-MSCs were isolated by fluorescence activated cell sorting (FACS). The way to identify and prospectively isolate a subset of MSCs (PDGFRa+Sca-1+CD45-TER119-) from adult murine bone marrow was previously reported. Then, pimonidazole-staining patterns in BM were analyzed in flow cytometry assay. Next, isolated BM-MSCs were cultured in normoxic condition (20% oxygen concentration) with or without N-acetylcysteine (NAC), and hypoxic condition (1% oxygen concentration) for one, two, and four weeks. In each passage, the number of cells and the capabilities to differentiate into osteocyte and adipocyte were evaluated. Moreover, the levels of ROS were measured by 2', 7' -dichlorofluorescein diacetate (DCFDA), a fluorogenic dye that measures hydroxyl, peroxyl and other ROS activity within the cell. The pimonidazole uptake was higher in MSCs than in other fraction of BM. Isolated BM-MSCs proliferated more numerously in hypoxic condition than in normoxic condition. However, supplementation of the culture media with NAC in normoxic condition caused an increase of cell proliferation to the same level in hypoxic condition. The reduction of ROS levels with NAC added condition was confirmed by flow cytometric analysis with DCFDA. In cell differentiation assay, it was difficult for BM-MSCs to differentiate into osteocyte and adipocyte in hypoxic condition. On the other hand, it was easy in normoxic condition. Our findings suggested that murine BM-MSCs are resident in hypoxic condition and ROS regulate their stemness.

F-3093

IS THE REDUCTION OF PRO-MIGRATORY CYTOKINE RELEASE THE REASON FOR IMPAIRED WOUND HEALING AFTER SULFUR MUSTARD EXPOSURE?

Schmidt, Annette, Thiermann, Horst, Steinritz, Dirk
Pharmacology and Toxicology, Munich, Germany

The effect of sulfur mustard (SM) with regard to the direct injured tissues of the skin, eyes and airways is well investigated. Little is known about the effect of SM to mesenchymal stem cells (MSC). However, this is an interesting aspect. The clinical picture of SM exposed patients show chronic wounds with an impaired wound healing for weeks till month. It is known today that MSC play an important role e.g. in chronic impaired wound healing. Therefore we wanted to investigate how SM affects MSC and if these findings might constitute a better understanding of the effect of sulfur mustard gas with respect to skin wounds. From our previous work we know that SM reduces the migratory activity of MSC dramatically. One aspect we analyzed in this relationship was the secretom of MSC after contact with SM. Therefore we used a slide-chip array that facilitates us to analyzed 275 cytokines simultaneously in a single probe. By analyzing the sectretom of MSC after contact with SM it was ostentatious that factors that are described to increase MSC migration are highly decreased. Is this reduction of pro-migratory cytokines the reason for the impaired wound healing?

F-3094

CHARACTERIZATION OF MICRORNA EXPRESSION PROFILING OF IN VITRO DE-DIFFERENTIATED HUMAN PANCREATIC ISLET CELLS

Sebastiani, Guido, Ventriglia, Giuliana, Nigi, Laura, Mancarella, Francesca, Valentini, Marco, Dotta, Francesco
Diabetes Unit, Fondazione Umberto Di Mario ONLUS - University of Siena, Siena, Italy

Beta-cell de-differentiation has recently been identified as a mechanism of beta-cell dysfunction both in type-1 and type-2 diabetes. Upon metabolic and/or inflammatory stress, beta-cells undergo a specific phenotypic re-arrangement which on one side protects them from apoptosis and, on the other, elicits functional impairment. Previously, we have set up (Gallo et al. Cell Death and Diff., 2007) a model of in vitro de-differentiation of human native islets (HI) that, when cultured in appropriate conditions, undergo an epithelial-mesenchymal like transition process (EMT), generating human pancreatic islet-derived mesenchymal cells (hPIDM), which in turn can be expanded and induced to redifferentiate into insulin-producing cells. MicroRNAs (miRNAs) are small endogenous RNAs, which negatively regulate gene expression by targeting 3'UTR of mRNAs. Recently, miRNAs have been demonstrated to control several biological processes including embryo development, stemness and cell differentiation. MiRNAs have also been proposed to contribute to the development of many disorders including diabetes mellitus. Here, we aimed at characterizing the miRNA expression profile during human pancreatic islet cell de-differentiation process. Human pancreatic native islets were collected from 3 multiorgan donors and cultured in RPMI-1640 with 10% FBS, antibiotics-antimicotics and glutamine for 15 days. De-differentiated proliferating hPIDM cells were collected and total RNA was extracted from human native islets and from hPIDM cells. Expression of 768 miRNAs was evaluated using Taqman Human microRNAs panel A and B arrays. Data and statistical analysis was performed using expression suite software 1.0.1 using 2-ddCt method. MiRNA prediction target analysis followed by gene ontology functional classification was performed using Targetscan 6.2 algorithm and DAVID Bioinformatic Resource 6.7. Among 768 miRNAs analyzed, 335 and 331 miRNAs were detected in HI and in hPIDM cells respectively. Following de-differentiation, 110 miRNAs resulted significantly decreased and 13 increased in hPIDM cells vs native human islets (unpaired t-test with FDR correction, $p<0.05$). Upregulated miRNAs included miR-100, miR-337-3p, miR-214, miR-199a-3p and -5p, miR-137, miR-708, miR-99a and miR-302s. To gain insights into the molecular pathways potentially regulated by these miRNAs, we looked at the predicted targets genes. Using Targetscan 6.2, we extrapolated a list of 196 genes computationally predicted as targets of the 13 upregulated miRNAs. In order to classify the predicted genes we used the online algorithm DAVID 6.7 which allowed us to perform a gene ontology analysis. We identified 11 functional categories with a significant p-value. Among these categories, most genes belonged to cell-cell adhesion mechanisms

(23.9%) and to differentiation process (16.8%); in addition, 9.5% of genes belonged to insulin signaling or secretion and to glucose uptake. In conclusion we detected a specific miRNA signature of in-vitro dedifferentiated human pancreatic islet-derived mesenchymal cells. We specifically identified 13 miRNAs strongly upregulated during this process, which may indeed function as regulators of the mesenchymal-like phenotype. Using a bioinformatic approach we uncovered a putative specific role for these miRNAs in exploiting several functions by regulating the expression of genes involved in stemness and in mesenchymal-like phenotype acquisition.

F-3095
IN VITRO STUDY BETWEEN HUMAN DEGENERATIVE DISC CELLS AND MESENCHYMAL STEM CELLS THROUGH PARACRINE STIMULATION

Shim, Eun-Kyung[1], Park, Jung-Chul[1], Kim, Ki-Joon[2], Jung, Byung-Joo[2], Kim, Tae-Wan[1], Choi, Eun-Young[1], Kim, Chang-Sung[1]
[1]Department of Periodontology, Research Institute for Periodontal Regeneration, College of Dentistry, BK21 Plus Project, Yonsei University, Seoul, Republic of Korea , [2]Department of Neurosurgery, Naeun Hospital, Anyang, Republic of Korea

Objectives: While mesenchymal stem cells (MSCs)-based therapies for regeneration of the intervertebral disc degeneration (IVD) have been proposed, the paracrine effect between degenerative disc cells and MSCs has not been fully investigated. Therefore, it is unclear whether implanted MSCs would stimulate endogenous disc cells. Here, we investigate the paracrine effect between degenerative disc cells and MSCs during coculture or culture in cell-conditioned medium. Materials and Methods: Human degenerative nucleus pulposus (NP), annulus fibrosus (AF) cells, and MSCs were indirectly coculture. Cellular proliferation, collagen synthesis of extracellular matrix and RNA expressions of selected genes were then evaluated after coculture. Furthermore, degenerative disc cells and MSCs were cultured in the presence or absence of cell-conditioned medium. We analyzed cellular proliferation and cell migration using scratch wound during culture of cell-conditioned medium. Results: Cell proliferation of NP, AF cells and MSCs were significantly upregulated in samples indirectly cocultured. In addition, extracellular matrix collagen of NP, AF cells and MSCs increased in the NP or AF/MSC coculture. Coculturing promoted the aggrecan, collagen I, II, SOX 9 gene expression of NP cells and the aggrecan, collagen I gene expression of AF cells. In the cell-conditioned medium, NP, AF cells and MSCs showed increased cell proliferation. NP and AF cells were increased cell migration by the MSC-conditioned medium cultured in the normoxia and hypoxia. Unlike NP and AF cells, however, migration of MSCs was increased by NP and AF cells-conditioned medium cultured in the hypoxia. Conclusion: The results showed a possible mechanism of interaction between degenerative disc cells and MSCs mediated by paracrine effect. The effect on degenerative disc cells was enhancement of cellular proliferation, extracellular matrix collagen and matrix protein gene expression. In addition, MSCs were increased cell migration and proliferation by degenerative disc cell-conditioned medium. These results imply that MSCs contributed to enhancing the wound healing process by increased cell migration and proliferation. This study provides the rationale for further investigation of the potential of MSCs therapy in treating IVD. This research was supported by the Bio and Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT and Future Planning(2012M3A9C6049862).

F-3096
REPAIR OF BLADDER FIBROSIS AND VOIDING FUNCTION USING DIRECT TRANSPLANTATION OF MESENCHYMAL STEM CELL INTO BLADDER WALL IN RATS WITH SPINAL CORD INJURY

Song, Yun Seob[1], Lee, Hong Jun[2], Choi, Sungsik[2], An, Jin[2], Doo, Seung Whan[1], Kim, Jae Heon[1], Kim, Sung U.[2]
[1]Urology, Soonchunhyang University Hospital, Seoul, Republic of Korea , [2]Medical Research Institute, Chung-Ang University College of Medicine, Seoul, Republic of Korea

Experiments employing reparative approaches for complete spinal cord injury (SCI) have largely focused on transplantation of stem cell into injured spinal cord for motor recovery neglecting to investigate bladder dysfunction. In spinalized rats, inefficient bladder function might lead to complete deterioration of bladder function, infections and other lower urinary tract complications. The present study was performed to investigate human mesenchymal stem cells (B10) directly transplanted to the bladder wall are capable of inhibiting collagen deposition and improve cystometric parameters in SCI rats. B10 were labeled with fluorescent silica magnetic nanoparticles (MNP) contained rhodamine B isothiocyanate (RITC) conjugated to terminal silanol groups. Forty 6-week old female Sprague-Dawley rats were divided into 4 groups (group 1: control, group 2: sham operation, group 3: SCI, group 4: SCI rats receiving B10). For SCI model, contusion was performed on the thoracic spinal cord very severe intensity weight drop. Four weeks after the onset of SCI, B10 were injected into the bladder wall. Serial T2-weighted MR images were taken immediately B10 injection (1×10^6 cells) and at 4 weeks post-transplantation. Locomotor behavioral tests were performed using rotarod, Basso-Beattie-Bresnahan (BBB) test and voiding response was assessed at 4 weeks after transplantation and bladder was harvested. Nissl staining of spinal cord sections was performed. The hindlimbs of all the animals with SCI were completely paralyzed one day after contusion injury. Both of rotarod and BBB score were 0 and the development of SCI was confirmed and still preserved until 8 weeks after SCI. Non-recovery from SCIs was still found in Nissl staining until 8 weeks after SCI. While weight of bladder and collagen deposition increased after SCI, transplantation of B10 induced recovery to the original weights. Immunofluorescence microscopic observation of MNP-labeled B10 cells demonstrated the abundant red RITC dots in the cytoplasm of the B10 cells. MR imaging of MNP-labeled B10 cells in the bladder showed hypointense signal intensities on T2-weighted images until 4 weeks post-transplantation. B10 cells positive for human mitochondria antigen were found in the transplantation site indicating that MNP-labeled B10 cells survived in the bladder at 4 weeks after transplantation. Transplanted B10 differentiated to smooth muscle cells. The SCI group showed a higher collagen deposition than the sham operation group ($P<0.05$). The group with transplantation of B10 hMSCs after SCI showed a lower collagen deposition than the group with SCI ($P<0.05$). Intercontraction interval decreased after SCI but it recovered after B10 treatment. Maximal voiding pressure and residual urine volume increased after SCI but it recovered after B10 treatment. MNP-B10 transplanted into rat bladder survived 4 weeks post-transplantation using antihuman nuclear antibody staining and MR imaging. Transplanted B10 directly transplanted to the bladder wall inhibited the bladder fibrosis and mediated recovery of bladder dysfunction in the rat SCI model. Transplantation of mesenchymal stem cells could be a novel therapeutic strategy against bladder dysfunction in patients with SCI.

F-3097

THE FUNCTIONS OF MICRORNAS IN SELF-RENEWAL AND DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS

Suh, Nayoung, Hwang, Supyong, Park, Seul-Ki, Lee, Ha Yeon
Asan Institute for Life Sciences, Asan Medical Center, Seoul, Republic of Korea

Human mesenchymal stem cells (hMSCs) have a great potential in stem cell therapy, but little is known about the molecular mechanisms controlling their therapeutic properties. MicroRNAs (miRNAs) are small non-coding RNAs that regulate diverse biological processes by repressing gene expression at post-transcriptional levels. To determine the functions of miRNAs in hMSCs, we deleted DGCR8, which encodes a RNA binding protein essential for miRNA processing. By performing a quantitative RT-PCR assay, we evaluated several highly expressed miRNAs in hMSCs and showed that the levels of selected miRNAs were greatly reduced in the DGCR8 knockdown hMSCs. Next, we tested the effects of DGCR8 deletion in hMSCs using growth rates and MTS assays and observed severe proliferation blocks. We further demonstrated that G1-S regulators such as Cdkn1a (p21) is highly upregulated in DGCR8 depleted hMSCs, similar to what has been known for embryonic stem cells. This suggests that the aspect of cell cycle control by miRNAs is common between the different stem cells for rapid proliferation. In addition, DGCR8 knockdown hMSCs expressed higher levels of subsets of cytokines and growth factors including bone morphogenic protein 2 (BMP-2) demonstrating their potential functions in paracrine capability of hMSCs. Taken together, these findings indicate that miRNAs function in regulating diverse properties of hMSCs which can be manipulated to enhance therapeutic potential.

F-3098

THE ROLES OF ALPHA5-BETA1 INTEGRIN-MEDIATED MECHANOTRANSDUCTION IN OSTEOGENSIS OF MESENCHYMAL STEM CELLS MODULATED BY MATRIX STIFFNESS

Sun, Meiyu, Hongwei, Lv, Yin, Zhang, Lisha, Li, Yulin, Li
The Key Laboratory of Pathobiology, Ministry of Education, Norman Bethune College of Medicine, Jilin University, Changchun, China

Multipotent mesenchymal stem cells (MSCs), which could differentiate into a variety of cell types, including adipocytes, neurons, myoblasts and osteoblasts are an important cell source for tissue engineering and regenerative medicine. Extracellular matrices with varying stiffness profoundly impact on the phenotype of MSCs because the cells sense and response to the extracellular mechanical stimuli. However, the mechanisms of integrin-mediated mechanotransduction regulated to influence osteogenesis on matrix stiffness are not well defined. The purpose of this study was to further explore how matrix stiffness affects α5β1 integrin and stimulates the downstream signaling and subsequently modulates MSCs fate toward osteogenic lineages. Tunable polyacrylamide hydrogels with different stiffness values, as measured by a biomechanical testing machine, were prepared by altering the concentration of acrylamide and bis-acrylamide, for use as an analogue of extracellular matrix. MSCs were cultured on different substrate coated with fibronectin for 0, 7, 14, 21 days, in the absence of any exogenous differentiation-inducing supplements and assessed by osteogenic markers related-transcription factor 2 (RUNX2), alpha-1 type I collagen, osteopontin, alkaline phosphatase(ALP), osteocalcin detected by quantitative real-time RT-PCR, ALP activity and alizarin red S staining for mineralization. Western blotting (WB) analysis was for expressions of PI3K, ERK and phospho-ERK1/2 on different substrates after 2 and 4 hours of induction. The expressions of α5 integrin and activated β1 integrin were determined 2 and 4 hours after seeding by WB and flow cytometry. Bis-acrylamide with the ratio of 0.1% and 0.7 % in 8% acrylamide gels resulted in two different stiffness values corresponding to Young's modulus of 11-17kPa and 62-68kPa, respectively. Osteogenic lineage commitment of MSCs was promoted when cultured on relatively stiff substrate with a modulus of 62-68kPa compared with relatively soft 11-17kPa substrate, as significantly up-regulated expressions of type I collagen, osteocalcin, osteopontin and Runx2 on the gene level, with the presence of ALP, and the evidence of rich calcium deposits on mineralized matrices. WB analysis demonstrated an increase in expressions of PI3K and phospho-ERK1/2 on stiffer matrices than soft matrix after 2 hours. After 4 hours of cell inoculation on stiffer substrate, the expression of phospho-ERK1/2 was higher than that after 2 hours, whereas, the expression of PI3K decreased. Furthermore, the levels of α5 integrin and activated β1 integrin on 62-68kPa substrate were higher than those on 11-17kPa substrate during osteogenic induction. Taken together, our results indicate that α5β1 integrin and the downstream of PI3K and ERK1/2 signaling are important regulators of mechanotransduction during MSCs osteogenic differentiation induced by matrix stiffness, despite that PI3K may only work during the early stage of differentiation.

F-3099

PROINFLAMMATORY RESPONSE OF TLR4 PRIMED MESENCHYMAL STEM CELLS

Sun Hwa, Kim, Kyoung Hwa, Jung, Kyoung Sun, Park, Mi Ran, Choi, Young Gyu, Chai
Hanyang University, Ansan, Republic of Korea

Human mesenchymal stem cells (hMSCs) mediate distinct immune modulating responses in specific toll-like receptors (TLRs) by inflammation stimulation. TLR4-primed MSCs (MSC1) by exposing low-level, short-term LPS induce anti- and pro-inflammatory cytokines that are mediated by TLR4. In the present study, we analyzed global gene expression in TLR4-primed MSCs using RNA-seq technique. TLR4-primed MSCs showed higher expression of pro-inflammatory cytokines such as IL1B, IL6 and TNF-alpha genes, adhesion molecules such as VCAM1 or ICAM genes and general stress response markers such as COX2, SOD2 and HO1 genes. In addition, the altered genes were analyzed using Pathway Studio, resulting in TLR4-primed MSCs inducing inflammatory responses, adhesion molecules and general stress response makers that are regulated by AKT and/or NADPH oxidase. These results show that stimulation of TRL4-primed MSCs promote the gene expression change associated with pro-inflammation cytokines, adhesion molecules and general stress response markers via ROS production mediated by AKT and/or NADPH oxidase.

F-3100

BONE MARROW DERIVED MESENCHYMAL STEM CELLS VERSUS BLASTEMA CELLS: PROMOTING REGENERATION AT EXPERIMENTALLY AMPUDATED DIGIT TIPS OF C57 ADULT MICE

Taghiyar Renani, Leila, Baghaban Eslaminejad, Mohamadreza
Developmental Biology at Cell Science Research Center, Royan Institute, Tehran, Iran

Introduction: The limb bud is excellent model toward understanding vertebrate regeneration. Successful digit tip regeneration is dependent on level of amputation in adult mouse. Amputation of the distal region of the terminal phalanx of mice causes an initial wound healing response followed by blastemal stem cell (blCs) formation. But amputation of the proximal region does not followed by regeneration because

of absence of BlCs. The objective of this study is to transplant either blCs or MSCs into experimentally-amputated mice digit tips in order to examine whether or not MSCs are able to promote regeneration. Method: The blCs were collected from the blastema formed at amputated digit tips of C57 neonatal mice and MSCs obtained from bone marrow of 4-week-old C57 mice. Passaged-3 cells from both sources were then characterized with respect to their in vitro differentiation potential into skeletal cell lineages as well as the expression of an assortment of surface markers. Furthermore, to track the cells following transplantation, both MSCs and blCs were transfected with GFP^+ vector. For regeneration assay, 4-week-old C57 mice were used. 24 hours before cell transplantation, the digit tip from finger 2, 3 and 4 were amputated at either distal or proximal levels. Then 3×10^4 cells from either blCs or MSC culture were injected into the amputated digits. Five weeks after transplantation, the digits were histologically and histomorphometrically examined and analyzed in terms of the formation of soft and hard tissue at amputation site compared with those of the control. 10 different digits from 8 mice were used for each time point. Studies were repeated three times. Results: Flow cytometry analysis indicated that more than 85% of both MSCs and blCs expressed CD90, CD73 and CD105. Both cells tended to be negative for CD44 and CD34 markers. Moreover blCs were positive for CD31 and Sca1 and vimentin. The cells from both sources were readily differentiated into osteogenic, adipogenic and chondrogenic cells lineages. According to our findings at distal amputation, both blCs and MSCs succeeded to regenerate the amputated part of digits. According to the histomorphometrical analysis, the thickness of epidermis and nail at digits with MSCs and blCs transplantation measured as 55% and 85% of the control respectively. Furthermore, the thickness of connective tissue under epidermis was 45% and 80% and the thickness of bone were 70% and 90% of the control at MSCs and blCs transplanted digits respectively. At proximal amputation, while blCs tended to create a regenerated tissue comparable to normal digits, MSCs failed to completely regenerate the lost tissues. Data are represented as mean ± SEM ($p < 0.05$). Conclusion: In this study, we demonstrated that transplantation of blCs could rescue the digit tip of experimentally-amputated adult mice. Furthermore, we found that MSCs could also be as an alternative substitute for blCs only at digit tip amputation at distal rather than proximal level.

F-3101
SKELETAL PHENOTYPES OF RUNX2 CONDITIONAL KNOCKOUT MICE

Takarada, Takeshi, Nakazato, Ryota, Tsuchikane, Azusa, Hinoi, Eiichi, Yoneda, Yukio
Laboratory of Molecular Pharmacology, Division of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan

Runt-related transcription factor-2 (Runx2; also known as Cbfa1, PEBP2α and AML3), a cell-specific member of the Runt family of transcription factors, plays a critical role in cellular differentiation processes from mesenchymal stem cells into osteoblasts and chondrocytes. In effort to further improve our understanding of when Runx2 becomes necessary during bone formation *in vivo*, we have generated conditional knockout mice lacking exon 4 of the *Runx2* gene, which was crossed with α1(I)-collagen-Cre (α1(I)-Cre;$Runx2^{flox/flox}$) or α1(II)-collagen-Cre transgenic mice (α1(II)-Cre;$Runx2^{flox/flox}$). In newborn *α1(II)-Cre;*$Runx2^{flox/flox}$ mice, mineralization impairment was restricted to skeletal areas undergoing endochondral ossification including long bones and vertebrae. In contrast, no apparent skeletal abnormalities were seen in mutant embryo, newborn, and 3- to 6-week old-mice in which Runx2 had been deleted with the *α1(I)-collagen-Cre* driver. These results suggest that Runx2 is absolutely required for endochondral ossification during embryonic and postnatal skeletogenesis, but that disrupting its expression in already committed osteoblasts as achieved here with the *α1(I)-collagen-Cre* driver does not affect overtly intramembranous and endochondral ossification. The Runx2 floxed allele established here is undoubtedly useful for investigating the role of Runx2 in mesenchymal stem cells at particular time periods of embryonic and adult stage.

F-3102
EXTRACELLULAR MATRIX PROTEIN COMBINATIONS MIMICKING DUCHENNE MUSCULAR DYSTROPHY MUSCLE ECM COMPOSITION MODULATE MYOGENIC DIFFERENTIATION IN ADIPOSE-DERIVED STEM CELLS

Thomas, Kelsey, Engler, Adam
University of California, San Diego, La Jolla, CA, USA

Duchenne Muscular Dystrophy (DMD) is caused by the lack of functional dystrophin, a protein that links the actin cytoskeleton and extracellular matrix (ECM). This results in sarcolemmal instability and increased skeletal muscle deterioration. While hyperactivation of muscle satellite cells (SCs) to repair degeneration depletes them at an elevated rate, therapeutic exogenous SC supplementation restores dystrophin expression and improves contractility. SCs are limited in both number and expansion capabilities; on the other hand, adipose-derived stem cells (ASCs) are a readily accessible, multipotent stem cell source capable of differentiating into mesodermal-derived lineages using growth factors, tissue-matched substrate stiffness, and individual ECM proteins. Here using liquid chromatography coupled with tandem mass spectroscopy (LC-MS/MS), we compared ECM composition in DMD to healthy muscle and found that decorin and collagens I and VI were upregulated, laminin and collagen IV were downregulated, and collagens II, III and V did not change. Next, using a high throughput ECM protein microarray printed onto hydrogels that mimic muscle stiffness, we created ECM protein combinations using the matrix composition data from LC-MS/MS. We found that collagens I, II, III, IV, and V, fibronectin, vitronectin, and tenascin-c allowed ASCs to adhere, proliferate, and differentiate over a 7 day period. When specifically examining normal and disease ECM compositions, we found that both DMD (collagen I + collagen VI) and healthy control conditions (laminin-111 + collagen IV) facilitated ASC adhesion and proliferation, but the addition of decorin to DMD matrix coatings decreased ASC adhesion. To further examine myogenic differentiation conditions, we cultured C2C12 mouse myoblasts on single ECM protein coatings on substrates of permissive (11kpa) and non-permissive (40kpa) stiffness, finding high Myf5 expression on laminin-111 and collagen I on permissive stiffness. These data provide an impetus for further examination of the role of ECM composition in myogenic differentiation, DMD progression, and stem cell plasticity.

F-3103
COMPARISON OF DIFFERENT TISSUES FROM THE SAME DONOR SHOWS THAT ONLY HUMAN ADIPOSE DERIVED PERICYTES INCREASED SIGNIFICANTLY LIFE SPAN IN MDX/UTR- MICE

Valadares, Marcos Costa, Gomes, Juliana, Castello, Giuliana, Assoni, Amanda, Pelatti, Mayra, Vainzof, Mariz, Zatz, Mayana
Human Genome Research Center, University of São Paulo, São Paulo, Brazil

Duchenne muscular dystrophy (DMD), is still an untreatable lethal X-linked disorder which affects 1 in 3500 male birth. It is caused by

the absence of muscle dystrophin due to mutations in the dystrophin gene. The potential regenerative capacity as well as immune privileged properties of mesenchymal Stem Cells (MSC) in an attempt to treat DMD has been under investigation for many years One of the questions to be addressed is whether stem cells from distinct sources have comparable clinical effects when injected in murine or canine muscular dystrophy animal models. Many studies comparing different stem cells from various sources were reported but these cells were obtained from different donors and thus with different genetic backgrounds. Here we investigated whether pericytes obtained from 4 different tissues (muscle, adipose tissue, fallopian tube and endometrium) but from the same donor have the same clinical impact when injected in double mutant Utrntm1Ked Dmdmdx/J mice, a clinically relevant model for DMD (life span 120-150 days). Following a protocol of intraperitoneal injections of 106 cells per 8 weeks we evaluated the motor ability and the life span of treated mice as compared to controls. Our results showed that only adipose tissue derived pericytes are able to increase significantly (39 days on average) the life span of affected mice. Microarray analysis showed an inhibition of the interferon pathway by adipose derived pericytes. Our results suggest that the clinical benefit associated with intraperitoneal injected human adult stem cells is related to immune modulation rather than tissue regeneration.

F-3104

BMP-2 INDUCED SIGNALLING MECHANISM AND DUAL ACTION IN HUMAN ADIPOSE STEM CELL DIFFERENTIATION

Vanhatupa, Sari, Ojansivu, Miina, Juntunen, Miia, Miettinen, Susanna
Institute of Biosciences and Medical Technology, University of Tampere, BioMediTech, Tampere, Finland

Recent years have witnessed a rapid increase in the number of clinical trials with mesenchymal stem cells (MSCs) investigating the efficacy in treating clinical conditions. *In bone tissue engineering, the development of an effective combination of stem cells with growth and differentiation inducing factors is an important and critical step to substitute the usage of allograft or autograft tissues.* Bone morphogenetic protein (BMP)-2 has previously been used in clinical applications to stimulate bone regeneration. Because of the contradicting results, there are no clear indications of the functionality and osteogenic impact of BMP-2 on MSCs, including adipose stem cells (ASCs). The aim of this study was to analyse the functionality of BMP-2 dependent Smad signalling mechanism in different culture conditions and in different ASC donor lines as well as differentiation potential of BMP-2 treatment *in vitro*. BMP-2 induced Smad1/5 activation was analysed in different serum conditions (foetal bovine serum; FBS and human serum; HS) with several ASC donor lines by Western Blotting of phosphorylated Smad1/5 protein (p-Smad1/5). The functionality of the actual signalling mechanism was analysed by immunofluorescence microscopy of the p-Smad1/5 nuclear translocation. The differentiation potential due to BMP-2 stimulus was analysed in basal growth medium (BM) and osteogenic medium (OM) by quantitative alkaline phosphatase assay (qALP), Alizarin Red mineralization assays, Oil Red O lipid formation assays, expression of osteogenic marker genes and adipogenic marker genes. Our results indicate that the functionality of BMP-2 is strongly dependent on serum and culture conditions. BMP-2 induced Smad1/5 activation and nuclear translocation in all cell lines studied, but Smad activation was prominent only in HS conditions compared to FBS conditions. Interestingly, BMP-2 exhibits a dual role in differentiation process of ASCs, and the effect on differentiation fate is donor cell line dependent. BMP-2 stimulated osteogenic effect in some cell lines by inducing ALP activity and mineralization, whereas BMP-2 induction clearly promoted adipogenic differentiation in other cell lines. These results indicate that although BMP-2 induces Smad signalling mechanisms, the actual effect of this signalling might have dual function and has to be individually pre-analysed in clinical applications to confirm the benefit of the usage of BMP-2 together with ASCs in bone healing process. These results also partially explain the existing contradictory of the reported BMP-2 studies and indicate to the variability in functional mechanism of BMP-2 stimulated ASCs.

F-3105

P53 STATUS IN MESENCHYMAL STEM CELLS PLAYS AN IMPORTANT ROLE IN BONE HOMEOSTASIS

Velletri, Tania, Wang, Ting, Chen, Xiaodong, Ningxia, Xie, Gan, Yurun, Huang, Yin, Chen, Qin, Lin, Liangyu, Shi, Yufang
Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Shanghai, China

The majority of investigations on p53 have been focused on tumor cells. Recent studies have revealed the importance of p53 in maintaining genomic stability, proliferation and immunosuppression of mesenchymal stem cells (MSCs). However, the role of p53 in regulating stemness and differentiation of MSCs is not known. We isolated MSCs from $p53^{-/-}$ mice and $p53^{+/+}$ mice, and found that, compared to $p53^{+/+}$MSCs, $p53^{-/-}$ MSCs exhibited increased osteoblast differentiation in conditional culture medium. Consistence with this finding, Osteorix and Runx2, the factors related to Osteogenesis were also increased significantly in $p53^{-/-}$MSCs. MSCs are an important cell type in the bone marrow. Various studies have demonstrated that MSCs regulate bone formation balance through differentiating into osteoblasts directly or regulating the function of osteoclasts via receptor activator of nuclear factor (NFκ B ligand (RANKL)-RANK interaction. When we detected the expression of RANKL in MSCs derived from $p53^{+/+}$ and $p53^{-/-}$ mice, we found that RANKL2 and RANKL3 were increased, while RANKL1 was decreased in $p53^{-/-}$ MSCs. Therefore, our studies provided novel information about the role of p53 in regulating bone metabolism through MSCs.

F-3106

MESENCHYMAL STROMAL CELL THERAPY IN DOBERMAN PINSCHERS WITH DILATED CARDIOMYOPATHY: PROMISES BEING DELIVERED

Vulliet, Richard
University of California Davis, Davis, CA, USA

Veterinary patients with terminal diseases offer unique opportunities for clinical trials with adult stem cells. In this vein, we have treated dogs with a heritable form of dilated cardiomyopathy (DCM) that closely parallel the human disease. In pilot trials, we find that allogeneic bone marrow stromal cell (MSC) therapy provides effective treatment for DCM. Doberman Pinschers with DCM were treated systemically with normal donor dog bone marrow MSCs. With the exception of the two of the most severely affected dogs euthanized before the cells had a chance to improve the DCM, all other dogs demonstrated improvement in activity, attitude and/or cardiac parameters. Comments from owners like "dog has more energy," "is back to stealing toys from sibs," or "is now dragging us around like he used to" are common. Several have survived longer than would be expected for their clinical signs. One dog with approximately 17,000 PVCs per 24 hrs had the number of PVCs reduced to less than 300 over the two month course of treatment with MSCs. This time course suggests that the decrease in PVCs was due to the administered cells rather than the concurrent anti-arrhythmic medication. If true, this suggests that MSCs may also be beneficial in treating DCM associated arrhythmias, as well as in dogs in congestive heart failure. Several adverse reactions were observed during these

studies that required modification of our treatment protocols. We believe that MSCs will be an effective treatment for DCM in Doberman Pinschers since we have improved both the quality and quantity of life of the treated dogs. Further optimization of treatment protocols is necessary since we observed several unknown or unforeseen adverse effects. We are now enrolling additional patients for formal clinical trials. Veterinary patients with naturally-occurring diseases, such as DCM, provide an ideal model intermediate to developing stem cell therapies in human patients.

F-3107
IMAGING OF TRANSFORMED RAT MESENCHYMAL STEM CELLS BY INTRAVITAL MICROSCOPY AND TOTAL BODY BIOLUMINESCENT DISTRIBUTION IN VITRO AND IN VIVO

Wang, Mengyu[1], Brustad, Tor[2], Fodstad, Oystein[1], Kvalheim, Gunnar[1]
[1]Cell Therapy, Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, [2]Radiation Biology, Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway

The transformed rat MSCs were still able to differentiate into fat, bone and neural like cells. In vivo the cells also gave tumor and metastasis in nude mice. The tumors formed had morphology like an immature sarcoma and importantly, the cells conserved their capability for ex vivo differentiation after several passages in the animals. TrMSCs over-expressed TGF-beta and TGF-beta related proteins, such as bone morphogenetic protein. Based on these findings we concluded that the TrMSCs bear some characteristics resembling tumor stem cells (Blood Abstract 2006 108. 4256). To further study the in vivo growth properties of the TrMSCs, with special emphasis on migration, angiogenesis and proliferation in vivo, we used a previous developed in-growth chamber model for vital microscopy in Balb/c nu/nu athymic nude mice (Falkvoll et al. Exp.Cell.Biol.52,1984). A single TrMSC sphere with cells transduced with EGFP reporter gene was introduced into the chamber and the growth was studied by vital microscopy employing a fluorescence microscope equipped with a camera. As a control normal EGFP positive MSCs from the same animal was used. Twenty four hours after implantation the TrMSCs proliferated and migrated from the center of the sphere to the blood vessel area. At the same time the vessel started to increase in diameter and shape. After 48 hours the cells invaded the capillary blood vessel area resulting in a further enlargement of the existing vessels along with a significant formation of new vessels. When the normal MSC were tested in the same model no detectable migration and vessel formation could be detected during a 72 hour observation time. To test and observe the growth pattern before and after differentiation dual reporter gene marked adipocytic and ostegenic linage-differentiated TrMSCs and undifferentiated TrMSCs were injected subcutaneously into the nude mice. To observe TrMSCs's stemness, growth and migration in vivo, sorted side populating cells were tested we found that $5x10^2$ SP+ cells gave growth in the animals with comparison of negative growth by $5x10^2$ SP- cells. For all in vivo assessment here, TrMSCs were transfected with retrovirus with dual reporter gene GFP- Leuciferase and imaged using a cooled charge-coupled device (CCD) camera. In conclusions the "stemness"properties of TrMSC both in vitro and in vivo could be potential useful for investigations of new treatment modalities targeting the tumor stem cells.

F-3108
BREAST TUMOR CELLS MODULATE PROGENITOR CELLS OF THE HEMATOPOIETIC BONE MARROW MICROENVIRONMENT

Wobus, Manja[1], Dhawan, Abhishek[1], List, Catrin[1], Dittrich, Tobias[1], Duryagina, Regina[2], Hofbauer, Lorenz[3], Ehninger, Gerhard[1], Bornhäuser, Martin[1]
[1]Hematology Research Unit, Department of Medicine I, Faculty of Medicine, Technical University of Dresden, Dresden, Germany, [2]University Hospital Dresden, Dresden, Germany, [3]Division of Endocrinology, Diabetes, and Bone Diseases Department of Medicine III, Faculty of Medicine, Technical University of Dresden, Dresden, Germany

The bone marrow hematopoietic niche is an anatomic site where hematopoietic stem and progenitor cells (HSPCs) can be sustained. A major cellular component is mesenchymal stromal cells (MSCs). The bone marrow niche can be hijacked by circulating malignant cells not only in patients with hematologic malignancies but also in patients with breast cancer early in the course of disease. These disseminated tumor cells (DTC) use similar molecules and mechanisms as HSPCs to lodge in the bone marrow and interfere with hematopoiesis. To investigate those events *in vitro*, primary MSCs were isolated from bone marrow aspirates of healthy donors and co-cultured with breast tumor cell lines MCF-7 or MDA-MB231 in different direct or indirect systems. The non-malignant epithelial cell line MCF-10A served as a control. After different time points of co-culturing, important MSC characteristics as proliferation, cytokine secretion and differentiation were investigated. For trans-well migration and adhesion experiments, CD34+ HSPCs were isolated from peripheral blood of healthy donors after mobilisation. Moreover, in an observational study of 78 breast cancer patients the number of circulating HSPCs was determined. We detected that both MCF-7 and MDA-MB231 breast tumor cell lines or their conditioned medium induce a significantly decreased expression and secretion of the chemokine SDF-1 (CXCL12) by MSCs with a consecutive Suppression of trans-well migration potential of HSPCs. In contrast, this effect was not observed with MCF-10A non-malignant cells. The SDF-1 down-regulation in MSCs was partly associated with increased TGFβ1 signaling since addition of a blocking anti-TGFβ1 antibody to MSC/tumor cell cultures completely rescued the SDF-1 secretion. Moreover, the TGFβ1 expression levels in MSCs were increased after contact to tumor cell conditioned medium. The detection of a higher number of circulating progenitor cells in patients with untreated breast cancer compared to healthy controls (13±3 vs. 5±1 colonies per $1x10^5$ mononuclear cells) may be the result of an altered SDF-1 signaling but also an out-competition of HSPCs by tumor cells. This effect could be demonstrated *in vitro* by simultaneous and sequential adhesion assays to a stromal layer. We observed that increasing numbers of MCF-7 cells significantly reduced the HSPC adhesion to MSCs in a dose dependent manner. The same result was obtained with MDA-MB231 but not with MCF-10A cells which indicates a tumor cell specific effect. Interestingly, MCF-7 cells increased MSC proliferation of 42% and MDA-MB231 cells supported MSC growth of 27%. Blocking with PD173074 or SU5402 abrogated this effect which suggests a regulation by basic FGF at least in part. Moreover, both tumor cell lines dramatically decreased the osteoblastic differentiation potential of MSCs which could be rescued by the addition of recombinant PDGF-BB. In summary, we provide evidence for a modulation of MSCs by breast tumor cells which may represent novel therapeutic targets early in the course of disease.

F-3109

PLURIPOTENCY AND DIFFERENTIATION OF HUMAN ADIPOSE DERIVED STEM CELLS CULTURED ON ECM GRAFTED HYDROGELS HAVING DIFFERENT ELASTICITY

Wu, Meng-Hsueh, Jia-Yi, Lin, Ching-Tang, Wang, Higuchi, Akon
Chemical Engineering and Materials Engineering, National Central University, Jhongli, Taiwan

Stem cells purified from human adipose tissue (hADSCs) via serial culture of stromal vascular fraction (SVF) on tissue culture plates show multilineage differentiation ability in vitro. In the past decades, extracellular matrices (ECMs) such as collagen, fibronectin, vitronectin, and laminin were used as coating or self-standing materials on 2D culture and 3D culture of stem cells, and the differentiation and proliferation ability of stem cells (e.g., ADSCs or bone marrow-derived mesenchymal stem cells) were investigated. However, the origin of these ECMs are mainly animal-derived or human recombinant, and, therefore, it is not suitable to use them for clinical applications due to xeno-origin contamination and high cost of ECMs. On the other hand, it has been shown that physical environmental factors, such as matrix elasticity, and small functional groups including oligopeptides can have significant effect in determining stem cell fate. To achieve our ultimate goal of affordable, personalized regenerative medicine, clinically safe and economical ways to proliferate stem cells are absolutely essential while maintaining their pluripotency as well as directing stem cell lineage specification without the use of induction chemicals. Here we investigated pluripotency and differentiation ability of hADSCs cultured on poly(vinylalcohol-co-itaconic acid), PVA-IA, hydrogels grafted with several ECMs (collagen type I, fibronectin, and vitronectin) as well as oligopeptides derived from collagne type I, fibronectin, and vitronectin. The stiffness of PVA-IA hydrogels can be controlled by the crosslinking time from 30 min to 48 h in glutaraldehyde solution, which generates the elasticity from a 3.7 kPa to 30.4 kPa storage modulus corresponding 11.1 kPa to 91.2 kPa elastic modulus from calculation of E=3G'. hADSCs were found to maintain their pluripotency on relatively soft hydrogels grafted with vitronectin and oligopeptide derived from vitronectin (KGGPQVTRGDVFTMP) from pluripotent gene expression (Oct 4, Nanog, and Sox2) but not with fibronectin nor CellStart (commercially available ECMs composed of BSA and fibronectin), whereas early differentiation marker of osteoblasts (Runx2) were found on hADSCs cultured on stiffer hydrogels grafted with collagen type I in expansion medium without any induction (differentiation) components. It is also found that the hydrogels grafted with oligopeptide derived from vitronectin using the concentration higher than 50 μg/ml is necessary to keep pluripotency of hADSCs where surface density of oligopeptide derived from vitronectin increases with increasing the reaction solution of the oligopeptide from surface analysis by XPS. Early ectoderm marker of nestin was found to express on softer hydrogels grafted with vitronectin and oligopeptide derived from vitronectin. It is concluded that physical cues such as stiffness of culture materials as well as biological cues of ECM components can guide and decide pluripotency and differentiation lineages of stem cells.

F-3110

EPIGENETIC ALTERATIONS OF MESENCHYMAL STEM CELLS IN 3D SPHEROIDS

Wu, Yaojiong
Graduate School at Shenzhen, Tsinghua University, Beijing, China

Mesenchymal stem cells (MSCs) hold profound promise in tissue repair/regeneration. However, MSCs undergo remarkable spontaneous differentiation and aging during monolayer culture expansion. In this study, we found that 2-3 days of 3-dimensional (3D) spheroid culture of human MSCs (hMSCs) that had been expanded in monolayer for 6 passages increased their clonogenicity and differentiation potency to neuronal cells. Moreover, in accordance with these changes, hMSCs in spheroids underwent epigenetic changes in the expression of miRNA involved with stem cell potency and elevated levels of histone H3 acetylation in K9 in promoter regions of Oct4, Sox2, Nanog and TERT. Moreover, intra-carotid infusion of 3D spheroid MSCs in rats with middle cerebral artery occlusion (MCAO) and reperfusion resulted in engraftment of the cells into the lesion and significant (over 70%) reduction of the infarct size along with restoration of neurologic function. In addition, the enhanced effect of spheroid MSCs was coincided with significantly increased differentiation of the hMSCs into neurons and markedly increased number of endogenous GFAP-positive neural progenitors in the peri-infarct boundary zone. In contrast, the similarly administered monolayer MSCs resulted in a modest functional improvement of the injured brain. Our results indicate that spheroid culture changes the epigenetic status of pluripotent genes in hMSCs and increases their multipotency and efficacy in tissue repair/regeneration.

F-3111

TRANSDIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS (HADSCS) INTO MOTONEURON-LIKE CELLS FOR CELL REPLACEMENT THERAPY IN SPINAL CORD INJURY

Gao, Shane[1], Gao, Zhengliang[2], **Xu, Jun**[3]
[1]Stem Cell Research Center, Shanghai, China, [2]Shanghai Tenth People's Hospital of Tongji University and Tongji University School of Medicine, Shanghai, China, [3]School of Medicine Stem Cell Research Center, Tongji University, Shanghai, China

Human adipose-derived stem cells (hADSCs) are increasingly presumed to be an ideal stem cell sources as an alternative to ESCs and iPSCs for cell-replacement therapies. In the present study, we have developed a stepwise hADSCs trans-differentiation protocol with retinoic acid (RA), sonic hedgehog (SHH) and neurotrophic factors. Our protocol can efficiently trans-differentiate hADSCs into electrophysiologically active motoneuron-like cells (hADSC-MNs) which expressed both a cohort of pan neuronal markers and various motor neuron specific markers. Importantly, when RA- and SHH- preconditioned hADSCs were transplanted to a SCI mouse model, hADSC-MNs survived well in mouse spinal cord and fully integrated into the host tissue. Except for a small subset stained GFAP positive, the transplanted cells persisted as MAP2 positive neurons with classical neuronal morphology. As well, Transplanted hADSC-MNs largely prevented the formation of injury-related cavities and exerted obvious immune-suppressive effect at the injured site. As such, the SCI mice from hADSC-MN transplanted group survived better than the PBS control group and gradually gained significant functional recovery. Assessment of hindlimb locomotor function by BMS indicated a dramatic behavior improvement in SCI-hADSC-MN group after transplantation. Our work suggests that hADSCs can be readily transformed into motoneuron like cells in vitro and stay viable in spinal cord when transplanted into mouse SCI model and exert therapeutic effect for spinal cord injury by rebuilding the broken circuit and suppressing the injury-induced inflammation.

F-3112
HEPATICALLY DIFFERENTIATED DENTAL PULP CELLS TREAT LIVER INJURY OR CIRRHOSIS

Yaegaki, Ken, Ishkitiev, Nikolay, Imai, Toshio, Tanaka, Tomoko, Okada, Mio, Tominaga, Noriko, Ishikawa, Hiroshi
Nippon Dental University, Tokyo, Japan

Objective: Acute or chronic liver injury, i.e. cirrhosis, is a major cause of liver failure and death. Recently we developed an in vitro method for hepatic differentiation of stem cells from SHED. In the present research we investigate the ability of in vitro hdSHED to repopulate an injured liver or one having secondary biliary cirrhosis, and to contribute to the healing process in a rat model in vivo. Methods: We obtained the primary culture from human SHED. CD117 positive cells fraction was magnetically separated, and then differentiated into hepatocyte-like cells following the protocol previously reported. The cells were transplanted into rats' liver with acute injury or induced secondary biliary cirrhosis. Regeneration of human liver tissue was determined immunohistochemically and by in situ hybridization. Recovery of function in liver cirrhosis was also examined with serological tests. Results: Injured livers were positive for immunohistochemical factors, and in situ hybridization confirmed engraftment of human hdSHED in the livers of rats with injury or cirrhosis. However control group demonstrative to be negative against those markers mentioned above. Serological tests confirmed the presence of human hepatic markers in the animals' blood and transplanted human cells' integration into liver function after cirrhosis. Accessibility and capability of producing a large number of stem cells and of differentiating hdSHED of 100% purity make SHED an appropriate stem-cell source for regenerative medicine. Conclusion: hdSHED are shown to engraft morphologically and functionally in livers with acute injury or secondary biliary cirrhosis and to contribute to the repopulation of the damaged organ. Moreover, the serological cirrhotic markers of the transplanted animals remained within the norm. SHED are a suitable and convenient source of MSC of non-embryonic origin.

F-3113
EXPRESSION PROFILING OF HUMAN MESENCHYMAL STEM CELL FROM BONE MARROW

Yang, Jinok, Koo, Namjin
Kobic, Kribb, Daejeon, Republic of Korea

Human mesenchymal stem cells (MSCs) are generally used to describe multipotent self-renewing cells and can be differentiated to make several type cells such as adipocytes, chondrocytes, and osteoblasts. Originally multipotent mesenchymal stromal cells cultured from a variety of tissue types have been shown to differentiate into cardiomyocytes, endothelial cells, hepatocytes, and neural cells. To further MSC research, we obtained the time-series resources from bone marrow and umbilical cord blood for the isolation, expansion, differentiation, and verification of multipotent MSCs. We have studied the expression profiling of differentiated nerve and myocardium. Consequently, we got the 342 and 552 up-regulated genes (fold change > 2) in nerve from bone marrow and umbilical cord blood. Also 365 and 334 up-regulated genes in myocardium from bone marrow and umbilical cord blood were investigated. Microarray analysis of MSC during nerve and myocardium differentiation identified candidate genes set for further examination and functional analysis. Based on a variety of resources we could show that MSC represent a cell population which can be expanded for therapeutic applications.

F-3114
ROS-MEDIATED MECHANISMS OF SENESCENCE IN HUMAN ADIPOSE-DERIVED MULTIPOTENT MESENCHYMAL STROMAL CELLS ON SELF-RENEWAL AND DIFFERENTIATION CAPACITY

Yen, Men-Luh[1], Chen, Pei-Min[1], Yen, B. Linju[2], Lee, Nan-Ting[1], Lee, Yu-Wei[2], Wu, Yao-Ming[3], Lin, Min-Tsan[4], Huang, Guan-Tarn[5]
[1]Deptartments. of Primary Care Medicine and Ob/Gyn, National Taiwan University, Taipei, Taiwan, [2]National Health Research Institutes, Zhunan, Taiwan, [3]Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan, [4]Departments. of Primary Care Medicine and Surgery, National Taiwan University, Taipei, Taiwan, [5]Department of Internal Medicine, National Taiwan University, Taipei, Taiwan

Mesenchymal stromal cells (MSCs) are therapeutically relevant multilineage and immunomodulatory progenitors, and adipose tissue is an easily accessible source for human MSCs. Ex vivo expansion of these rare cells is necessary for clinical application and can result in detrimental senescent effects, with mechanisms still being elucidated. Understanding of the mechanisms involved in replicative senescence of human adipose tissue-derived MSCs (hAMSCs) is therefore important to further therapeutic use of these versatile progenitors. We found that vigorous ex vivo expansion of hAMSCs results in proliferative decline, cell cycle arrest, and altered differentiation capacity of increased adipogenesis but decreased osteogenesis. This senescent phenotype was associated with reactive oxygen species (ROS) accumulation, and with increased expression of p16INK4a-pathway and adipogenic genes including PPARγ and leptin, but decreased expression of osteogenic genes such as Runx2 and the pluripotency genes Oct-4, Sox-2, Nanog, and Klf-4. These global changes in the transcriptional and functional programs of proliferation, self-renewal, and differentiation were all mediated by ROS, and could be reversed with antioxidant agents. Our findings implicate the strong effects of ROS on multiple MSC functions and point to the importance of understanding ROS-mediated senescence mechanisms in stem cells. Further research is ongoing to elucidate the molecular mediator of ROS-induced global changes in MSC functions.

F-3115
COMPARATIVE ANALYSIS OF CELL PROLIFERATION, IMMUNOSUPPRESSIVE ACTION, AND MULTILINEAGE DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS FROM BONE MARROW, ADIPOSE TISSUE, AND UMBILICAL CORD BLOOD

Yin, Dezhong[1], Wells, Joy[1], Curley, Jeanmarie[1], Grady, Kevin[2]
[1]ATCC, Gaithersburg, MD, USA, [2]ATCC, Manassas, VA, USA

Mesenchymal stem cells (MSCs) can be isolated from multiple tissue sources and then used as research tools leading to potential cellular therapies. A better understanding of the differences in characteristics of MSCs prepared from different tissue sources is critical for developing appropriate cell applications. To address this issue, we have conducted a comparative study of bone marrow (BM)-, adipose tissue (AT)-, umbilical cord blood (UCB)-derived MSCs along with an immortalized AT-MSC line (ASC52telo). In the course of this study, we investigated differences in surface marker expression, immunosuppressive action, cell proliferation, and adipogenic, osteogenic and chondrogenic differentiation potential. Regarding surface marker expression, the four cell types had similar expression levels of CD29, CD44, CD73, CD90, CD105, CD166, CD14, CD19, CD34, and CD45. These markers were chosen to meet the International Society for Cellular Therapy (ISCT) guidelines. Compared to other MSCs, BM-MSCs had a higher efficiency of

osteogenic differentiation potential while there was no marked difference in adipogenic and chondrogenic differentiation potential among the four cell types. Differences in proliferation capacity and T cell suppression among the four cell types are currently being evaluated. It is hoped that this comparative data and analysis will provide direction regarding the choice of starting material as it relates to desired research application.

F-3116
INTERCELLULAR ADHESION MOLECULE-1 INHIBITS OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS AND IMPAIRS BIOSCAFFOLD MEDIATED BONE REGENERATION IN VIVO

Xu, Fenfen[1], Zhu, Heng[1], Li, Ximei[1], Yang, Fei[2], Chen, Jide[1], Tang, Bo[1], Chu, Yanan[1], Liu, Yuanlin[1], Zheng, Rongxiu[1], Wang, Lisheng[3], **Zhang, Yi**[1]

[1]Cell Biology, Basic Medical Sciences, Bejing, China, [2]Chemistry, Bejing, China, [3]Cell Biology, Radiation Medicine, Bejing, China

Mesenchymal stem cell (MSC) loaded bio-scaffold transplantation is a promising therapeutic approach for bone regeneration and repair. However, growing evidence shows that pro-inflammatory mediators from injured tissues suppress osteogenic differentiation and impair bone formation. To improve MSC-based bone regeneration, it is important to understand the mechanism of inflammation mediated osteogenic suppression. In the present study, we found that synovial fluid from rheumatoid arthritis (RASF) patients and pro-inflammatory cytokines including interleukin-1α, interleukin-1β and tumor necrosis factor α, stimulated intercellular adhesion molecule-1(ICAM-1) expression and impaired osteogenic differentiation of MSCs. Alkaline phosphatase (ALP) activity and bone matrix mineralization were remarkably suppressed after MSCs were incubated with RASF and pro-inflammatory cytokines. The mRNA of key bone forming markers, Runt-related transcription factor 2 (Runx2)and osteocalcin (OCN), was significantly down-regulated in a dose-dependent manner. Interestingly, overexpression of ICAM-1 in MSCs using gene transfection approach also inhibited osteogenesis. In contrast, ICAM-1 knock-down significantly reversed the osteogenic suppression. Both ALP activity and bone matrix mineralization were partially rescued, the mRNA expression of Runx2 and OCN were recovered. In addition, after transplanting a traceable MSC-PLGA construct in rat calvarial defects, we found that overexpression of ICAM-1 suppressed MSC osteogenic differentiation and matrix mineralization in vivo. Bone-like tissue filled the defect engrafted by both untransfected MSC-PLGA constructs and vector transfected MSC-PLGA constructs, only small bone nodules were observed in the calvarial defect that was repaired with ICAM-1- MSC-PLGA constructs. New born bone formation in the defect engrafted by untransfected MSC-PLGA (55.0±7.0%) and vector transfected MSC-PLGA (53.0±10.58%) was higher than that in defects grafted by the ICAM-1 -MSC-PLGA(22.33±3.06%) using Image-Pro Plus method. Mechanistically, we found that ICAM-1 enhances MSC proliferation but causes stem cell marker loss. ICAM-1 high-expression decreased the expression of Nanog, Oct4, and Sox2 in MSCs. Furthermore, overexpression of ICAM-1 stably activated the MAPK and NF-κB pathways but suppressed the PI3K/AKT pathway in MSCs. More importantly, specific inhibition of the ERK/MAPK and NF-κB pathways or activation of the PI3K/AKT pathway partially rescued osteogenic differentiation, while inhibition of the p38/MAPK pathway caused more serious osteogenic suppression. In summary, our findings reveal a novel function of ICAM-1 in osteogenesis and suggest a new molecular target to improve bone regeneration and repair in inflammatory microenvironments.

F-3117
INTERCELLULAR ADHESION MOLECULE-1 SUPPRESSES PREADIPOCYTE DIFFERENTIATION

Zheng, Chunxing[1], Yang, Qian[1], Liu, Keli[2], Shou, Peishun[1], Chen, Qing[1], Jiang, Menghui[1], Cao, Gang[1], Cao, Wei[1], Xie, Ningxia[1], Li, Fengying[1], Wang, Ying[1], Shi, Yufang[1]

[1]Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Shanghai Jiao Tong University School of Medicine, Shanghai, China, [2]School of Life Science And Technology, ShanghaiTech University, Shanghai, China

Intercellular adhesion molecule-1 (ICAM-1) is a type of intercellular adhesion molecules present on the membrane of various cell types. It plays important roles in stabilizing cell-cell interaction and regulating leukocytes transmigration through endothelial cells. We found that ICAM-1negatively regulates adipogenesis. In the stromal and vascular fractions (SVFs) of adipose tissue, the CD31-CD45-Sca1+ cells are capable of spontaneous adipogenic differentiation. These cells can be further divided into ICAM-1+ subset and ICAM-1- subsets. Interestingly, the spontaneously differentiated adipocytes are mainly derived from the ICAM-1+ subset with perivascular localization in adipose tissue. Importantly, the markers of preadipocytes, such as Zfp423, PPARγ and C/EBPβ, are highly expressed in ICAM-1+, but not ICAM-1- subset. Therefore, preadipocytes are in the CD31-CD45-Sca1+ICAM-1+ subset. It has been reported that the induction of C/EBPβ and C/EBPδ at the early stage leads to the expression of C/EBPα at the late stage. This cascade of reaction decides the adipogenesis process. Intriguingly, in primary SVFs cultures, the ICAM-1 level decreased at the late stage of adipogenic differentiation, which inversely correlated with increased C/EBPα level, and the ICAM-1 expressing cells barely expressed C/EBPα, and vice versa. Thus, our data strongly suggests a negative relationship between ICAM-1 and adipogenesis.

F-3118
NESTIN IS ESSENTIAL FOR TENO-LINEAGE DIFFERENTIATION OF TENDON STEM CELLS

Zi, Yin, Hu, Jiajie, Chen, Xiao, Ouyang, Hongwei

Zhejiang University, Hangzhou, China

Tendon Stem Cells (TSCs) plays a critical role in tendon regeneration, however, the key factors that regulate TSCs maintenance and differentiation is still unclear. Nestin has important functions in various adult stem cells. In this study , we found that NESTIN play an important role in human tendon stem cells maintenance and tenogenesis. It is demonstrated that NESTIN knockdown had no significant influence on proliferation and multi-differentiation (including osteogenesis, chondrogeneis and adipogenesis) potential of tendon stem cells. However, NESTIN expression level was related to the survival of tendon stem cells when exposed to stress environment. Most importantly, NESTIN knockdown in tendon stem cells downregulated tendon specific gene expression, such as Scx, Mkx, Collagen I. Also in vitro engineered tendon constructed by NESTIN knockdowned TSCs, showed significant inferior in collagen fibril assembly. Furthermore, in vivo postnatal Nestin knockdown also resulted in reduced collagen maturation level, evidenced by smaller collagen fibrils and polarized light analysis. However, NESTIN function were not related to cytoskeleton. These data demonstrate that NESTIN is essential to teno-lineage differentiation of tendon stem cells, and has a distinct cytoprotective effect.

PRE-CLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL CELLS

F-3121
VITAMIN C IMPROVES THERAPEUTIC EFFECTS OF ADIPOSE-DERIVED STEM CELLS TRANSPLANTATION IN MOUSE TENDINOPATHY MODEL

Kang, Kyung-Ku, Kim, Ah-Young, Lee, Eun-Mi, Lee, Eun-Joo, Min, Chang-Woo, Lee, Myeong-Mi, Kim, Sang-Hyeob, Sung, Soo-Eun, Hwang, Meeyul, Ghim, Soong-Koo, Jeong, Kyu-Shik
Department of Veterinary Pathology, College of Veterinary Medicine, Kyungpook National University, Daegu, Republic of Korea

Tendon disorders are frequent and are responsible for much morbidity both in sport and the daily life. But no effective therapies for tendon injury are currently available. Recently stem cell therapy raises one type of new therapy for tendinopathy after tendon injury, but therapeutic effects was low. Therefore, we hypothesized that a combined therapy using both Adipose-derived stem cells (ASC) and Vitamin C might improve the tendon regeneration on tendinopathy. To determine the combined effects of ASC transplantation with Vitamin C, we used Senescence marker protein 30 (SMP30) knockout mouse that cannot biosynthesize vitamin C by themselves. Ten-week-old, male, SMP30 KO mice were used in this study and animal were divided into four groups (Control group, Vitamin C group, ASC group, and Vitamin C + ASC group). Mice were induced tendonitis in achilles tendon by collagenase type-I injection. After 1 week, 2 x 10^5 cells per leg were injected into intratendon. After 30 days, all mice were sacrificed and achilles tendons were isolated. ASC transplantation and vitamin C treatment combined mice were show better regeneration of tendonitis than other groups in both gross and microscopic findings. Gene expresseion of Collagen I/III ratio, IGF-1, TGF-β, VEGF and PDGF also upregulated in combined group considering as better regeneration in tendon. VC group showed higher than V group in serum vitamin C level indicating VC group less use vitamin C due to the normalized tendon recovery. Thus, this study showed that Vitamin C improved effect of ASC transplantation for tendinopathy through inducing better stem cell niche.

F-3122
IL-1RA SECRETED BY MESENCHYMAL STEM CELLS EXERTS A KEY IMMUNOMODULATORY ROLE ON THE MATURATION OF B CELLS AND MACROPHAGES

Luz-Crawford, Patricia Alejandra[1], Djouad, Farida[1], Toupet, Karine[1], Franquesa, Marcella[2], Martin, Hoogduijn[3], Jorgensen, Christian[1], Noel, Daniele[1]
[1]INSERM U844, Montpellier, France, [2]Department of Internal Medicine, Section Nephrology and Transplantation,, Erasmus MC, University Medical Center, Rotterdam, Netherlands, [3]Department of Internal Medicine, Section Nephrology and Transplantation, Erasmus MC, University Medical Center, Rotterdam, Netherlands

The efficacy of interleukin-1-receptor antagonist (IL-1RA) treatment has been documented in several autoimmune diseases and in particular, in rheumatoid arthritis (RA). IL-1RA is a member of the interleukin 1 cytokine family mainly secreted by activated monocytes and macrophages. It is an endogenous specific receptor antagonist that competes with the pro-inflammatory signals triggered by IL-1α or IL-1β and participates to the immune system homeostasis through the maintenance of IL-1/IL-1RA balance. IL-1RA is also secreted by mesenchymal stem cells (MSCs) that are potent immunosuppressive cells with promising results for RA. The aim of this study was therefore to evaluate whether IL1RA may contribute to the therapeutic effect of MSCs. MSCs were isolated from the bone marrow of mice knockout for IL-1RA (IL-1RA-/- MSCs) and wild type mice (wt MSCs). One million of cells were injected twice (day 18 and 24) via the tail vein in collagen induced arthritic mice (CIA). In contrast to wt MSCs that significantly inhibited CIA progression, the injection of IL1RA-/- MSCs did not decrease the percentage of Th1 and Th17 cells in spleens and lymph nodes of treated mice. The loss of therapeutic effect could not be associated to the incapacity to inhibit the proliferation of T cells since in vitro, IL1RA-/- and wt MSCs exerted a similar suppressive effect on concanavalin A-activated T cells. However, IL1RA-/- MSCs were less efficient than wt MSCs to reduce the maturation of macrophages in coculture as evidenced by a significantly higher percentage of F4/80+CD86+MHCII+ cells, which expressed higher levels of TNF-α and lower amounts of IL-10. Moreover in contrast to wt MSCs, IL1RA-/- MSCs were not able to reduce the proliferation and maturation of IgG-secreting B lymphocytes. We also observed lower amounts of IL-10-secreting B cells when cocultured with IL1RA-/- MSCs. Indeed, IL-1RA is involved both in the switch from a pro-inflammatory M1 macrophage polarization state to an anti-inflammatory M2 macrophage subtype and the generation of IL-10-secreting B cells.In summary, the present data showed a new insight into the mechanism of immunosuppression exerted by MSCs to inhibit arthritis progression and highlighted the role of IL-1RA in this effect.

F-3123
SYSTEMIC THERAPEUTIC EFFECT OF PLACENTAL EXPANDED (PLX) CELLS FOLLOWING LOCAL ADMINISTRATION

Pinzur, Lena[1], Zahavi, Efrat[1], **Ofir, Racheli**[1], Aberman, Zami[1], Volk, Hans-Dieter[2], Reinke, Petra[2], Akyüz, Levent[2], Gaberman, Elena[3], Gorodetsky, Raphael[3], Chatterjee, Piyali[4], Mitchell, Brett[4]
[1]Pluristem Therapeutics Inc., Haifa, Israel, [2]Charité - Universitätsmedizin Berlin Institute of Medical Immunology and the Brandenburg Center of Regenerative Therapy (BCRT), Berlin, Germany, [3]Sharett Institute of Oncology, Hadassah - Hebrew University Medical Center, Jerusalem, Israel, [4]Texas A&M Health Science Center/Scott and White Healthcare, Temple, TX, USA

The main dogma in cell therapy postulates that delivery of the cells to the region of interest is an essential prerequisite for the success of the treatment. Thus, direct injection of cells into the injured site is considered a preferred method of their introduction to the organism. We propose here that a systemic effect following intramuscular (IM) cell administration is useful for treatment of distal injured tissues via an endocrine effect of the cell secretome. This systemic therapeutic effect was demonstrated using PLX cells administered IM in a variety of animal models. The systemic therapeutic effect of IM injected PLX was studied in the following animal models: 1) The hind limb ischemia (HLI) C57BL/6 mouse model, in which ischemic damage to one of the hind limbs was caused by femoral artery ligation and dissection. One day after the dissection, PLX cells were administered to either the site of damage in the injured limb or to the contralateral limb; 2) The acute radiation syndrome (ARS) C3H mouse model, in which PLX cells were administrated 1 day and 5 days following an exposure to lethal total body irradiation; 3) Toll like receptor (TLR)-3 or TLR-7 stimulation induced preeclampsia (PE) C57BL/6J mouse model, in which one day following PE induction PLX cells were introduced to gestational day 14 pregnant mice. In the HLI study, both local and contralateral PLX IM treated mice manifested a significant increase in blood flow

rate in the ischemic limb, regardless of the site of injection. When tested in the ARS study, PLX injected IM were able to mitigate the radiation induced bone marrow (BM) damage as evidenced by an increase in survival and body weights of the irradiated mice as well as by increased cellularity of all 3 blood lineages and of BM. The plasma of the experimental animals was collected and studied for the presence of specific human proteins. Human cytokines were transiently elevated in the plasma of PLX-treated irradiated mice compared to PLX-treated non-irradiated mice. In the case of the PE model, PLX introduction IM resulted in normalization of hypertension, proteinuria, endothelial dysfunction and placental necrosis. In conclusion, IM administration of PLX cells, resulting in a systemic therapeutic effect, presents a significant advantage over local cell administration to the site of injury, which is often complicated since the target tissue/organ may be hard to access, requires specialized technical skills, and might be painful. Another benefit over IV administration is that cells may be cleared more slowly thus leading to an increased and prolonged paracrine effect.

F-3124
HUMAN DENTAL PULP STEM CELLS IN PLATELET-RICH PLASMA SCAFFOLD FOR THE TREATMENT OF FULL-THICKNESS ARTICULAR CARTILAGE DEFECTS IN A RABBIT MODEL

Payão, Spencer Luiz Marques[1], Yanasse, Ricardo[1], de Labio, Roger William[1], Orcini, Wilson[2], Kinoshita, Angela[2], Matsumoto, Mariza Akemi[2], Igarashi, Daniel[1], dos Santos, Ricardo Ribeiro[3]
[1]Genetics, Faculdade de Medicina de Marília, Marília, Brazil, [2]Universidade Sagrado Coração, Bauru, Brazil, [3]Hospital São Rafael, Salvador, Brazil

PURPOSE: This study aims to evaluate the capacity of human dental pulp stem cells in a PRP scaffold to regenerate full-thickness cartilage defects in rabbits. METHODS: Full-thickness cartilage defects were created in the patellar groove of 18 rabbits. Two experimental groups were studied: a critical defect group (CTL), and a group in which the defect was filled with stem cells in a PRP scaffold (PRP+SC). The synovial membrane was evaluated histologically. The reparative tissue was evaluated macroscopically and histologically (ICRS Visual Histological Assesment Scale). RESULTS: Enlargement of the Sinovial Lining Layer was greater in the CTL group. Both groups exhibited a low-grade sinovitis at 6 weeks and no sinovitis at 12 weeks. At 12 weeks the CTL group presented a mixture of hyaline cartilage and fibrocartilage and PRP+SC group showed fibrocartilage. There was a tendency of the articular surface of the knees in the PRP+SC group at 12 weeks to be continuous and regular (p=0.06). The PRP+SC group had more repaired cartilage surface covered by Lamina Splendens at 6 and 12 weeks CONCLUSION: Full-thickness cartilage defects treated with human dental pulp stem cells seeded in a PRP gel scaffold showed a cartilage surface repair with the presence of Lamina Splendens and a tendency to be smoother an more regular, however, the repair was limited to fibrocartilage tissue.

F-3125
BONE MARROW AUTOLOGOUS MONONUCLEAR CELLS INFUSION IN CIRRHOTIC PATIENTS DOES NOT IMPROVE LIVER FUNCTION OR HISTOLOGY

Torres, André[1], Bica, Rafael[1], Paula, Tatiana Pereira de[1], Kasai Brunswick, Tais Hanae[2], Gutfilen, Bianca[3], Coelho, Henrique Sérgio Moraes[1], Campos de Carvalho, Antonio C.[4], Goldenberg, Regina[4], **Rezende, Guilherme Ferreira da Motta**[1]
[1]Internal Medicine, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [2]UFRJ, Rio de Janeiro, Brazil, [3]Radiology, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [4]Federal University Rio de Janeiro, Rio de Janeiro, Brazil,

Introduction: Studies in experimental cirrhosis suggest that bone marrow mononuclear cells (BMMC) therapy can improve liver function and reduce liver fibrosis, but it has not been proven in humans. Aim: To evaluate the effect of peripheral infusion of BMMC in liver function and histology of patients with liver cirrhosis due to chronic hepatitis C virus. Methods: BMMC were isolated from bone marrow of 10 patients presenting cirrhosis and moderate liver failure, a mean number of 5.43 x 10^8 cells was infused into a peripheral vein, and BMMC comprised 70.5% of the total cell population. Clinical and laboratory parameters of liver function (albumin, bilirubin, coagulation tests, liver function scores) and samples of liver tissue obtained previously and 30 days after cells infusion were compared. The samples were stained with Hematoxylin and Eosin and Picrossírius Red for morphological analysis; α-SMA, Ki67 and MMP9 were compared by immunohistochemical staining quantification. Results: Two patients were excluded from the analysis due to intense fragmentation of the hepatic tissue. The median age of the 8 patients was 64.5 years, and 50% were male. All the liver samples collected before therapy presented regenerating cirrhotic nodules rimmed by dense fibrosis and variable degrees of Inflammatory activity. One month after cells infusion there was no significant change in any of the analyzed variables, including liver function parameters, degree of inflammation or fibrosis, intensity of stellate cells activation, cell proliferation or extracellular matrix degradation in liver parenchyma. Conclusion: The peripheral infusion of BMMC did not improve liver function or histology of cirrhotic patients with chronic hepatitis C.

F-3126
ATOPIC DERMATITIS COULD BE AMELIORATED BY AUTOLOGOUS ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELL THERAPY

Yoo, Tai June[1], Park, Kee Woan[2], Chae, Byoung Gy[1], Song, Iljun[1]
[1]Yoo Clinic and StemGen Stem Cell Therapy Center, Seoul, Republic of Korea , [2]Line Dermatology Clinic, Seoul, Republic of Korea

The symtoms of Atopic dermatitis were ameliorated by autologous adipose tissue derived mesenchymal stem cells Atopic dermatitis is very difficult disease. This is chronic, itchy, superficial inflammation of the skin, usually occurring in individuals with a personal or family history of allergic disorders. The cause is unknown however the immunologic dysregulation could be the cause of this disorder like many other immunologic illnesses. Adipose tissue derived mesenchymal stem cells do their functions by regeneration of tissues damaged and do their function by immunomodulation through up regulating Treg cell function and down regulating Th1/Th17 cytokines and other chemokines. We have treated 7 AD patients with ADAMSC and obtained great results and we like to pursue this therapy for more patients with severe atopic dermatitis. The initial case who was treated by this therapy received 100 million ADAMSC first and second therapy was done 8 weeks after the initial therapy

and 6 other Patients received 200 million of above ADAMSC three times intravenously. They have all improved their symtoms and signs of atopic dermatitis. The first case patient was allergic to house dust mite, peanut allergy and also soybean allergy. She was not treated for these conditions by her local doctor. She had 7 yrs of history before the infusion of stem cells for the therapy. Patients noted improvement, not itchy and less lesions three days after the infusion of stem cells. Skin was found to be less dry, her total IgE decreased from 243 units to 153 units and eosinophil count decreased from 905 to 420. The interferon gamma level decreased to from 2257 units to 551 unit and TNF alpha level decreased from 34,27 units to zero unit level. IL-6 was decreased from 15.6 units to zero units. The IL-1 beta level was also decreased from 5.63 units to zero units. There were marked improvement of skin lesions and almost all of the skin lesions were disappeared and returned to normal appearance. It is a great success case. The rest of the cases were improved in all cases in varying degrees. Thus, though it is not a rigorous clinical study setting, our preliminary study indicates that the autologous adipose tissue derived stem cells therapy might be a reasonable choice for severe form of atopic dermatitis, provided that if we have a similar results after a well designed clinical trial.

F-3127
THERAPEUTIC EFFICACY OF ALLOGENEIC UMBILICAL CORD BLOOD THERAPY DEPENDING ON THE COMBINED USAGE OF ERYTHROPOIETIN FOR CHILDREN WITH CEREBRAL PALSY RELATED TO PERIVENTICULAR LEUKOMALACIA

Min, Kyunghoon[1], Kim, MinYoung[2]
[1]Rehabilitation Medicine, Bundang CHA Medical Center, Seongnam-si, Gyeonggi-do, Republic of Korea, [2]Rehabilitation Medicine, CHA University, CHA Bundang Medical Center, Seongnam, Republic of Korea

Background:Umbilical cord blood (UCB) therapy has been studied as a restorative avenue for children with cerebral palsy (CP). Erythropoietin (EPO) is known to be neuroprotective to alleviate the neurologic impairments in neonatal encephalopathy. Allogeneic UCB therapy combined with EPO was reported to be beneficial for them. CP can be resulted from various risk factors and different responses would be expected according to the relevant etiologies. This study aims to investigate the effects of combined administration of EPO in allogeneic UCB therapy in children with CP related to periventicular leukomalacia (PVL). Methods:1) Patients:The medical records of children with CP from 2010 until 2013 were reviewed retrospectively and UCB therapy was done in 282 children with CP. Among 282, 83 patients were included with the criteria of (1) CP treated with allogeneic UCB therapy, (2) PVL in brain MRI, and (3) age: below 10 years old or below. The numbers of those who received the combination therapy or sole allogeneic UCB therapy were 73 and 10, respectively. Allogeneic UCB was selected which included at least 3 x 10^7/kg total nucleated cells with being matched for at least 4 of 6 human leukocyte antigen types A, B, and DRB1. EPO was used as an adjuvant to potentiate the effects of UCB therapy. UCB group received EPO intravenously at a dose of 500 IU/kg 2 times and 250 IU IU/kg subcutaneously twice per week for 4 weeks. To enable survival of transfused cells and prevent graft-versus-host disease in allogeneic UCB group, cyclosporin had been used for 4 weeks. Cyclosporin was given intravenously at 3 mg/kg per day for 6 hours twice a day during the first week and oral cyclosporin solution (12 mg/kg per day) was continued for the following 3 weeks. The dose of cyclosporin was adjusted to maintain the target blood level as 100 to 200 ng.ml. The study was approved by the local ethics committee. 2) Outcomes:Changes of motor and cognitive function were measured with Gross Motor Function Measure (GMFM), Gross Motor Performance Measure (GMPM), Bayley Scales of Infant Development II (BSID-II) mental and motor raw scales. Those outcomes were assessed 3 times at baseline and Months 3 and 6. Adverse events had been monitored during 6 months post-UCB therapy. 3). Results: The mean ages of Combination group, who received UCB and EPO, and sole UCB group were 46.2 and 37.0 months respectively. There were no significant differences of age, gestational age, baseline GMFM, GMPM, and BSID-II scores between 2 groups. Compared to sole UCB group, Combination group showed significant better improvements in GMFM at 3 months post-therapy (p=0.004). BSID-II mental and motor raw scores were meaningfully higher at 3 months post-therapy (ps<0.021). There were no serious adverse events during the study period. Conclusion: These results suggest that the combined usage of EPO might be required to augment the efficacy of allogeneic UCB therapy. Further studies with randomized, prospective, larger sample size are required to determine the combined EPO is synergistic to the therapeutic efficacy of allogeneic UCB therapy.

F-3128
HYPOXIC CONDITIONING ENHANCES THE PARACRINE ACTIVITY OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS (ADMSCS)

Mirlashari, Mohammad Reza[1], Josefsen, Dag[1], Wang, Meng Yu[1], Hasvold, Grete[2], Landsverk, Kirsti Solberg[2], Gullestad, Hans Petter[3], Kvalheim, Gunnar[1]
[1]Department of Cellular Therapy, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, [2]Department of Radiation Biology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway, [3]Department of Plastic Surgery, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway

Introduction: Human Mesenchymal Stem Cells (MSCs) were originally proposed for stem cell therapies in regenerative medicine due to their propensity to differentiate into specific cell types. However, MSCs were found to be more supportive of engineering functional tissue constructs through secretion of a spectrum of growth factors and cytokines, termed paracrine factors, which are angiogenic and cytoprotective. MSCs are present in adult tissues, including bone marrow and adipose tissue. For many years, bone marrow-derived stem cells were the primary source of stem cells for tissue engineering applications. However, recent studies have shown that Adipose-Derived Mesenchymal Stem Cells (ADMSCs) provides a clear advantage over other stem cell sources due to higher expression of genes involved in angiogenesis and the ease with which adipose tissue can be accessed as well as the ease of isolating stem cells from harvested tissue. Typically, MSCs are cultured under ambient, or normoxic, conditions (21% O2). However, the physiological niches for MSCs in the adipose tissue and other sites have much lower oxygen tension (<3%). Several studies have investigated effects of reduced oxygen tension on human ADMSCs with remarkably contrasting results. We therefore examined the effects of hypoxia on the production of cytokines, chemokines, growth factors and expression of Hypoxia inducible factor 1α (HIF-1α), Hypoxia inducible factor 2 α (HIF-2α). Further we analysed the effect of hypoxia on the expression of pluripotency transcription factors (Nanog, Oct4 and Sox2). Methods: Automatic Stromal vascular fraction (SVF) extraction was achieved by the Celution system (Cytori Therapeutics, Inc., San Diego, CA, http://www.cytori.com). ADMSCs were isolated from SVF and cultured under normoxia (21% O2) and hypoxia (1% O2) for 48 h. Western blot and flow cytometry were used to analyse HIF-1α, HIF-2α, Nanog, Sox2, Oct4 and expression of surface antigens, recpectively. The Bio-Plex analysis was also used to determine profiles of secreted cytokines (TNF-α, IFN-g, IL-6, IL-8, IL-10) , growth factors (VEGF and bFGF) and chemokines (MCP-1, Eotaxin) in response to hypoxia.

Results: Our result shows that, hypoxia induced stabilization of HIF-1α and increased the secretion of growth factors, chemokines and cytokines from ADMSCs (1.5-2.0 fold compared to normoxia level). But hypoxia did not affect the phenotype, morphology of ADMSCs, and expression of HIF-2α, Nanog, Oct4 and Sox2. Conclusions: In this study we have demonstrated that short term culturing of ADMSCs under hypoxic condition increased secretion of cytokines, chemokines, and growth factors from ADMSCs. In vivo experiments are ongoing and results will be presented. If these data turn out to be positive, ADMSCs cultured under hypoxic conditions will be used for treatment of chronic wounds developing after curative radiotherapy.

F-3129

IMPACT OF TISSUE-ENGINEERED BIOLOGICAL DRESSINGS PRODUCED FROM HUMAN ADIPOSE-DERIVED STEM CELLS ON CUTANEOUS WOUND HEALING IN VIVO

Morissette Martin, Pascal, Maux, Amandine, Fradette, Julie
Centre LOEX de l'Université Laval, Centre de recherche du CHU de Québec - Axe Médecine Régénératrice, Département de Chirurgie, Faculté de Médecine, Université Laval, Québec City, QC, Canada

In a context of an aging population with an increasing proportion of diabetic patients, chronic wounds and skin ulcers are a major issue for health care systems. Several teams investigate the use of stem cells to stimulate wound healing and a lot of interest is given to the effects of their secretome. Previous works have shown that the self-assembly approach of tissue engineering can be used to produce manipulable cell-sheets using adipose-derived stem cells (ASC). During that process, the ASC contained into those cell-sheets can be differentiated into adipocytes; therefore two types of reconstructed tissue may be produced: connective tissues containing ASC and adipose tissues containing differentiated adipocytes. We consider that these reconstructed tissues could be used successfully as biological dressings for the treatment of skin ulcers. However preclinical studies are needed before designing human clinical studies. The hypothesis of our work is that both types of biological dressings would have enhancing effects on skin healing using a murine model. In the present study, we used a K14-H2B-GFP mouse strain (which features a fluorescent epidermis) to follow precisely the migration of keratinocytes into a cutaneous silicone-splinted full-thickness excisional wound model. Experimental groups comprised: i) ASC dressings placed in the wound beds (changed every 3 days); ii) adipose dressings placed in the wound beds (changed every 3 days); and iii) untreated wounds. Objectives: 1 -) Adaptation of the murine silicone-splinted full-thickness excisional wound model compatible with the use of biological dressings; 2-) Determination of wound reepithelialisation kinetics by following keratinocyte migration with a non invasive LuminaIVIS imagery system; 3-) Determination of bioactive molecule profiles produced by the two types of biological dressings before being applied to wounds. Results: 1-) The adaptation of the silicone-splinted full-thickness excisional wound model for dressing changes was achieve with the joint use of Mepitel® , Normlgel® and TegadermFilm®; 2-) Results of two experiments showed that the kinetics of wound reepithelialisation did not differ between the three experimental groups (between 12 and 18 wounds per group); 3-) The preliminary quantification of secreted bioactive molecules by ELISA Duosets kits (R and D systems) revealed higher levels of leptin (3,63 fold), angiopoietin-1 (1,94 fold) and similar levels of HGF, VEGF and PAI-1 in differentiated adipocytes dressings supernatants compared to those of ASC dressings supernatants. These similar secretion profiles are in accordance with the kinetics reepithelialisation results. In conclusion, these results show that the biological dressings do not delay nor accelerate wound reepithelialisation during unimpaired healing. We thus provided an in vivo «proof of safety» for these ASC-based reconstructed tissues. Ongoing analyses will evaluate the quality of the regenerated skin. It will now be important to evaluate their capacity to enhance the healing of chronic ulcers.

F-3130

CELL THERAPY PRODUCTS: DEFINING THE COMMERCIALIZATION PATHWAY

Moyer, Mary Pat
INCELL Corporation LLC, San Antonio, TX, USA

The development path from the lab bench to clinical use of a cell or tissue therapy is complex. Results from our group and others show that the process is accelerated if key elements such as quality systems, Good Manufacturing Practices, raw materials and sourcing, procurement, excipients, pre-clinical in vitro and animal testing, labeling, (cryo) storage, quality control and product release criteria are integrated into a Quality by Design strategy. Proper formulations and a quality assurance approach saves time and costs. These approaches are critical to the goal of developing cost-effective regenerative medicine products that address unmet medical needs of patients around the world.

F-3131

NOVEL THERAPEUTIC STRATEGY FOR DIABETIC NEPHROPATHY USING OPTIMALLY PRECONDITIONED SELF BONE MARROW DERIVED MESENCHYMAL STEM CELLS IN MICE

Nagaishi, Kanna, Konari, Naoto, Ataka, Koji, Nakano, Masako, Ishikawa, Kozoh, Fujimiya, Mineko
Sapporo Medical University, Sapporo, Japan

Diabetic nephropathy is the most detrimental complication for diabetic patients, which comprises renal failure and increased risk of cardiovascular disease. Despite strict glycemic and hypertension controls, 30 to 40% of diabetic patients develop diabetic nephropathy. Major functions of mesenchymal stem cells (MSCs) are repair and regeneration of target tissue and immune-modulation through production of trophic factors, cell supplementation, and cell-cell interaction. Although MSCs have been implicated in renal disorder, the therapeutic mechanism through which they contribute to diabetic nephropathy has not been clarified. Furthermore, MSCs which are derived from diabetic animals (DM-MSCs) decrease the proliferative potency and various cell functions. In this study, we investigated the effects of MSC therapy on diabetic nephropathy with a focus on the role of bone marrow-derived cells (BMDCs) that infiltrate the kidney and its mechanism such as paracrine effects via trophic factors. In addition, we compared the therapeutic effect of non-optimized DM-MSCs and optimized DM-MSCs with novel methods using biologics. Bone marrow derived MSCs, which were isolated from normal rat, were administered to high-fat diet (HFD)-induced type 2 diabetic mice and streptozotocin (STZ)-induced insulin-deficient diabetic mice. MSC-conditioned medium (MSC-CM) was also administered to examine the trophic effects of MSCs on the nephropathy. The therapeutic effects of MSCs were analyzed by assessing urinary albumin-creatinine ratio and histological findings. Kinetics and molecular profile of BMDCs in the kidney were evaluated using bone marrow-chimeric mice which were performed bone marrow transplantation in wild type mice by GFP transgenic mice. DM-MSCs were isolated from streptozotocin (STZ)-induced insulin-deficient diabetic rat and were also administered to diabetic mice. MSC therapy ameliorated the aggravation of urinary albumin-creatinine ratio in spite of very low numbers of MSCs distributing in the kidney. The curative effects of the MSC

and MSC-CM therapies were similar at 8 weeks of treatment despite persistent hyperglycemia in diabetic mice. Both therapies suppressed the abnormal infiltration of BMDCs into the kidney, reversed the excessive expression of proinflammatory cytokines in interstitial cells and prevented glomerular and tubular histopathologic changes, such as mesangial expansion, podocytes damage, endothelial cell degenerations, thickening of basement membrane, tubular dilatation and interstitial fibrosis. MSC-CM included not only various bioactive substances, such as cytokines and growth factors, but also large quantity of exosomes. However DM-MSC did not show therapeutic effects for diabetic nephropathy in both HFD-diabetic mice and STZ-diabetic mice, DM-MSCs which were optimally pre-conditioned with biologics derived from human tissues reverted proliferative potency and regenerative power of damaged kidney. MSC therapy is a powerful tool for ameliorating diabetic nephropathy by inhibiting the inflammatory reactions induced by BMDCs and enhancing tissue regeneration. These effects are likely due to paracrine effects of secretor factors of MSCs. The novel method to optimize abnormal DM-MSCs may provide promising potential to enhance curative effect of auto transplantation of MSCs in diabetic patients.

F-3132
ROLE OF MESENCHYMAL STEM CELLS IN HEPATOCARCINOGENESIS IN RATS

Atta, Hazem Mahmoud[1], **Noorwali, Abdulwahab**[1], Faidaah, Mamdooh[2], Damiati, Laila[2], Filimban, Najlaa[2], Al-Grigry, Mihal[2], Habib, Hamid[3], Radwi, Amer[4], Ali, Naushad[5]

[1]Clinical Biochemistry, King Abdulaziz University, Faculty of Medicine, Jeddah, Saudi Arabia, [2]King Fahd Center for Medical Research, King Abdulaziz University, Stem Cell Unit, Jeddah, Saudi Arabia, [3]Pediatrics, King Abdulaziz University, Faculty of Medicine, Jeddah, Saudi Arabia, [4]Oncology, King Abdulaziz University, Faculty of Medicine, Jeddah, Saudi Arabia, [5]Radiology, King Abdulaziz University, Faculty of Medicine, Jeddah, Saudi Arabia

The use of mesenchymal stem cells (MSCs) in regenerative medicine is expanding. Cell-based therapy is rapidly developing and the recent reports of successful clinical trials add momentum to explore their ability to overcome intractable diseases. It was previously reported that bone marrow-derived MSCs can ameliorate hepatic fibrosis and may improve the molecular signals involved in HCC induction in experimental animals. We intended to confirm and elucidate the role played by MSCs in this process. In this work, we investigated the effect of injection of MSCs, labeled and unlabeled with iron oxide nanoparticles on the molecular signals and the tumor mass in advanced hepatic fibrosis and HCC, in the rat model. Pathologic evidence of hepatic fibrosis and HCC was confirmed. Magnetic resonance imaging (MRI) was used to track MSCs in the affected and control liver. We detected a significant tumor-mass reduction in the group which received MSCs compared to the control group. Time course tracking of labeled MSCs using MRI enabled the confirmation of their role in the therapeutic process. The results of this work may confirm the possible therapeutic potential of MSCs on HCC in rats. Further work is needed to investigate the molecular role played by MSCs in the carcinogenic process.

F-3133
INTRANASAL INSTILLATION OF MESENCHYMAL STEM CELLS FOR IDIOPATHIC PULMONARY FIBROSIS THERAPY: AN ALTERNATIVE EFFECTIVE ROUTE

Silva, Luisa H.[1], Silva, Jaqueline R.[2], Chaves, Sacha B.[1], Lima, Emilia[3], Ricardo, Azevedo B.[1], **Oliveira, Daniela M.**[1]

[1]Genetics and Morphology, Universidade de Brasilia, Brasilia, Brazil, [2]Faculdade de Ciencias Da Saude, Universidade Federal do Para, Brasilia, Brazil, [3]Instituto de Quimica, Universidade Federal De Goias, Goiânia, Brazil

It is known that pulmonary alveoli - the structures responsible for gas exchange - are very delicate and frequently exposed to certain substances (e.g. chemicals, medicines, and smoking) that may injure them. As a result of successive lesions in lung alveoli, many patients develop Idiopathic Pulmonary Fibrosis (IPF): an infirmity that is unresponsive to treatments with steroids and immunosuppressive agents, unlike other inflammatory and fibrotic lung diseases. Thus, lung transplantation is the only possibility that provides a real chance of cure; otherwise, the survival of the patient lasts on 3-4 years. Recently, some papers have suggested the therapeutic potential of Mesenchymal Stem Cells (MSC), demonstrating that these are able to migrate toward the injured sites and to attenuate disease evolution through its anti-inflammatory and immunomodulatory mechanisms. However, there is no agreement about the amount of arriving and remaining MSC in lungs after their administration; which casts doubt on some traditional routes of MSC administration (intravenous or intra-arterial). Therefore the present study aimed to evaluate the efficacy of intranasal administration of MSC in a murine model of IPF, comparing it with intravenous route. Initially, saline solution (control group) or bleomycin sulphate solution (6 mg/kg) were administered intratracheally into black mice (C57BL6) in order to induce of pulmonary inflammation. Then, 24 hours later, MSC labelled with iron oxide nanoparticles coated with DMSA (Fe-DMSA) were injected into the tail vein or administrated by intranasal instillation ($5x10^5$ MSC per animal). During seven days, the weight of the mice were measured. On the eighth day, the animals were euthanized and their lungs were collected for histological analysis; moreover, the staining techniques employed were hematoxylin-eosin (H and E) (to verify alveolar structure changes), Gömöri trichrome (collagen deposition) and Prussian blue staining (to detect MSC labelled with Fe-DMSA). Our results firstly showed a significant weight loss of the animals that received MSC intravenously in comparison with the control group; on the other hand, animals treated intranasally had their weight constant. Since this reduction suggests IPF progression in animals, histological analysis was then performed in order to confirm it: the alveolar structure in intravenously treated animals was thickened; furthermore, there were several lymphocytes infiltrates. Secondly, as expected, were found more MSC in intranasally treated mice's lungs, usually next to the bronchioles. The alveolar integrity verified in these animals suggests the MSC effectiveness in diminishing the inflammatory effects. Interestingly, intravenously treated animals had marked MSC in abundance in spleen and lymphnodes, suggesting that this route of administration fails by retaining large quantities of cells in these organs, thus preventing their arrival in the injured lung tissue and permitting the IPF advancement. A fact that does not happen in intranasally treated animals, since this route is probably the most direct. In summary, administration of MSC by intranasal instillation is a promising alternative for the treatment of IPF considering that MSCs to migrate more easily and directly towards lesioned lung alveoli. This work was supported by CNPq.

F-3134

CONSERVATION OF SIALYLLACTOSAMINYL GLYCAN EXPRESSION IN HUMAN MESENCHYMAL STEM CELLS DERIVED FROM DIFFERENT SOURCES

Pachon Peña, Olga Gisela, Ruiz-Cañada, Catalina, Sackstein, Robert
Dermatology, Brigham and Women's Hospital/Harvard Medical School, Boston, MA, USA

Introduction. Human Mesenchymal stem cells (hMSC) from bone marrow have therapeutic potential in augmenting hematopoietic recovery following bone marrow transplantation, as well as in tissue engineering and regenerative medicine. Concurrently, other sources of hMSCs (e.g., from adipose tissue) are emerging for use in regenerative medicine. However, there is little known regarding the glycome of hMSCs from marrow versus adipose sources. In particular, we sought to determine whether adipose-derived MSC (AMSC) express E-selectin ligands and, if not, if they have similar expression of sialylated lactosaminyl glycans as compared with marrow-derived MSC (MMSC). Methods. AMSC were isolated from adult human subcutaneous adipose tissue according to published protocols (Pachón et al., 2011). To evaluate E-selectin ligand expression, cells were analyzed by flow cytometry for expression of sLex determinants (antibody HECA452) and for E-selectin-Ig (E-Ig) chimera reactivity. To enforce expression of E-selectin ligands on AMSC, we used the α-(1,3)-fucosyltransferase VII (FTVII) in presence of fucose substrate (GDP-Fucose). Surface markers expression regarding MSC yield, expression of sLex epitopes and E-Selectin-ligand activity was analyzed after enforced α-1,3-fucosylation. Capacity of AMSC to support lymphocyte binding was also analyzed by Stamper-Woodruff Asssay as well as proliferation capacity, osteogenic and adipogenic differentiation potential were therefore tested for AMSC cultured after FTVII treatment. Results. Flow cytometry analysis showed that AMSCs expressed surface markers characteristic of MMSC (CD90, CD105, CD73, CD44; no CD45 or CD34). Similar to MMSC, AMSC had no reactivity with E-Ig or with HECA452, but FTVII treatment induced marked HECA452 and E-Ig reactivity; western blot showed enforced expression of the CD44 glycoform "Hematopoietic cell E-/L-selectin Ligand" (HCELL), with abrogation of E-Ig and HECA452 reactivity following with N-glycosidase F treatment. Moreover, our findings demonstrate that Bromelain digestion on FTVII treated AMSCs, decreased CD44 expression and HECA452 reactivity significantly (data were confirmed by flow cytometry and western blot); all of these data together indicate that sialofucosylated determinants have been created principally on membrane glycoproteins, not glycolipids. Interestingly, we have also found an adipogenic and osteogenic differentiation potential increased on FTVII treated AMSCs compared with untreated cells as well as a significant increase of the cell proliferation capacity. Conclusion. AMSC have similar expression of sialyllactosaminyl glycans as MMSCs, and equally convert native CD44 into HCELL following exofucosylation.

F-3135

PLACENTA-DERIVED MESENCHYMAL STEM CELLS-INDUCED REJUVENATING ACTION AND SUBSEQUENT MICROBIOTA CHANGES LED TO THE PREVENTION OF HELICOBACTER PYLORI-INDUCED GASTRIC CANCER

Park, Jong-Min[1], Han, Young-Min[1], Chung, Mi-Kyung[1], Kwon, Sung-Hun[1], Kim, Hojin[2], Choi, Yong-Soo[3], Hahm, Ki Baik[1]
[1]*Department of Gastroenterology and Cancer Prevention Research Center, CHA University School of Medicine, Seongnam, Republic of Korea,* [2]*CHA Stem Cell Institute, CHABio and Diostech Co., Ltd., Seoul, Republic of Korea ,* [3]*Department of Applied Bioscience, CHA University, Seongnam, Republic of Korea*

H. pylori infection triggered diverse clinical entities including mild gastritis to serious gastric cancer, in which eradication is prerequisite for either preventing recurrence of gastroduodenal ulcers or remission of MALT lymphoma, but the contribution to revert premalignant lesion through eradication is still under debate. *H. pylori* associated chronic atrophic gastritis (CAG) was known to be precancerous lesions associated with the changes of gastric microenvironment including microbiota. We have hypothesized mesenchymal stem cells (MSCs) administration might rejuvenate *H. pylori*-associated atrophic changes, potentiate microbiota, and prevent gastric cancer. We have administered MSCs called Plastem established from placenta chorioallantoic membrane or conditioned media (CM) around 24 weeks in *H. pylori* infected and high salt diet administered mice model and sacrificed at 36 weeks, when CAG and gastric tumors were developed. Plastem MSCs given *iv* and CM, *ip*, effectively ameliorated the degree of *H. pylori*-associated CAG as well as incidence of tumorigenesis, in which significant amelioration of inflammatory mediators as well as significant enforcement of regenerations were noted. Atrophic changes of stomach after *H. pylori* led to overt changes of gastric microbiota, whereas no significant changes were seen in MSC groups correlated with pathologic changes. In conclusion, rejuvenation of *H. pylori*-associated atrophic changes with MSC administration drastically improved gastric microenvironment and resulted in significant prevention of *H. pylori*-associated gastric cancer in mice model.

F-3136

TREATMENT OF COLLAGEN-INDUCED ARTHRITIS USING IMMUNE MODULATORY PROPERTIES OF HUMAN MESENCHYMAL STEM CELLS

Park, Kyu-Hyung, Mun, Chin Hee, Kang, Mi-Il, Lee, Sang-Won, Lee, Soo-Kon, **Park, Yong-Beom**
Division of Rheumatology, Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea

Objectives: Mesenchymal stem cells (MSCs) have immune modulatory properties. We investigated the potential therapeutic effects of human bone marrow (BM)-, adipose tissue (AD)-, and cord blood (CB)-derived MSCs in an experimental animal model of rheumatoid arthritis (RA), and explored the mechanism underlying immune modulation by MSCs. Methods: We evaluated the therapeutic effect of clinically available human BM-, AD- and CB-derived MSCs in DBA/1 mice with collagen-induced arthritis (CIA). CIA mice were injected intraperitoneally with three types of MSC. Treatment-control animals were injected with 35 mg/kg methotrexate (MTX) twice weekly. Clinical activity in CIA mice, degree of inflammation, cytokine expression in the joint, serum cytokine levels, and regulatory T cells (Tregs) were evaluated. Results: Mice treated with human BM-, AD-, and CB-MSCs showed significant improvement in clinical joint score, comparable to MTX-treated mice. Histologic examination showed greatly reduced joint inflammation and damage in MSC-treated mice

compared with untreated mice. Microcomputed tomography also showed little joint damage in the MSC-treated group. MSCs significantly decreased serum interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, and interferon-γ and increased IL-10 and transforming growth factor-β levels. Tregs were increased in mice treated with MSCs compared to untreated or MTX-treated mice. Conclusion: Human BM-, AD-, and CB-MSCs significantly suppressed joint inflammation in CIA mice. The cells decreased pro-inflammatory cytokines and upregulated anti-inflammatory cytokines and induced Tregs. Therefore, our study suggests that the use of human BM-, AD-, and CB-MSCs could be an effective therapeutic approach for RA. This study was supported by a grant of the Korean Health Technology R and D Project, Ministry of Health and Welfare, Republic of Korea. (HI13C1270)

F-3137
IDENTIFICATION OF INFLAMMATORY CYTOKINES RELEASED BY HUMAN TUBE MESENCHYMAL STROMAL CELLS

Pelatti, Mayra[1], Gomes, Juliana[2], Perin, Paulo[3], Czeresnia, Carlos[4], Rodrigues, Elaine[1], Zatz, Mayana[2], Jazedje, Tatiana[2]

[1]Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo, São Paulo, Brazil, [2]Human Genome and Stem Cell Research Center, Institute of Bioscience, University of São Paulo, São Paulo, Brazil, [3]Specialized Center for Human Reproduction, São Paulo, Brazil, [4]Celula Mater, São Paulo, Brazil

Recent studies have shown that Mesenchymal Stromal Cells (MSCs) can have a therapeutic effect against cancer, releasing pro-inflammatory molecules that would act directly in the tumor microenvironment, inhibiting its growth. In opposition, other studies report increase in tumor volume and worsening of symptoms when MSCs are co-injected with tumor cells. These antagonistic effects indicate that the relationship of MSCs and tumor microenvironment are still poorly understood. The discordance between observed results could be explained by the different origins of MSCs that were injected, the different methods of cell inoculation (co-injections, intravenous or intraperitoneal injections) and the variances between the used animal models. The aim of this study is to analyze the cytokines released by "in vitro" co-cultivation of murine mammary adenocarcinoma cells (4T1 lineage) and human breast adenocarcinoma cells (MCF7 lineage) with human tube MSCs (htMSCs). Three htMSCs lineages obtained from tubal ligation/resection samples collected during the proliferative phase from fertile women, which were characterized by cytometry and differentiation potential in vitro were used in this study. htMSC lineages were co-cultured for 48 hours with tumor cells in the proportion 1:1. Inserts were used in order to avoid the contact between the tumor cells and htMSCs. Human tube MSCs RNA was extracted for analysis by PCR-array and the supernatant was collected for cytokine levels by multiplex immune assay. Our preliminary results suggest that the co-culture of htMSCs and tumor cells stimulate the release of cytokines with pro-inflammatory profile. When compared to MCF7 cells cultured alone, co-cultures of MCF7 and htMSCs showed increased levels of TNF-a, IFN-y, IL-13. We also observed a reduction of cytokines related to angiogenesis, such as VEGF, PDGF-BB and bFGF, in co-cultures, while MCF7 cells cultivated alone released a larger amount of these cytokines. Compared to 4T1 cells cultured alone, co-cultures of 4T1 and htMSCs further increased production of pro-inflammatory cytokines. It was not possible to measure cytokines released only by 4T1 murine cells, since the available assay is specific for human molecules. Our results support previous results from our group, where we observed that htMSCs intraperitoneally injected into immune-competent mice decreased tumor growth, increased their survival, reduced the number of metastases and the levels of inflammation in the lungs.

F-3138
IN VIVO FLUORESCENCE IMAGING AND ENDOMICROSCOPY FOR TRACKING OF MESENCHYMAL STEM CELLS IN POST-RADIOTHERAPY LUNG INJURY RAT MODEL

Perez, Jessica Rika[1], Ybarra, Norma[2], Maria, Ola[2], Chagnon, Frederic[3], Lesur, Olivier[3], El Naqa, Issam[4]

[1]Biomedical Engineering, McGill University, Montreal, QC, Canada, [2]Medical Physics, Montreal General Hospital, Montreal, QC, Canada, [3]Sherbrooke University Hospital, Sherbrooke, QC, Canada, [4]Medical Physics, McGill University Montreal General Hospital, Montreal, QC, Canada

Purpose: Mesenchymal stem cells (MSCs) are a very promising tool for protection and recovery of radiation-induced lung injury in lung cancer patients receiving radiotherapy. However their clinical translation is hindered by our poor understanding of their mechanism of action, homing properties, and ideal routes of injection. In this work, we used two in vivo imaging techniques, whole body fluorescence imaging (WBFI) and endomicroscopy to uncover the in vivo properties of these cells that can lead to clinical translation. Methods: MSCs labeled with membrane dyes DiD or DiR were expanded and imaged in vitro with an optical imaging system (In-Vivo Xtreme, Bruker). DiR-labeled MSCs were injected intraperitoneally or intravenously in Sprague Dawley rats for WBFI. Subsequently, organs were harvested and imaged ex vivo. Images were acquired at excitation and emission wavelengths of 730 nm and 790 nm respectively. Regions of interest were highlighted and the mean fluorescence intensity (MFI) was recorded. Endomicroscopy (Cellvizio, Mauna Kea Technologies) was performed on a separate set of control animals with the fiber optic probe inserted by tracheotomy. DiD-MSCs were injected at the time of imaging and images were acquired in real time as the probe moves in the lung cavity. Important imaging features such as the number of MSCs and their localization within the tissue were computed from those images.In parallel we have established a rat model of lung damage involving hemithorax radiation of 16 Gy. Results:In vitro results showed that MSCs could be efficiently labeled in culture. DiR was the preferred dye for WBFI due to its near infrared optical properties giving better light tissue penetration. Another dye, DiD was required for endomicroscopy as its optical properties were compatible with available channels (660 nm). We observed dye dilution overtime but no effect on cell survival compared to control. Our in vivo WBFI results showed that we were able to image DiR-MSCs injected intraperitoneally in a rat model with a MFI of 2.7×10e8 P/s/mm2. Even though we were unable to observe a WBFI signal when MSCs were injected intravenously, we observed a fluorescence signal ex vivo in the lungs with a MFI of 8.5×10e7 P/s/mm2. Despite a successful use of DiR for WBFI, the sensitivity of this technique for MSCs tracking in vivo remains challenging. We therefore investigated the use of microendoscopy for real time MSCs fluorescence imaging within the lungs. In contrast to WBFI, which does not provide cellular resolution, endomicroscopy provides a confocal fluorescence microscopy lateral resolution of 3.3 um within the lung. Using a fluorescent blood vessel marker, we established that the endomicroscope has the required sensitivity to detect lung injury and treatment efficacy as we were previously able to detect vasculature architecture and permeability variations. We are currently investigating the in vivo behavior of DiD-labeled MSCs in the lung upon intravascular or endotracheal injection. Conclusion: In this work, we have evaluated fluorescent cell labeling methods to image MSCs in vivo. We established the superiority of endomicroscopy to WBFI for in

vivo MSCs tracking at the cellular level. As real time imaging allows us to follow the distribution of MSCs within the lung, endomicroscopy is a promising new method that will help monitor efficacy and determine optimal delivery routes for MSC-mediated lung regeneration post-radiotherapy in our rat model of radiation induced lung damage.

F-3139
HUMAN ESC DERIVED MESENCHYMAL STEM CELLS ATTENUATE EXPERIMENTAL AUTOIMMUNE UVEITIS

Qin, Yu[1], Chan, Ann[1], Kouris, Nicholas[2], Nastke, Maria-Dorothea[2], Kimbrel, Erin[2], Ashki, Negin[1], Wang, Wei[1], Lanza, Robert[3], Levinson, Ralph[1], Gordon, Lynn[1]

[1]Ophthalmology, University of California Los Angeles, Los Angeles, CA, USA, [2]Advanced Cell Technology, Marlborough, MA, USA, [3]Advanced Cell Technology, Worcester, MA, USA

Purpose: Mesenchymal stem cells (MSCs) have significant tissue regeneration potential as well as immunomodulatory properties, exerted by direct contact and in a paracrine fashion; therefore MSC therapy is explored as a promising treatment for autoimmune disease. Uveitis is a category of inflammatory diseases that affects humans and is a significant cause for vision loss. Here, we investigate the effects of human embryonic stem cell-derived MSCs (hESC-MSCs) on experimental autoimmune uveitis (EAU), a murine model of uveitis. Methods: EAU was induced in mouse by peptides of the interphotoreceptor retinoid binding protein (IRBP). B10RIII mice were immunized with 50μg IRBP 161-180 and C57BL6 mice with 500μg IRBP 1-20 in the presence of 1.5μg immune adjuvant pertussis toxin. Intraperitoneal injections of 5 million hESC-MSCs were performed on day 0 or day 7. Clinical exams were performed at the peak of EAU and mice were euthanized for histology analysis. Results: Treatment of hESC-MSCs on day 0 significantly decreased EAU histology scores in B10RIII ($p=0.04$) and C57BL6 ($p=0.0001$) mice, and clinical exam scores in C57BL6 ($p=0.0002$) mice compared to untreated control EAU mice. Treatment of hESC-MSCs on day 7 had a tendency of reducing EAU inflammation in mice; however, the effect was not statistically significant. Conclusion: Early systemic treatment of hESC-MSCs ameliorated both severe (B10RIII) and mild (C57BL6) EAU in murine models. Additional work is needed to fully understand the mechanism of attenuation and whether MSCs can be used during periods of active uveitis to control disease.

F-3140
HUMAN MULTIPOTENT ADULT PROGENITOR CELLS STIMULATE THE HEMATOPOIETIC COLONY FORMATION CAPACITY IN PATIENTS WITH BONE MARROW FAILURE

Roobrouck, Valerie[1], Broekaert, Dorien[1], Vanwelden, Thomas[1], Sels, Kathleen[1], Delforge, Michel[2], Verfaillie, Catherine[1]

[1]Development and Regeneration (Stem Cell Institute), Catholic University Leuven, Leuven, Belgium, [2]Hematology, University Hospitals Leuven, Leuven, Belgium

Myelodysplastic syndromes (MDS) represent a heterogeneous group of clonal hematopoietic stem cell (HSC) disorders characterized by ineffective hematopoiesis and peripheral cytopenias. The etiology of MDS is not fully elucidated, but appears to be multifactorial based on two crucial elements: intrinsic lesions in the hematopoietic stem/progenitor cell (HS/PC) and an aberrant bone marrow (BM) microenvironment. The majority of MDS patients receive supportive care therapy, including blood transfusions, growth factors, epigenetic and immunomodulating drugs. However, lack of efficacy in a significant percentage of patients together with the high cost for society and the long-term toxic effects of these drugs, indicate the need for better therapies. Human Multipotent Adult Progenitor Cells (hMAPC) are non-hematopoietic stromal stem cells derived from BM which secret various growth factors and have potent immunomodulatory effects. Evaluate the potential of hMAPC as a novel stem cell-based therapy for patients with BM failure. The hypothesis is that hMAPC will increase blood cell production by secretion of growth factors, by suppressing proinflammatory cytokines and by improving stromal interactions with HSCs, in order to reduce cytopenias in MDS patients. BM samples from the iliac crest of MDS patients and age-matched controls were collected and mononuclear cells isolated by density gradient centrifugation. A colony forming cell (CFC) assay was initiated by plating the mononuclear cell fraction in methylcellulose, supplemented with growth factors, with or without the addition of hMAPC provided in transwells above the culture. Hematopoietic colonies were scored 14 days later. The frequency of primitive hematopoietic progenitor cells was calculated by plating 15,000 $CD34^+$ cells on stromal AFT feeders for five weeks, with or without the addition of hMAPC in different conditions. Then, cells were replated in methylcellulose and colonies scored after 14 days. In addition, DNA was collected from colonies of female patients and used for clonal analysis. Here, we evaluated the X inactivation pattern of the human androgen receptor gene on the maternal and paternal allele of the X chromosome. Finally, irradiated hMAPC were used as stromal feeders to evaluate their potential in maintaining the hematopoiesis of diseased BM cells. The CFC assay with MDS BM cells showed an increase in granulocyte/macrophage colonies when hMAPC were added to the culture. Similarly, a higher percentage of primitive hematopoietic progenitor cells was obtained when $CD34^+$ cells from these patients were plated on feeders in the presence of hMAPC. Clonal analysis of the colonies is ongoing to determine whether hMAPC do not stimulate the proliferation of MDS blasts. Furthermore, hMAPC can maintain the hematopoiesis of MDS patients, albeit at low levels and only supporting the differentiation of granulocyte/macrophage progenitors. Human MAPC exert a positive effect on the *in vitro* colony forming capacity of hematopoietic cells derived from MDS patients, mainly on the granulocyte/macrophage progenitors. This effect was seen when the cells were provided in a transwell above the culture, indicating the importance of secreted factors. Clonal analysis of these colonies is needed to exclude leukemic evolution of blasts. Human MAPC, provided as feeders, were also able to sustain the differentiation of granulocyte/macrophage progenitors.

F-3141
EQUINE PLATELET LYSATE AS ALTERNATIVE TO FETAL BOVINE SERUM IN EQUINE MESENCHYMAL STROMAL CELL CULTURE

Russell, Keith[1], Koch, Thomas G.[2]

[1]Biomedical Science, University of Guelph, Guelph, ON, Canada, [2]University of Guelph, Guelph, ON, Canada

Mesenchymal stromal cells must be expanded to sufficient numbers *in vitro* for emerging tissue regeneration therapies. Presently, expansion media for mesenchymal stromal cells is supplemented with fetal bovine serum. However, potential issues associated with fetal bovine serum are high cost, high variability in batch to batch composition, and risk of complications from bovine antigens. Platelet lysate has shown promise as an alternative supplement to fetal bovine serum in human studies. To determine how equine mesenchymal stromal cells grow in medium enriched with platelet lysate at various concentrations in comparison to fetal bovine serum. A dose titration study was performed comparing platelet lysate to fetal bovine serum in mesenchymal stromal cell expansion medium using two proliferation assays: resazurin and WST-1.

Platelet concentrate was generated from 5 equine whole blood samples through a double centrifugation method, followed by a freeze/thaw cycle to produce platelet lysate, and subsequently pooled. A pool of 5 batches of fetal bovine serum was also prepared. Proliferation assays were performed in 96 well plates with cord blood-derived mesenchymal stromal cells from 5 lines after 4 days in expansion medium with 2.5% to 30% platelet lysate or fetal bovine serum. Platelets counts varied considerably among the samples before standardizing the platelet concentrates at 1×10^6 platelets/μL. Cells cultured in platelet lysate showed higher proliferation levels when compared to those cultured in fetal bovine serum up to a 20% concentration. Both treatments showed a dose-dependent response up to a concentration of 20%, whereas proliferation plateaued in the platelet lysate treatment beyond this concentration. We determined that expansion medium enriched with platelet lysate can support the proliferation of equine mesenchymal stromal cells. It must be acknowledged that when PC and MSC are applied simultaneously as regenerative agents, their effect may not be additive as one might expect. However, overall, these results suggest platelet lysate could be recommended as a suitable supplemental alternative as it performed, at typical working concentrations, equal to fetal bovine serum.

F-3142

IL-8 ENHANCES ANGIOGENESIS OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS BY INCREASING VASCULAR ENDOTHELIAL GROWTH FACTOR

Ryu, Chung Heon, Kim, Seong Muk, Jeong, Chang Hyun, Jeun, Sin Soo

Postech-Catholic Biomedical Engineering Institute, Seoul, Republic of Korea

The beneficial effects of mesenchymal stem cells (MSCs) are mediated partly by the paracrine production of cytoprotective and trophic factors. Vascular endothelial growth factor (VEGF) is released from MSCs as a paracrine trophic factor and contributes to the therapeutic effects of the stem cell by regulating angiogenesis and promoting revascularization in injured tissues. Interleukin-8 (IL-8), an inflammatory chemokine with potent proangiogenic properties, is upregulated in the ischemic brain and has been shown to promote homing of bone marrow-derived cells to injured sites. However, the effect of IL-8 on MSCs paracrine function remains unknown. In this study, we found that IL-8 induced VEGF production and phosphorylation of Akt and ERK. Both effects could be blocked by inhibitors (LY294002, PD098059) or siRNA-mediated silencing of Akt and ERK in human bone marrow MSCs (hBM-MSCs). IL-8-induced VEGF production in hBM-MSCs significantly increased tube formation on Matrigel compared with basal secreted VEGF. In a rat stroke model, administration of IL-8-treated hBM-MSCs decreased the infarction volume and increased angiogenesis in the ischemic boundary zone compared with hBM-MSC treatment alone. In conclusion, these results demonstrate that IL-8 stimulates VEGF production in hBM-MSCs in part via the PI3K/Akt and MAPK/ERK signal transduction pathways and that administration of IL-8-treated hBM-MSCs increases angiogenesis after stroke. This approach may be used to optimize MSC-based therapies for numerous diseases including stroke, myocardial ischemia, and spinal cord injury.

F-3143

SAFETY AND DOSING OF AUTOLOGOUS MESENCHYMAL STEM CELL NEURAL PROGENITORS INJECTED INTRATHECALLY IN MULTIPLE SCLEROSIS PATIENTS: RESULTS OF A PILOT STUDY

Sadiq, Saud, Harris, Violaine

Tisch Multiple Sclerosis Research Center of New York, New York, NY, USA

Multiple sclerosis (MS) is a chronic demyelinating autoimmune disease that afflicts more than 2.3 million people worldwide, and is the most common cause of disability in young adults, excluding trauma. The pathological course of MS involves an early predominantly inflammatory demyelinating disease phase (relapsing remitting MS) for which most current MS treatments are approved. Over time, the disease evolves into a progressive degenerative stage (secondary progressive MS) associated with axonal loss and scar formation, causing sustained physical and cognitive disability. Furthermore, a subset of patients display progressive disease from the onset (primary progressive MS). Thus, there is a critical unmet need for therapies that can stop or reverse the progression of MS through strategies of remyelination, neuronal repair, and regeneration. Mesenchymal stem-cell derived neural progenitors (MSC-NPs) represent an autologous, neural subpopulation of bone marrow MSCs. Intrathecally injected MSC-NPs resulted in improved neurological function in mice with experimental autoimmune encephalomyelitis (EAE), the mouse model of MS. The objective of this study was to optimize the clinical translation of autologous intrathecal MSC-NPs through a chart review of initial clinical experiences with this treatment in MS patients. This preliminary clinical study was conducted as an exploratory procedure to inform safety and dosing of this novel cellular therapy. Retrospective data on the short-term and long-term safety of intrathecal injections of autologous MSC-NPs in 7 patients with progressive forms of MS was analyzed as an IRB-approved chart review. All patients gave informed consent. Subjects were evaluated for degree of disability and adverse events before and after each treatment. The seven MS patients received between 2 and 5 treatments of intrathecal autologous MSC-NPs. Dose ranged from $5x10^4$ to $1.6x10^7$ cells, and treatments were spaced an average of 5 months apart. All patients have been followed to the present time (mean 6.6 years). There were no serious adverse events reported. Of the 22 treatments, mild fever was reported once, mild headache was reported 4 times, and a moderate headache lasting approximately 1 week was reported once, all resolving with an analgesic. Five of the 7 patients showed a measureable clinical improvement following MSC-NP injections. Based on these preliminary results, it was determined that MSC-NP doses of between 2 and 10 million cells would be consistently safe and possibly effective. Preliminary data obtained from this treatment resulted in FDA approval for a phase I study of MSC-NP treatment for 20 MS patients.

F-3144

THERAPUTIC EFFECT OF BDNF-EXPRESSING HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL IN THE CONTUSED RAT SPINAL CORD

Sang In, Park

Institute of Catholic Integrative Medicine, Incheon, Republic of Korea

Mesenchymal Stem Cells (MSCs) transplantation has been proposed as a strategy for spinal cord injury (SCI) repair. The aim of this study was to evaluate the efficacy in adult rat contused spinal cord of adenovirus vector-mediated brain-derived neurotrophic factor (BDNF) gene transfer to human umbilical cord blood (hUCB)-derived MSCs. We efficiently were engineered MSCs secreted of BDNF via

adenoviral transduction mediated by cell-permeable peptides. SCI was induced by contusion using a weight-drop at the level of T9 and then the MSC-BDNF cells were transplanted into the boundary zone of injured site at 1week after injury. BDNF production by MSC-BDNF cells was greater than seen in uninfected MSCs. MSC-BDNF showed significantly more functional recovery than did control rats following SCI. MSC-BDNF cells at 8 weeks after transplantation were found to survive and preferentially localize to near the injury site. Also these cells were differentiated lineage. These data suggest that MSC transfected with BDNF gene may be useful in the treatment of SCI and may represent a new strategy for the treatment of SCI.

F-3145
INTRAPERITONEAL ADMINISTRATION OF HUMAN MESENCHYMAL STEM CELLS RESTRAINS STATUS EPILEPTICUS INDUCED NEURODEGENERATION AND INFLAMMATORY REACTION IN THE HIPPOCAMPUS

Shetty, Ashok K., Hattiangady, Bharathi, Shetty, Geetha, Mishra, Vikas, Choi, Hosoon, Yu, Ji Min, Prockop, Darwin J., Kodali, Maheedhar, Shuai, Bing, Rao, Xiolan

Institute for Regenerative Medicine, TAMHSC College of Medicine at Scott and White, Temple, TX, USA

Status epilepticus (SE), a common neurological disorder typified by severe and prolonged seizure activity, can result from many origins including head trauma, stroke and tumors. Hippocampal neurodegeneration and inflammation, the most noticeable structural modifications induced by SE, is often implicated in initiating epileptogenesis. Administration of a combination of antiepileptic drugs is generally efficient for containing acute seizures. However, they fail to thwart SE-induced neurodegeneration, inflammation and development of chronic temporal lobe epilepsy (TLE). Although numerous causes have been proposed for SE-induced development of TLE, its link to hippocampal alterations such as the loss of GABA-ergic interneurons and failure of inhibition, neuroinflammation typified by increased levels of pro-inflammatory cytokines, reactive astrocytes and activated microglia, and aberrant neurogenesis have received considerable support. Memory and mood impairments after SE are mostly linked to the loss of hippocampal principal neurons and altered hippocampal neurogenesis. Hence, an ideal interventional therapy for SE ought to be capable of protecting the remaining hippocampal principal neurons and subclasses of GABA-ergic interneurons, diminishing the inflammatory reaction and preserving the normal pattern of hippocampal neurogenesis. We investigated the efficacy of intraperitoneal (i.p.) administration of human mesenchymal stem cells (hMSCs) for preventing SE-induced inflammatory reaction and neurodegeneration in the hippocampus. We induced SE in young male F344 rats via graded i.p. injections of kainic acid (3 mg Kg/hour for 3-4 hrs). An hour after the induction of SE, cohorts of rats exhibiting SE received an i.p. injection of either vehicle (VEH) or hMSCs (10 million cells in 1 ml). An injection of diazepam (5 mg/Kg) terminated behavioral seizures in both VEH and hMSC treated animals two hours after the induction of SE. In comparison to naive controls, rats receiving VEH after SE exhibited increased levels of pro-inflammatory cytokines (IL-1ß, TNF-α and IL6, $p<0.01$-001) and myeloperoxidase (MPO derived from infiltrating neutrophils, $p<0.0001$) in the hippocampus at 24 hours post-SE. They also displayed significant loss of neuron-specific nuclear antigen+ principal neurons ($p<0.01$-0.001) and GABA-ergic interneurons expressing parvalbumin ($p<0.001$) and neuropeptide Y ($p<0.001$) in the dentate gyrus at 7 days post-SE. In contrast, rats receiving hMSCs after SE displayed pro-inflammatory cytokines and hippocampal principal neurons to levels seen in naive controls, decreased MPO levels, and reduced loss of GABA-ergic interneurons expressing parvalbumin and neuropeptide Y. Quantitative PCR showed enhanced expression of genes encoding anti-inflammatory cytokines (IL4 and IL10) and alternative activation of microglia (Chi3l3, MRC1 and ARG1) in rats receiving hMSCs. These rats also exhibited reduced numbers of ED1+ activated microglia than VEH treated rats in the hippocampus ($p<0.05$) at 7 days post-SE. Thus, early hMSC treatment after SE is efficacious for restraining the surge of pro-inflammatory cytokines and MPO, the loss of principal and GABA-ergic neurons, and activation of microglia in the hippocampus. As both inflammation and loss of GABA-ergic interneurons contribute towards the evolution of SE-induced injury into TLE, hMSC treatment after SE appears beneficial for restraining SE-induced TLE and related co-morbidities.

F-3146
USAGE OF WHARTON'S JELLY-MSC FOR TREATMENT OF OSTEOPOROSIS IN NOD MICE

Shyu, Jia-Fwu[1], Cheng, Jung-Tzu[1], Chu, Tzu-Hui[1], Chen, Wei-Yu[1], Wang, Hwai-Shi[2], Chen, Tien-Hua[2]

[1]Biology and Anatomy, National Defense Medical Center, Taipei, Taiwan, [2]Anatomy and Cell Biology, National Yang Ming University, Taipei, Taiwan

Osteoporosis (OP) and diabetes both cause significant impairments to the patients. Growing number of studies have confirmed the association between these two diseases. Impaired bone formation has been proposed as a major factor in Type 1 diabetes mellitus (T1DM), probably because of the lack of insulin and insulin-regulated bone anabolic factors. In this study, we transplanted Wharton Jelly mesenchymal stem cells (WJ-MSCs) into hyperglycemic non-obese diabetic (NOD) mice. We hypothesized that systemic infusions of WJ-MSCs promote a bone anabolic effect in osteoporotic T1DM rodents. In NOD mice received WJ-MSCs transplantation, the blood glucose was decreased and the blood insulin was increased as compared to saline control group. The result of micro-CT analysis of trabecular bone of the fifth lumbar vertebrate and distal femur showed decrease bone volume in hyperglycemic NOD mice as compared with euglycaemia group. WJ-MSC-treated NOD mice showed increased bone volume. In these mice, increased bone formation was found as indicated by increase bone formation marker P1NP and dynamic bone histomorphometry. In addition, we found decreased sclerostin and increased Wnt10b in blood of WJ-MSC-treated NOD mice. In conclusion, we demonstrated that transplantation of WJ-MSCs is able to reverse hyperglycemia and increase bone volume by increasing bone formation in NOD mice. Because of MSCs from the umbilical cord can be easily isolated and expanded in culture, these cells may be a useful new source of cellular therapies for OP with T1DM.

F-3147
TRANSPLANTATION OF HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS LEADS TO BEHAVIORAL IMPROVEMENT AND HISTOLOGICAL RESTORATION IN STROKE-DAMAGED RATS

Oh, Seung-Hun[1], Roh, Jeong-Eun[2], Choi, Chunggab[2], Jeong, Yongwoo[2], Lee, Nayeon[2], Chang, Da-Jeong[2], Jeon, Iksoo[2], Kim, Hyun Sook[1], Lee, Youngjun[3], Choi, Yong-Soo[3], Kim, Ok-Joon[1], **Song, Jihwan**[2]

[1]Department of Neurology, CHA Bundang Medical Center, CHA University, Gyeonggi-do, Republic of Korea , [2]CHA University, Seoul, Republic of Korea , [3]Department of Applied Bioscience, CHA University, Gyeonggi-do, Republic of Korea

Mesenchymal stem cells (MSCs) are attractive allogeneic cell source for treating various intractable diseases. In this study, we investigated

therapeutic effects of GMP-grade human umbilical cord-derived MSCs (hUC-MSCs) in a rodent model of middle cerebral artery occlusion (MCAo). hUC-MSCs were injected intravenously at 24 hr after MCAo, and the transplanted animals were examined up to 8 weeks using various behavioral tests, and immunohistochemical analysis was performed afterwards. First of all, transplanted animals exhibited significant behavioral improvements in rotarod, stepping and modified neurological severity score (mNSS) tests. We also found that transplanted hUC-MSCs can contribute to the reduction of infract volume, glial scar formation and apoptosis. They were also shown to play an important role in the modulation of inflammatory response. We also observed that the number of proliferating neuroblasts significantly increases in the subventricular zone (SVZ), implying that transplanted hUC-MSCs also promotes some aspects of neurogenesis. Taken together, these results provide strong evidence that hUC-MSCs are functional in vivo and may serve as useful tools to treat various brain diseases, including stroke. This study was supported by a grant of the Korea Healthcare technology R and D project, Ministry for Health, Welfare and Family Affairs (A121964)

F-3148
EX VIVO EXPANDED ALLOGENEIC MESENCHYMAL STEM CELLS WITH BONE MARROW TRANSPLANTATION IMPROVED OSTEOGENESIS IN PATIENTS WITH SEVERE HYPOPHOSPHATASIA

Taketani, Takeshi[1], Mihara, Aya[2], Oyama, Chigusa[2], Tanabe, Yuka[2], Yamamoto, Kei[2], Kobayashi, Hironori[2], Kanai, Rie[2], Onigata, kazumichi[2], Fukuda, Seiji[2], Yamaguchi, Seiji[2], Katsube, Yoshihiro[3], Oda, Yasuaki[3], Tadokoro, Mika[3], Sasao, Mari[3], Yuba, Shunsuke[3], Ohgushi, Hajime[3]

[1]Division of Blood Transfusion, Shimane University Hospital, Shimane, Japan, [2]Department of Pediatrics, Shimane University Faculty of Medicine, Shimane, Japan, [3]Research Institute for Cell Engineering, National Institute of Advanced Industrial Science and Technology, Hyogo, Japan

Hypophosphatasia (HPP) is one of the bone metabolic disorders caused by mutations of the tissue-nonspecific alkaline phosphatase (TNSALP) gene. The disease is characterized by the deficit of bone and tooth mineralization. Symptoms of severe HPP patients with extremely low ALP activity occur within perinatal period and the patients show gradual reduce of bone mineralization, resulting in fatal course due to respiratory disturbance. These findings suggest that restoration of osteogenesis (bone mineralization) is the key for their prognosis. However, there has been no curative treatment for HPP. Mesenchymal stem cells (MSCs), which reside in bone marrow (BM), umbilical cord blood, and adipose tissue, have multipotency that differentiate into various mesenchymal lineages. These include bone, cartilage, tendon, muscle, and adipose tissue. MSCs can easily be isolated and expanded in vitro while maintaining genetic stability, furthermore the MSCs also modulate immune system and support growth of hematopoietic stem cell. Due to these distinctive properties of MSCs, they are clinically available for treatments of graft versus host disease (GVHD) and regeneration of various tissues. However, MSCs have never been used to facilitate osteogenesis in patients with severe HPP.Our preliminary report (J Pediatrics, 2009, 154:924) showed that the patient was rescued and shown to possess osteoblasts/osteocytes from donor origin, however serum ALP remained to be low. Based on our initial experience, we performed transplantation of allogeneic BM-derived MSCs in patients with severe HPP who underwent preceding BMT to determine effects of the MSCs on bone mineralization. Two patients with severe HPP were studied. Both patients were diagnosed as fatal HPP based on perinatal onset, respiratory disturbance requiring mechanical ventilator, and *TNSALP* mutations with extremely low ALP activity. Their donors were asymptomatic relatives of the patients harboring heterozygous mutation of the *TNSALP* gene. ALP activities and mineralization of all donors were normal. Myeloablative conditioning regimen including buslfan and cyclophosphamide was used for BMT. Tacrolimus and short cource of methotrexate were used for GVHD prophylaxis. Small amount (about 20ml) of BM were used for culture expansion of MSCs in a flask containing α-minimum essential medium with 15% fetal bovine serum and 10 μg/mL kanamycin sulfate. We performed BMT at first, and then intravenously infused the cultured expanded MSCs. The BMT was done once and MSC infusions were done for several times. Five-times MSC transplantation (MSCT) was performed in one patient, and 9-times MSCT in the other. The number of infused MSCs was 10^6/kg. Two patients have survived over 3 year old. Their physical development and respiratory status improved. One patient could wean from respirator in the daytime. The bone mineral density and roentgenologic analyses revealed that the mineralization has gradually ameliorated. Serum bone-type ALP elevated in only one patient. Chimerism analysis of mesenchymal fraction isolated from BM showed the existence of MSCs derived from donor. Adverse events of BMT were tolerated and those of MSCT were not identified. Interestingly, steroid-refractory GVHD was improved by MSCT. Our data suggests that multiple MSC infusions followed by single BMT can be considered as an effective treatment to facilitate bone mineralization in patients with severe HPP.

F-3149
THE VOLUME OF MESENCHYMAL STEM CELLS IN THE PERIPHERAL BLOOD IN HORSES ASSOCIATED WITH PHYSICAL STRESS

Trunda, Miroslav, Pavelcova, Katerina, Klubal, Radek

Czech Gene Bank, Prague, Czech Republic

Introduction and hypothesis/objectives: Peripheral blood is one of easy accessible source of hematopoietic stem cells and mesenchymal stem cells (MSCs). In regenerative medicine we are interested especially in mesenchymal stem cells. However, the number of mesenchymal stem cells in peripheral blood is very low. Our objective was to determine whether physical exercise triggers release of MSC precursors in the peripheral blood of horses. In our study we used five warmblooded sports horses. Peripheral blood was collected from the vena jugularis. Peripheral blood was collected in five horses in three phases. The first phase was at rest (horses were not exercised for three days, peripheral blood was collected in the morning in the stable). The second phase was two hours after exercise and the third phase was 20 hours after exercise. In the third phase were only two horses. The exercise was one hour trotting and galloping with a rider. Collection of peripheral blood was 10 ml in a syringe with heparin. The peripheral blood was transported to the laboratory within one hour. In the laboratory, the peripheral blood was separated. The mononuclear cells (MNCs) were separated from the peripheral blood immediately after the collection with help of Histopaque. Afterwards the mononuclear cells were cultured in A-MEM 89%, FBS 10%, PenStrep 1% (37°C, 5% CO2). Number, viability, and CD markers (CD44, CD90, CD105) were determined using Bürker´s chamber and flow cytometry (FACS). Change of cultivation medium was performed after 3-4 days. During 3 weeks of culture the morphology of mesenchymal stem cells (MSCs) was controlled. After 3 weeks of culture trypsinized MSCs were subsequently counted in a Bürker chamber, and viability analysis of the expression of CD markers (CD44, CD90, CD105) was performed on the FACS. Results: The first phase (at rest): MSCs were isolated in vitro only in one horse. Number of MSCs was 0.8 x 106 and CD44+

CD90+, CD105+. The horse suffered with a hoof abscess 10 days before. In the remaining four horses MSCs were not isolated. The second phase (2h after exercise): MSCs were isolated in vitro in the two horses which had negative in the previous MSCs cultivation. Number of MSCs was 0.7 x 106, 1.2 x 106 and CD44+ CD90+, CD105+. MSCs were not isolated from the horse which was positive cultivation of MSCs in the first phase. The third phase (20h after exercise): The pheripheral blood was collected only two horses in this phase. One horse had positive cultivation of MSCs in the second phase and one horse had negative cultivation in the first but also the second phase. MSCs were isolated in vitro in both horses. Number of MSCs was 1.4 x 106, 1.9 x 106 and CD44+ CD90+, CD105+.

F-3150
USAGE OF UNDIFFERENTIATED WHARTON'S JELLY MESENCHYMAL STEM CELLS TO TREAT NOD MICE INVOLVES INDUCTION OF INSULIN-PRODUCING CELL DIFFERENTIATION AND SUPPRESSION OF T CELL MEDIATED AUTOIMMUNITY

Tsai, Pei-Jiun[1], Wang, Hwai-Shi[2], Weng, Zen-Chung[3], Shyu, Jia-Fwu[4], Chen, Tien-Hua[2]

[1]Institute of Clinical Medicine, National Yang Ming University, Taipei, Taiwan, R.O.C., Taipei, Taiwan, [2]Institute of Anatomy and Cell Biology, School of Medicine, National Yang Ming University, Taipei, Taiwan, R.O.C., Taipei, Taiwan, [3]Division of Cardiovascular Surgery, Department of Surgery, Taipei Medical University Hospital, Taiwan, R.O.C., Taipei, Taiwan, [4]National Defense Medical Center, Taipei, Taiwan

Type 1 diabetes mellitus (T1DM) is caused by T cell mediated autoimmune destruction of pancreatic β-cells. Recently stem cell therapeutic approach has been implicated to treat T1DM. Systemic administration of mesenchymal stem cells (MSCs) has been shown to be incorporated into a variety of tissues and have immunosuppressive effects, results in regeneration of pancreatic islets. We had showed that insulin-producing cells (IPCs) generated from human umbilical cord (Wharton's Jelly, WJ)-MSCs are potential cell sources to treat diabetic animals. However, the underlying mechanisms are unclear. The purpose of this study was to discern whether the undifferentiated WJ-MSCs can differentiate into IPCs in pancreas and modify immunological responses in NOD mice. Undifferentiated WJ-MSCs were transduced with green fluorescent protein (GFP) by lentiviral vector and injected into retro-orbital venous sinus in NOD mice. Seven days after transplantation of WJ-MSCs-GFP, fluorescent islet-like cell clusters in pancreas could be tracked. WJ-MSCs-GFP-treated NOD mice had significant lower blood glucose and higher survival rate than phosphate buffered saline-treated mice. We also found systemic and local reduction in autoaggressive T cell population, including T helper 1 cells and IL-17-producing T cells, together with an increase in regulatory T cells population in treatment group. Furthermore, we observed increases of antinflammatory cytokines and decrease of dendritic cells. At 23 days after transplantation, higher human C-peptide and mouse insulin in serum as well as improved glucose tolerance were found. Additionally, the undifferentiated WJ-MSCs-GFP were differentiated into IPC as shown by colocalization of human C-peptide and GFP in the pancreas. Significantly more intact islets and less severe insulitis were also found. In conclusion, the undifferentiated WJ-MSCs can treat NOD mice by differentiating into insulin-producing cells in vivo, conducting immunomodulatory effects and repairing the destroyed islets.

F-3151
A NOVEL ANIMAL COMPONENT-FREE CULTURE SYSTEM FOR EFFICIENT DERIVATION AND EXPANSION OF HUMAN MESENCHYMAL CELLS FROM BONE MARROW AND ADIPOSE TISSUE

Wagey, Ravenska[1], Yau, Jacky[1], Hadley, Erik[1], Wong, Matthew[1], Duronio, Chris[1], Elliott, Melissa[1], Sampaio, Arthur[1], Miller, Cindy[1], Thomas, Terry E.[1], Eaves, Allen C.[2], Louis, Sharon A.[1]

[1]STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Human mesenchymal progenitor cells (MPCs) are commonly derived from bone marrow (BM) and adipose tissues (AD) and expanded in culture media containing animal serum. To minimize the potential risks associated with exposure to animal serum, development of a fully-defined animal component-free (ACF) culture system for deriving and expanding MPCs is critical. However, currently available ACF media for culturing MPCs do not support isolating MPCs directly from primary tissues and are therefore limited to use with MPCs previously cultured in serum-containing media. Here we characterized MPCs in MesenCult™-ACF, a novel ACF culture medium and matrix system for deriving and expanding MPCs from primary BM and AD without the use of serum. MPCs were examined for clonogenic growth, long-term expansion, phenotype and differentiation potential. Clonogenic growth was evaluated using the Colony-Forming Unit-Fibroblast (CFU-F) assay. CFU-F assays from primary BM mononuclear cells were initiated by plating cells at 1 - 5 x 10^4 cells/cm^2 in MesenCult™-ACF or in serum-containing medium (control). To evaluate MPC expansion from primary BM, cells were plated at 3 - 5 x 10^4 cells/cm^2 in MesenCult™-ACF or 5 x 10^4 - 1 x 10^5 cells/cm^2 in control medium. Subsequent subcultures were plated at 1500 - 3000 cells/cm^2. For expansion cultures derived from AD tissues, cells were initiated at 500 - 1250 cells/cm^2 in either MesenCult™-ACF or control medium. The proliferative potential of MPCs in either MesenCult™-ACF or control medium was examined by determining cell number at each passage (P) for up to P8. Total CFU-F and average fold expansion in MesenCult™-ACF versus control media were compared using the paired t-test. Flow cytometric analysis of MPC markers (CD105, CD73, CD90) was performed at P2 and P4. Total CFU-F derived per 1 x 10^6 BM mononuclear cells was significantly higher in MesenCult™-ACF than in control medium (51 ± 8 versus 29 ± 5; mean ± SEM; n=6; $p<0.05$). Average fold-expansion at each passage of MPCs from BM mononuclear cells from P1 to P8 (31 to 46 days) in MesenCult™-ACF was significantly higher than in control medium (6.6 ± 1.0 versus 4.0 ± 0.8; mean ± SEM; n=6; $p<0.05$). Similarly, the average fold-expansion of AD-derived MPCs from P1 to P8 (25 to 39 days) was significantly higher in MesenCult™-ACF than in control medium (10.6 ± 1.2 versus 2.1 ± 0.2; mean ± SEM; n=3; $p<0.01$). Greater than 95% of BM and AD-derived MPCs cultured in MesenCult™-ACF medium expressed MPC markers and did not express hematopoietic markers (CD45, CD34, CD11b,CD19 and HLA-DR) at P2 and P4 (n=2). MPCs derived both from primary BM mononuclear cells (n=8) and AD (n=3) cultured in MesenCult™-ACF medium were able to differentiate efficiently at both early (P2) and late (P6) passages, into adipocytes, osteogenic cells and chondrocytes as visualized by Oil Red O, Von Kossa and Alcian Blue staining, respectively. In summary, the MesenCult™-ACF culture system efficiently derives and expands MPCs directly from primary tissues under strictly animal component-free culture conditions.

F-3152

CAN LOW CURRENT ELECTRICAL STIMULATION OF THE ANAL SPHINCTER ATTRACT EXOGENOUSLY ADMINISTERED MESENCHYMAL STEM CELLS?

Zutshi, Massarat[1], Li, Sun[1], Yeh, Judy[1], Damaser, Margot S.[2], Penn, Marc[3]

[1]The Cleveland Clinic, Cleveland, OH, USA, [2]Cleveland Clinic Foundation, Cleveland, OH, USA, [3]Summa Health, Akron, OH, USA

Purpose: We have previously optimized electrical stimulation (ES) parameters of current and duration to significantly upregulate gene and protein expression of Stromal Derived Factor 1(CXCL12/SDF-1) and, a mesenchymal stem cell homing cytokine in the anal sphincter in a rat model. The aim of this study was to investigate if low current ES can chemoattract mesenchymal stem cells (MSC) to the anal sphincter. Methods: 42 virgin female age and weight-matched Sprague Dawley rats were randomly allocated into three groups: intravenous (IV), (n=20), intraperitoneal (IP) (n=8), and intramuscular (IM) (n=14) based on routes of luciferase-labeled MSC delivery. IV and IP injections were performed daily for 3 consecutive days preceded by either ES (0.25mA, 60 minutes duration, 1 hour time lapse) or sham stimulation (SS, electrode placement without active current) on the same days; IM injection was performed once following ES. Within each group rats were randomly allocated into two subgroups: ES group using the optimized parameters and SS group. To assess MSC homing response *in vivo* and *ex vivo* imaging was performed . This was done 12 and 24 hours after ES and IV or IP MSC injection and 48 hours after ES and IM MSC injection to quantify the bioluminescence in the trunk and pelvic region as well as in the anal sphincter complex of each rat. Statistical significance ($p<0.05$) was determined using paired t-test. Results: No statistically significant difference was detected between the ES group and the SS group in in vivo and ex vivo bioluminescence after serial ES and IV ($p = 0.33$) or IP ($p = 0.13$) MSC delivery. However, statistically significant difference ($p = 0.03$) between the ES group and the SS group in MSC retention was detected following a one-time IM MSC injection (Fig). Conclusions: In this animal study, ES of anal sphincter complex using optimized parameters preceding local IM injection of MSC may significantly promote MSC retention. Future studies will incorporate an injury model to study muscle regeneration following ES and MSC injection. Clinical applications include anal sphincter regeneration using ES with cell based therapies at a time remote from injury.

POSTER BOOK PRESENTERS INDEX

LAST NAME	FIRST NAME	POSTER NUMBER
Abbasi	Sepideh	W-3090
Abdelalim	Essam	T-2070
Abdelhady	Shaimaa	T-2017
Abdul Jalil	Rufaihah	F-2102
Aberdam	Daniel	F-2048
Abud	Helen	W-1023
AbuSamra	Dina	F-1058
Adair	Jennifer	F-1059
Adamzyk	Carina	T-3128
Agarwal	Jasmin	T-2134
Agathocleous	Michalis	F-1061
Ahmadian Baghbaderani	Behnam	T-2173
Ahn	Eun Hyun	W-3091
Aho	Joy	T-2174
Ahuja	Akshay	F-2049
Akaike	Toshihiro	F-2103
Akhtar	Aslam	W-2134
Akwaa	Frank	F-1092
Al Harbi	Ayman	F-1062
Alaiya	Ayodele	F-1055
Alaiya	Ayodele	W-1055
Alajez	Nehad	W-3092
Al-Aqeel	Aida	T-1115
Alatar	Shemim	F-2104
Alcayaga-Miranda	Francisca	T-3129
Alder	Olivia	F-1015
Aldonyte	Ruta	W-3093
Alexeyenko	Andrey	F-2241
Alexson	Tania	F-2051
Alharbi	Samah	W-1024
Ali	Hamad	W-3003
Allison	Tom	F-2052
Alsobaie	Sarah	T-2089
Alworth	Samuel	W-2135
An	Su Yeon	W-2072
Anand	Akshay	F-1063
Andersen	Peter	T-3058
Anderson	David	W-3060
Anderson	John	T-3130
Anderson	Nickesha	T-2018
Andres	Lissette	T-2019
Andronikou	Nektaria	F-2105
Ang	Cheen Euong	W-3004
Araki	Ryoko	T-2175
Arefian	Ehsan	F-2053
Arenas	Ernest	T-3003
Arnold	Antje	W-2073
Assinck	Peggy	W-2074
Atkins	Harold	F-1064
Azami	Takuya	T-2010
Azizi	Hossein	W-2136
Aznar-Benitah	Salvador	T-2235
Babaeijandaghi	Farshad	W-2075
Babona-Pilipos	Rob	W-2076
Babos	Kimberley	T-3004
Babovic	Sonja	F-1065
Badner	Anna	T-3131
Baek	Jin Ah	T-1116
Baggiolini	Arianna	T-2011
Baghabaneslaminejad	Mohamadreza	W-3094
Bahia	Harsharon	F-2106
Baik	June	F-1066
Balani	Sneha	T-1033
Banovich	Nicholas	W-2229
Bao	Yunhua	T-2205
Barash	Itamar	T-1034
Barboza	Carlos	W-3078
Bardag-Gorce	Fawzia	F-3049
Bardy	Cedric	F-2107
Barreto	Mardem	T-2090
Barrett	Robert	W-1025
Barruet	Emilie	T-2157
Barta	Tomas	F-2054
Basak	Pratima	T-1092
Basu	Joydeep	T-2091
Basu Roy	Upal	F-1093
Batchelder	Cynthia	W-1047
Battle	Stephanie	F-1094
Bayin	Nermin	T-1093
Bayo Fina	Juan Miguel	T-3143, T-3132
Bazarek	Stanley	T-3005
Beck	Sam	F-2055
Beclin	Christophe	T-3006
Beer	Philip	F-1067
Beeravolu	Naimisha Reddy	T-2020
Beerman	Isabel	T-1057
Beh-Pajooh	Abbas	T-2176
Belenguer-Sánchez	Germán	T-3007
Bengtsson	Niclas	F-3050
Bertin	Enrica	F-2056
Best	Sarah	F-1032
Bhat	Vasudeva	F-1033
Bhatia	Sonam	F-2057

Bhutani	Nidhi	W-3095
Bi	Kun	T-2206
Billing	Anja	W-3079
Bilousova	Ganna	W-2138
Biran	Alva	F-1095
Biswas	Moumita	T-3008
Blanchard	Joel	F-2058
Blanchet	Marie-Renee	F-3051
Blanner	Patrick	T-2207
Blit	Patrick	F-2109
Block	Gregory	F-3052
Blong	Ian	T-2022
Boccaccio	Carla	T-1094
Bodhak	Subhadip	T-2092
Boroviak	Thorsten	T-2012
Bosio	Andreas	F-2110
Bowers	Megan	T-3009
Boyer	Leah	T-1117
Brady	Colleen	W-2077
Brady	Lee	F-2111
Braid	Lorena	T-3133
Breda	Laura	T-1058
Brick	David	T-2208
Brito	Felipe	W-3080
Brodie	Chaya	F-1096
Bronstein	Robert	T-3010
Brosh	Ran	F-2112
Brown	Andreas	T-1059
Bruckner	Annalise	F-2113
Brumbaugh	Justin	T-2139
Brunham	Liam	T-2209
Bruveris	Freya	F-1049
Budjan	Christoph	F-1034
Bulaeva	Elizabeth	F-1068
Burkert	Karsten	T-3059
Burks	Scott	T-3134
Buzanska	Leonora	W-2230
Bystrykh	Leonid	F-1069
Caglic	Dejan	T-2210
Calafiore	Marco	T-2211
Cameron	Kate	F-1016
Campagnaro	Bianca	F-1070
Cantero Peral	Susana	T-3060
Cao	Suying	F-2009
Cao	Yenong	W-3096
Carlson	Coby	T-2212
Casteels	Tamara	T-2159
Castro-Diaz	Nathaly	F-2237
Cha	Sang-Ho	W-3097
Chambers	Sarah	W-2078
Chan	Charles	W-3081
Chan	Leo	F-2114
Chan	Sunny	T-2023
Chander	Praveen	T-2024
Chang	Natasha	F-3053
Chang	Wing	W-2141
Chatterjee	Sumanta	F-1035
Chen	Chang-Yi	W-3061
Chen	Chen-Yun	F-2059
Chen	Christine	T-2160
Chen	Guibin	F-1050
Chen	Jiamei	T-1012, F-1013
Chen	Kevin	W-2014, F-1013
Chen	Min	T-1095
Chen	Rui	W-3099
Chen	Show Li	W-3062
Chen	Shuai	F-2116
Chen	Xiao	W-3098
Chen	Ying-Ting	W-3038
Chen	You-Tzung	F-2115
Cheng	Chun-Chun	T-3011
Chi	Chao	W-3121, T-3121
Chiang	Chi-Ling	F-1071
Chidgey	Ann	F-1036
Chien	Chian-Shiu	T-3062
Chien	Chiao-Yun	F-1017
Chiou	Shih-Hwa	T-2213
Cho	Hyun-Ju	W-3100
Cho	JaeJin	W-3101
Cho	Seung-Ju	F-2117
Choi	Chunggab	W-3103
Choi	Eunhyun	W-3076 , T-3122, F-2133
Choi	Jae-Il	F-1072
Choi	Na Young	W-2001
Choi	Seong-Mi	T-3135
Choi	Seung Ah	T-3136
Chong	Mark	T-2093
Choompoo	Narawadee	T-2177
Chou	Athena Vai Lam	T-3012
Chou	Bin-Kuan	T-2178
Chou	Shih-Jie	F-1018
Christiansen-Weber	Trudy	W-2142
Chung	Sangmi	T-2025
Chung	Stephen	F-1097
Chung	Tae Nyoung	T-3013
Ciuffreda	Maria Chiara	T-3137
Clarke	Raedun	T-2026
Clevenger	Tracy	T-2094

Codega	Paolo	T-3014
Cohen	Rick	W-2143
Coleman	Natalia	T-3015
Coleman	Ronald	T-3016
Coles-Takabe	Brenda	W-3039
Conder	Ryan	W-1026
Conway	Anne	F-2060
Cornacchia	Daniela	T-2214
Corradetti	Bruna	T-2086, F-3076
Coutinho	Fiona	T-3017
Couture	Sylvana	T-3018
Cox	Brian	T-2027
Crane	Ana	T-2216
Crapper	Liam	T-2217
Crean	Jennifer	F-1073
Crews	Leslie	F-1098
Cromwell	Evan	T-3063
Crook	Jeremy	T-3019
Crowder	Spencer	W-3077, T-3076
Cruz	Angelica	T-2179
Dah-Ching	Ding	T-2218
Daheron	Laurence	W-2144
Damaser	Margot	T-3138
Dariolli	Rafael	T-3139
Daughtry	Brittany	F-2061
Davila	Jonathan	W-2145
Davis	Brian	T-2180
De	Debojyoti	T-2028
de Morrée	Antoine	F-3054
de Peppo	Giuseppe Maria	W-2086, F-2100
de Rham	Casimir	T-2181
Decembrini	Sarah	W-3040
Degheidy	Tamer	W-3082
Dementyeva	Elena	T-2219
Devaraju	Karthikeyan	W-2146
Deveau	Todd	W-2079
Dhaliwal	Navroop	T-2029
Dias da Silva	Valdo Jose	W-3058, T-3123
Ditadi	Andrea	T-2030
Do	Hyo-Sang	T-2221
Doctor	Sean	F-2118
Doerr	Jonas	T-3020
Dolezalova	Dasa	T-2031
Domev	Hagit	T-3141
Dorsett	Laurel	T-2182
dos Santos	Rodrigo	W-2147
Douglas	Kathryn	T-3021
Downey	Jennifer	W-3104
Drouin	Geneviève	W-3105
Drowley	Lauren	T-3064
Drukala	Justyna	T-1041
Dubart-Kupperschmitt	Anne	T-2220
Dutta	Devanjali	T-1022
Eade	Kevin	W-2148
Easley	Charles	T-2032
Eaton	Erik	T-3142
Edenhofer	Frank	W-2149
Edward	Christina	W-1092
Eggenschwiler	Reto	T-2222
Egli	Dieter	W-2150
Eisner	Christine	F-1076
Elbaz	Judith	T-2183
Ellerby	Lisa	T-2223
Ellerstrom	Catharina	T-1119
Elsafadi	Mona	W-3106
Emani	Maheswara Reddy	F-2062
Engler	Adam	W-3107
Enzmann	Volker	T-3023
Erceg	Slaven	T-3024
Eremin	Ilya	F-3055
Ergin	Kemal	F-1100
Ericsson	Jesper	F-2119
Esfandiari	Behnaz	T-2095
Esmailzadeh	Sharmin	T-1096
Eto	Koji	F-1077
Evantal	Naveh	F-2063
Evers	Daniela	T-2135
Evseenko	Denis	T-2033
Faghihi	Faezeh	W-3108
Fahmy	Ahmed	T-2184
Fairchild	Michael	F-2002
Faiz	Maryam	T-3025
Faltus	Timo	W-2243
Fathi	Fardin	T-2185
Fedele	Stefania	T-2186
Felgentreff	Kerstin	T-2224
Feltham	Casey	T-2044
Feng	Bo	F-2120
Feng	Jian	T-2225
Fergus	Jeffrey	T-2187
Fernandes	Tiago	F-2121
Fernandez-Moure	Joseph	T-2096
Ferreccio	Amy	T-2136
Ferro	Zaquer	W-2080
Feuer	Ralph	T-3026
Fichadiya	Akash	W-3110
Field	Andrew	T-2161
Filippi	Celine	F-1019

Fina	Bayo	T-3143
Finger	Jared	T-2198
Fingrut	Warren	T-2242
Firpo	Meri	F-1008
Florian	Maria Carolina	F-1078
Fogarty	Lauren	T-3027
Forni	Maria Fernanda	T-3118
Forrester	Lesley	F-1079
Frank	Joseph	T-3144
Frank	Nathan	F-2122
Fratini	Paula	F-2065
Freude	Kristine	T-2188
Freund	Christian	T-2162
Frisca	Frisca	T-2163
Frobel	Joana	T-2164
Fuehrmann	Tobias	T-2097
Fujimori	Yuko	T-2189
Fujiwara	Naruyoshi	T-2190
Fukazawa	Takahiro	F-2123
Fukushima	Hiroyuki	W-3063
Füllgrabe	Anja	T-1042
Funakoshi	Shunsuke	T-2191
Funato	Kosuke	T-3001, F-3001
Fung	Moses	W-2244
Furukawa	Gabriela	W-1093
Fusaki	Noemi	T-2192
Futakuchi-Tsuchida	Akiko	W-3064
Gadhoum	Samah Zeineb	F-1080
Gajovic	Srecko	T-3028
Galat	Vasil	T-2226
Galic	Zoran	T-1120
Gallego Romero	Irene	W-3065
Gallo	Marco	F-1101
Gandre-Babbe	Shilpa	T-2193
Ganesh Pandian	Namasivayam	T-2137
Garbuzov	Alina	W-2003
Garcia	Bradley	F-2124
Garcia-Castro	Martin	T-2034
Gargett	Caroline	F-1038
Gargiulo	Ciro	W-3111
Garnier	Delphine	F-1102
Garza	Johan	F-2125
Geens	Mieke	T-2237
Geng	Jian-Guo	T-1023
Geng	Yijie	F-2126
Georges	Adriana	F-2127
Geum	Dongho	T-3029
Ghaedi	Mahboobe	F-1029
Ghaffari	Saghi	T-2233, F-1056
Gharibi	Borzo	W-3112
Ghasemzadeh-Hassankolaei	Maryam	W-3113
Ghazalli	Nadiah	F-1009
Ghazvini	Mehrnaz	T-2194
Giambra	Vincenzo	W-1094
Gill	Lisa	T-2165
Gilmour	Jane	F-1081
Ginani	Fernanda	W-3083
Giulitti	Stefano	F-2128
Gluck	Jessica	T-3065
Go	James	F-2129
Goh	Saik	W-2015, F-1007
Goldenberg	Regina	T-2098
Gomez-Aristizabal	Alejandro	T-3145
Gornalusse	German	T-1060
Gotoh	Shimpei	F-1030
Goyal	Nidhi	F-1020
Goyer	Benjamin	T-2099
Gracias	Aileen	T-1097
Graef	Isabella	T-2035
Grealish	Shane	T-3030
Grise	Kenneth	W-3041
Grompe	Markus	W-1016
Gruenert	Dieter	T-2166
Gu	Yansong	T-3146
Guan	Qingdong	T-3147
Guemez Gamboa	Alicia	T-3031
Guidi	Novella	F-1082
Gumpert	Anna	W-3066
Gunel-Ozcan	Aysen	W-3084
Guo	Lin	W-2231
Gupta	Manoj	T-2195
Gupta	Sarita	W-3114
Guzman	Marcela	F-2233
Haag	Daniel	T-3032
Hacibekiroglu	Sabiha	W-3042
Hadland	Brandon	F-1083
Hadley	Erik	F-2130
Hagiwara	Natsumi	T-2036
Hagner	Andrew	W-3115
Haigh	Jody	T-2196
Hall	Vanessa	F-2066
Hamilton	Brad	T-2140
Hamilton	William	T-2037
Hammer	Bonnie	T-2227
Hammoud	Lamis	T-2038
Han	Ho Jae	T-2071, F-2071
Han	Inbo	T-3148
Han	Jiyou	W-1095

Han	Sei-Myoung	W-3116
Hao	Hsiao-Nan	T-3033
Harbi	Shaghayegh	F-1051
Hargan Calvopina	Joseph	W-2004
Harkness	Linda	W-3117
Harris	Violaine	T-3149
Hasegawa	Yuki	T-2197
Hatta-Ohashi	Yoko	T-3034
Hawkins	David	F-2234
Hay	David	T-2039
Hayashi	Taro	W-3067
Hayward	Julie	T-3150
He	Lixiazi	F-2131
Hegab	Ahmed	F-1031
Hemmi	Natsuko	W-3068
Henderson	Kira	W-3118
Hendriks	William	F-2132
Heravi-Moussavi	Alireza	F-2067
Hernandez	Javier	T-2141
Hesse	Robert	W-2081
Heylman	Christopher	W-3069
Higuchi	Akon	T-2100
Hirabayashi	Yoko	T-1061
Hirai	Masako	W-2103
Hisatake	Koji	T-2142
Hitoshi	Seiji	T-3035
Hiyama	Taiki	F-3056
Ho	Mirabelle	T-2199
Ho Sui	Shannan	W-2104
Hoffman	Matthew	T-1035
Holder	Julie	T-2200
Holmgren	Gustav	W-3070
Holst	Bjørn	T-2201
Honda	Thomas	T-1099
Hong	Dengli	W-1096
Hong	So Gun	W-2205
Hong	Soomin	T-2040
Hoo	Nicholas	T-2041
Hood	Rebecca	W-2206
Hori	Keiko	W-3005
Horiguchi	Ikki	T-2202
Hosoya	Tomonori	T-1062
Hou	Juan	T-1036
Hovatta	Outi	T-1121
Hrstka	Sybil	W-2207
Hsi	Chang	T-3050
Hsieh	Jenny	W-3006
Hsu	Li-Wen	W-2105
Hsu	Wei-Ting	F-2068
Hsu	Ya-Chieh	T-1043
Hsu	Yi-Chao	W-3007
Hu	Guang	F-2069
Hu	Ping	T-3051
Huang	Jijun	T-2048
Huang	Junjiu	T-2047
Huang	Kevin	F-2010
Huang	Patricia	F-2015
Huang	Yue	W-2016
Huang	Yu-Jen	W-3119
Huggins	Ian	T-2049
Hughes	Virginia	W-2245
Huhn	Stephen	W-3008
Hui	Lijian	W-2088
Hulin	Julie-Ann	T-3052
Hung	Shih-Chieh	W-3085
Huo	Jeffrey	W-2232
Hursh	Deborah	F-2235
Hussein	Samer	T-2229
Hwang	Dong-Youn	W-2171, T-2171, F-2171
Hwang	Ki Chul	T-2143
Hwang	Meeyul	T-3053
Hysolli	Eriona	T-2203
Ibarra-Ibarra	Blanca Rebeca	T-3088
Ibrahim	Ahmed	W-2082
Ibrahim	Rawa	T-1063
Ihn	Han	T-3089
Ikeda	Hiroki	T-2204
Iki	Takehiro	F-1039
Ikuno	Takeshi	F-1052
IL-KWON	Kim	W-2106
Ina	Kurth	T-1100
Inagaki	Emi	W-3043
Inamura	Mitsuru	T-1064
Iqbal	Sharif	T-1024
Irion	Camila	T-3151
Irion	Stefan	W-1115
Isasi	Rosario	W-2240, T-2243, F-2243
Ishigami	Shuta	T-3152
Ishiy	Felipe	T-3090
Ishizuka	Toshiaki	T-2167
Itakura	Go	F-2173
Itakura	Yoko	W-1116
Iwata	Eiichiro	W-3127
Iyer	Dharini	W-2017
Jacob	Karen	F-2239
Jacob	Samson	T-2168
Jagasia	Ravi	W-3009
Jahani-Asl	Arezu	F-1103

Jan	Majib	T-3054
Janebodin	Kajohnkiart	W-3088, T-2087
Jang	Jiwon	T-2050
Jaros	Josef	W-3128
Jayakumaran	Gowtham	T-2144
Jazedje	Tatiana	W-3129
Jenkins	Catherine	F-1104
Jensen	Kim	T-1044
Jensen	Thomas	W-2005
Jeong	Hochang	W-1117
Jeong	Jaemin	F-2174
Jeong	Myong-Ho	W-2018
Jeyakumar	Jey	W-2107
Ji	Shuyi	T-2145
Ji	Youngmi	W-3087
Jiang	Linjia	W-2083
Jin	Ying	T-2051
Jo	Junghyun	T-2146
Johannesson	Bjarki	F-2175
Johnson	Wade	T-2052
Jones	Eugenia	W-2208
Jones	Mark	F-3077
Jonlin	Erica	W-2246
Ju	Ji Hyeon	W-2210
Ju	Younghee	W-2209
Judge	Luke	T-3066
Jung	Ji Hye	T-2053
Jung	Ji Won	F-2176
Jung	Young Hyun	W-2084
Kabiri	Zahra	T-1025
Kaid	Carolini	W-1097
Kaini	Ramesh	F-2157
Kaji	Keisuke	F-2177
Kallur	Therese	W-2108
Kamath	Anant	F-2178
Kamei	Kenichiro	W-2109
Kamenova	Kalina	F-2240
Kami	Daisuke	T-3091
Kanazawa	Sanshiro	T-3092
Kang	Catherine	T-1037
Kang	Eunju	F-2011
Kang	Jin-A	T-1066
Kang	Kyung-Ku	W-2133, F-3121
Kang	Phil Jun	F-2179
Kanke	Kosuke	W-2019
Kanki	Yasuharu	F-1053
Kannan	Nagarajan	T-1038
Kardel	Melanie	T-2054
Kareta	Michael	T-2147
Karimi	Rasoul	W-2006
Karimzadeh	Fereshteh	W-2020
Karl	Robert	W-3010
Karsten	Stanislav	W-2110
Karumbayaram	Saravanan	T-3038
Kasashima	Hiroaki	W-1098
Kasper	Maria	T-1045
Kats	Ilona	W-2211
Katsman	Yulia	W-2111
Kaul	Himanshu	W-2089
Kawabata	Kenji	F-1054
Kawabata	Soya	F-2180
Kawahara	Yumi	T-3093
Kawamura	Takuji	F-2181
Kennelly	Helen	T-2074
Kent	David	F-1084
Kenyon	Jonathan	T-2075
Keogh	Andrea	T-3094
Kerscher	Petra	W-3071
Khalili	Mitra	T-2148
Khalili	Saeed	T-3039
Kharazi	Ludmila	T-3040
Khazaei	Mohamad	W-3011
Kheolamai	Pakpoom	T-1067
Khoo	Melissa	T-1068
Khoury	Maroun	W-3130
Kiem	Hans-Peter	T-1069
Kieslinger	Matthias	T-1070
Kim	Ah-Young	T-3055
Kim	Daehwan	T-3095
Kim	Deok-Ho	F-2185
Kim	Dong-Wook	W-2213
Kim	Eunhye	T-2055
Kim	Hye Joung	T-3096
Kim	Hyung-Sik	W-3133
Kim	Jee Young	W-3135
Kim	Jin-Su	W-2101, F-2046
Kim	Jiyeon	W-1118
Kim	Jiyoung	T-3077
Kim	Jonghun	T-1013
Kim	Joo Yeon	T-2076
Kim	Junghee	W-3086
Kim	Jungho	F-2183
Kim	Kye-Seong	T-3056
Kim	Mee-Hae	F-2182
Kim	Mi Jin	T-2077
Kim	Moo Woong	T-2244
Kim	Pyung-Hwan	W-2112
Kim	Sang-Gyung	T-2149

Kim	Seong Muk	W-3131
Kim	Shin-IL	F-2184
Kim	Sungmin	W-3012
Kim	Sun-Mi	W-3132
Kim	Won-Jae	W-2021
Kim	Yeji	W-2212
Kim	Yu Mi	W-3134
Kim	Yun Hee	T-1046
Kingham	Paul	W-3136
Kirita	Yuhei	F-2086
Kiskinis	Evangelos	W-3013
Kitani	Tomoya	T-2078
Kizilay Mancini	Ozge	W-3137
Kizilyer	Aysen	T-1073
Klepsch	Sebastian	T-3097
Klim	Joseph	W-2214
Klimmeck	Daniel	F-1085
Klincumhom	Nuttha	F-2186
Knapp	David	T-1074
Knight	Charlotte	T-3078
Knoebel	Sebastian	T-2056
Knyazev	Oleg	W-3138
Kobayashi	Junko	W-2215
Koechlein	Claire	F-1086
Koide	Hiroshi	T-1101
Kokubu	Yuko	F-2087
Komorowska	Karolina	F-1087
Konecna	Zaneta	T-2057
Kong	Chiou Mee	W-1099
Kong	TaeHo	W-3139
Kong	Wuyi	T-2079
Kono	Ken	T-3079
Konoplyannikov	Anatoly	W-3141
Konoplyannikov	Mikhail	W-3140
Koong	Victor	W-2113
Korraa	Soheir	T-1075
Kostallari	Enis	W-3049
Kouroupi	Georgia	W-2216
Kouskoff	Valerie	T-1049
Kramer	Philipp	T-1029
Kramm	Anneke	T-3098
Krause	Matthew	T-3080
Krentz	Nicole	W-2022
Krepuska	Miklos	W-3142
Ku	Chia-Chen	F-2187
Ku	Jonjei	T-1102
Kubiura	Musashi	T-2230
Kubo	Naoki	W-2007
Kuljanin	Miljan	W-3143
Kumar	Vijay	T-1076
Kuninger	David	W-2114
Kunova	Michaela	T-2058
Kuo	Ting-yu	W-1100
Kuppers-Munther	Barbara	T-1014
Kuppusamy	Kavitha	T-3067
Kureshi	Alvena	F-2088
Kuroda	Aoi	T-1030
Kuroda	Takuya	F-2188
Kurokawa	Yosuke	W-3072
Kurtovic	Silvia	T-1050
Kusunose	Mayumi	W-2241
Kutsuzawa	Koichi	W-3014
Kwok	Chun Ting Davis	T-2059
Kwon	Sarah	W-2217
Kwon	Yoo-Wook	T-2231
Kz	Kshitiz	W-2218
Lacaria	Melanie	W-3015
Lackford	Brad	T-2060
Laco	Filip	W-3073
Lahvic	Jamie	T-1077
Lai	Courteney	T-1104
Lai	Damian	T-1103
Lai	Dongmei	T-2001
Lakshmipathy	Uma	F-2189
Lala-Tabbert	Neena	W-3050
Lalit	Pratik	T-3068
Lalli	Matthew	W-2219
Lam	Jeffrey	T-1078
Lam	Pk	W-3144
Lan	Chen-wei	W-2023
Laplane	Lucie	T-2240
Laricchia-Robbio	Leopoldo	T-2014, F-2134
Larrain	Juan	T-2081
Larsen	Hjalte	W-3016
Lassiter	Chelsea	W-3017
Lau	Shong	T-2150
Leach	Lyndsay	T-3042
Leal	Maria	W-1119
Lee	Bum-Kyu	T-2061
Lee	Choon-Soo	T-1051
Lee	Emily	T-1015
Lee	Eun Joo	W-3145
Lee	Eun-Mi	F-2191
Lee	Eveline	W-2220
Lee	Hae Kyung	W-3148
Lee	Hye Jeong	T-2002
Lee	Hyun Jik	T-2082
Lee	Hyun-Joo	W-3147

Lee	Jae Young	W-2024
Lee	Jaehun	T-1079
Lee	Jeoung Eun	W-2046, F-2047
Lee	Joonseong	F-2190
Lee	Man Ryul	T-2151
Lee	Miyoung	T-3081
Lee	Narae	W-3146
Lee	Nayeon	F-2193
Lee	Qian Yi	T-2152
Lee	Seung Bum	W-1040
Lee	Seunghee	F-2236
Lee	Sunray	T-2062
Lee	TingFang	W-1101
Lee	Vivian	F-2158
Lee	Wonyoung	T-2003
Lee	Yi Yen	T-1105
Lee	Yin Lau	F-2192
Leeb	Martin	W-2025
Leeder	Rachel	T-2083
Leeman	Dena	W-3018
Lepage	Sarah	F-2159
Leung	Man Hong	T-2063
Leung	Wai Lun	W-2026
Levine	Aaron	W-2242
Levine	Aaron	T-2241
Lewicki	Jakub	F-2089
Lewis	Coral-Ann	T-1080
Li	Aiqun	W-2221
Li	Jiang	T-3101
Li	Li	W-2115
Li	Lingna	F-2090
Li	Linheng	T-1081
Li	Lisha	T-3082
Li	Mo	F-2160
Li	Nianzhen	F-2194
Li	Xuan	F-2161
Li	Yichen	W-2222
Li	Yumei	T-3100
Liang	Lisa	W-1103
Liang	Yuh-Jin	W-1102
Lichtenberger	Beate	T-2153
Lien	Wen-Hui	W-1041
Lim	Daniel	W-1120
Lim	Jae-Yol	T-3083
Lim	Rebecca	T-1032, F-1014
Lima	Maria Joao	F-1010
Limb	G. Astrid	T-3043
Lin	Chih-Jen	F-2012
Lin	Hanyang	T-1106
Lin	Jia-yi	T-2064
Lin	Sung-Jan	W-1042
Linderoth	Emma	T-2154
Ling	Qing-dong	W-3019
Lippmann	Ethan	W-3020
Liu	Fang	T-1052
Liu	Hua	T-3084
Liu	Jessica Ai-jia	T-1026
Liu	Jing	F-2195
Liu	Xiaohu (Will)	F-1105
Liu	Yuan-Hung	F-3060
Loeken	Mary	W-2009
Loh	Sharon	F-2162
Lombaert	Isabelle	W-1032
Lorthongpanich	Chanchao	F-1106
Low	Marcela	T-3102
Lu	Catherine	W-1043
Lu	Joyce (Jean)	T-3103
Lu	Min	T-2155
Lu	Xibin	W-2027
Lu	Xinyi	T-2065
Lu	Yi-Fen	W-2028
Lubitz	Sandra	W-2223
Lujan	Ernesto	T-2156
Luz-Crawford	Patricia	W-3122, T-3124, F-3122
Lyublinskaya	Olga	T-3085
Ma	Hong	T-2232
Ma	Jie	W-3021
MacArthur	Chad	F-2135
Maddah	Mahnaz	W-3059, F-2172
Madhoun	Nour	T-1107
Mah	Nancy	F-2136
Mahmud	Nadim	T-1082
Mahmud	Neemat	W-3022
Main	Heather	W-2029
Majumdar	Anish	T-3104
Makhija	Harshyaa	W-2116
Mallon	Barbara	F-2137
Man	Jennifer	W-2117
Mandegar	Mohammad	W-2224
Manias Rothberg	Janet	T-1084
Mannerström	Bettina	T-3086
Mannie	Chelsea	T-2004
Manochantr	Sirikul	W-3150
Mao	Bin	T-1085
Marchetto	Maria Carolina	W-2225
Marchildon	Francois	W-3051
Margariti	Andriana	F-2138
Marko	Karoly	W-3151

Markouli	Christina	W-2030
Maroof	Asif	W-3023
Marshall	Julia	W-2118
Martin	Francisco	F-2108
Martin	Martin	T-1027
Martinez Cardozo	Constanza	T-3105
Masamoto	Yosuke	T-1086
Mascetti	Victoria	W-2031
Mashimo	Yasumasa	W-2119
Mason	Rebecca	F-2139
Massumi	Mohammad	F-2196
Masumoto	Hidetoshi	F-3061
Mathew	Shibin	W-2032
Mathieu	Julie	F-2140
Matosevic	Sandro	W-2120
Matrone	Gianfranco	F-2141
Matsumoto	Yoshihisa	W-2226
Matsuura	Katsuhisa	F-3062
Mattar	Citra	T-1087
Matthews	Kirstin	T-2239
Matzner	Michael	F-2072
McCarrey	John	F-2197
McDonald	Angela	W-2033
McFarlane	James	T-2005
McKee	Christina	W-2034
Md Isa	Muhammad Lokman	T-2006
Mehta	Ashish	F-3063
Meissner	Alexander	W-2035
Menkova	Inna	T-1088
Menon	Vilas	T-3037
Merkle	Florian	W-2227
Mesa	Kai	W-1044
Meyer	Lionel	W-1105
Michielin	Federica	W-2121
Middendorp	Sabine	T-1028
Miele	Evelina	W-2233
Miettinen	Susanna	W-3152
Migliaccio	Anna Rita	T-1089
Miller	Christine	T-1090
Miller	Paul	W-1057
Milovanoval	Tatyana	W-3153
Min	Chang-Woo	W-3123, T-2133
Min	Kyunghoon	F-3127
Minonzio	Greta	F-3064
Mirlashari	Mohammad Reza	F-3128
Mitani	Ko	W-2122
Mitchell	Jennifer	T-2066
Miura	Taichi	W-2036
Miura	Takumi	F-2142
Miyaoka	Yuichiro	W-2228
Miyazaki	Kaoru	F-2092
Möbus	Selina	T-1017
Moghiminasr	Reza	W-3024
Mohamadi	Parvaneh	W-1045
Mohammadnia	Abdulshakour	W-3025
Mohanty	Sujata	W-3089, T-2072, F-3058
Moon	Jisook	W-3026
Moon	Youn joo	T-1053
Moore	Jennifer	F-2143
Mora-Castilla	Sergio	F-1011
Morante-Redolat	Jose Manuel	W-3027
Moreira	Steven	W-2037
Mori	Hideki	W-2123
Morissette Martin	Pascal	F-3129
Morita	Ken	W-1106
Moriyama	Hiroyuki	T-3106
Moriyama	Mariko	T-3107
Morozova	Olena	F-1107
Mortensen	Luke	F-2073
Mostajo Radji	Mohammed	W-3028
Motono	Makoto	W-3029
Moyer	Mary Pat	F-3130
Mueller	Franz-Josef	W-2124
Muir	Kenneth	T-3108
Muller	Albrecht	T-2067
Mullin	Nick	T-2068
Mun	Chin Hee	W-1058
Murayama	Hideyuki	F-2144
Murray	Alexander	F-2145
Murrell	Julie	T-2101, F-3088
Musheer Aalam	Syed Mohammed	F-2198
Mussar	Kristin	T-1007
Muto	Kaori	T-2245
Nadkarni	Rohan	T-1031
Nag	Kakon	F-2163
Nagaishi	Kanna	F-3131
Nagamoto	Yasuhito	T-1018
Najimi	Mustapha	T-1019
Najm	Fadi	W-3030
Nakano	Rei	T-3109
Nakhaei-Nejad	Maryam	T-1054
Nampe	Daniel	W-2125
Narvaiza	Iñigo	F-2199
Natividad-Diaz	Sylvia	F-2200
Naujock	Maximilian	F-2205
Navara	Christopher	T-2069
Navarro	Sonia	W-1029
Nayler	Samuel	F-2206

Nazareth	Emanuel	W-2048
Nefzger	Christian	F-1022
Nevin	Zachary	F-2207
Ng	Ray	W-2038
Ng	Shiyan	F-2208
Ng	Yi Han	F-2146
Ngan	Elly	T-1108
Nguyen	Ha	W-2049
Nguyen	Long	W-1033
Nguyen	Phuong	W-1059
Ni	Zhenya	W-1060
Niakan	Kathy	W-2050
Nicholas	Cory	F-3037
Nikravesh	Mastaneh	F-3065
Ninomiya	Naoto	W-2039
Nishikii	Hidekazu	F-1088
Nishimura	Emi	W-1046
Nishiyama	Yuichiro	F-2201
Nishizawa	Masatoshi	F-2164
Noggle	Scott	W-2126
Nolan	Daniel	W-2127
Noorwali	Abdulwahab	F-3132
Norddahl	Gudmundur	F-1108
Nosi	Ursula	F-2147
Nowak-Imialek	Monika	T-2007
Nuschke	Austin	T-3110
O'Connor	Michael	T-3044
Ofir	Racheli	F-3123
Ofir	Racheli	W-3124
Ogle	Molly	W-1061
Oh	Hyemin	W-3032
Oh	Sang Yub	F-2074
Oh	Sangmin	W-2040
Oh	Steve	W-2041
Ohnishi	Yu-ichiro	W-3033
Ohsako	Seiichiroh	W-2042
Ohta	Kunimasa	F-2148
Ohta	Ryo	W-1049
Ojansivu	Miina	T-3111
Okada	Mio	T-3112
Okuyama	Michihiro	F-2093
Oliveira	Daniela	F-3133
Omelyanenko	Anna	W-3034
Omori	Shigenari	T-3113
Ong	Wee Kiat	T-3114
Onuma	Yasuko	W-2128
Oostendorp	Robert	W-1062
Ordonez	Paulina	F-3036
Org	Tonis	W-1063
Ortiz	Mariaestela	W-2129
O'Shea	Orla	F-2202
Osipovich	Anna	W-2234
Ostblom	Joel	W-2051
Ostermann	Laura	W-3035
Otani	Tomoki	W-3036
Otsuji	Tomomi	W-2130
Ott de Bruin	Lisa	F-2209
Ouji	Yukiteru	F-2094
Owens	D. Jason	F-2165
Pachon Peña	Olga Gisela	F-3134
Pajenda	Gholam	W-1121
Palomares	Karina	F-3066
Palpant	Nathan	T-2015, F-3059
Paluh	Janet	W-2043
Pankonin	Aimee	W-2131
Panula	Sarita	F-2001
Parachoniak	Christine	T-1020
Park	Christopher	W-1064
Park	Hyun-Sook	T-3116
Park	Jongjin	W-2052
Park	Jong-Min	F-3135
Park	Jung-Chul	T-3115
Park	Kook In	W-3037
Park	Kwang-Sook	W-1056, T-2172
Park	Myung Rae	F-2203
Park	Sangbum	F-1040
Park	Tea Soon	W-2172
Park	Yong-Beom	F-3136
Park	Young-Il	F-2166
Park	Yu Mi	W-2044
Patel	Nisha	T-1008
Patel	Parthive	F-1023
Paul	Sharan	W-2173
Payão	Spencer	W-3125, F-3124
Paylor	Ben	F-3067
Payne	Samantha	F-2075
PB	Megha	W-2053
Pearse	Richard	F-2245
Pease	Jim	W-2132
Pedroza	Rene	F-1115
Pelatti	Mayra	F-3137
Pellacani	Davide	W-1107
Pellicano	Francesca	F-1089
Peng	Kah-Whye	T-2102
Peng	Warner	F-1109
Peng	Weng Chuan	F-1024
Pereira	Thiago	F-2076
Perez	Cristian	F-2204

Perez	Jessica	F-3138
Perkins	Edward	T-2103
Perlin	Julie	W-1065
Perrier	Anselme	F-1116
Perry	John	T-1109
Petersson	Monika	F-1025
Petrie	Timothy	W-2045
Peyer	James	W-1066
Pezeshkian	Bahareh	W-1050
Pfisterer	Ulrich	F-2149
Piekarczyk	Marian	W-2174
Pierce	Halley	W-1067
Pijuan-Galitó	Sara	T-2104
Pilsworth	Jessica	W-1068
Pinho	Sandra	W-1069
Pinto	Mariana	W-1051
Pir	Pinar	W-2010
Pisciotta	Alessandra	T-3117
Porto	Marcella	F-3078
Pourasgari	Farzaneh	W-2054
Prasad	Pankaj	W-1108
Price	Feodor	W-3052
Prieto	Daniel	F-3003
Prodromidou	Kanella	F-2167
Proulx	Maryse	F-2095
Prowse	Andrew	F-1074
Puttonen	Katja	W-3053
Qi	Yuchen	T-2042
Qiao	Yunbo	F-2016
Qin	Yu	F-3139
Qizhou	Lian	F-3038
Qu	Qiuhao	F-3004
Quan	Xiaoyuan	F-2150
Quigley	Anita	F-2096
Rabu	Gabrielle	W-2235
Rahman	Shamim	F-2168
Rahmani	Waleed	F-3079
Rajanahalli	Pavan	W-2102, T-2009
Rajasekhar	Vinagolu	T-2105
Rane	Jayant	W-1034
Rashedi	Iran	F-2077
Rasmussen	Mikkel	F-2212
Redpath	Andia	W-1071
Rentas	Stefan	W-1072
Rezania	Alireza	F-2017
Rezende	Guilherme	T-3125, F-3125
Rhee	Catherine	F-2018
Richter	Jenna	W-1073
Riedel	Michael	F-2019
Rieger	Michael	W-1074
Robert	Jason	F-2246
Roberts	Casey	W-2175
Robillard	Julie	W-2239
Rodin	Sergey	F-1117
Rodini	Carolina	T-1110
Rodrigues	Bianca	W-1075
Rodriguez-Pardo	Viviana Marcela	W-1076
Roig-Lopez	Jose Luis	F-3005
Rojas	Nina	F-3090
Rolando	Chiara	F-3006
Rompolas	Panteleimon	F-1041
Ronaghi	Mohammad	F-2020
Roobrouck	Valerie	F-3140
Ross	Jason	W-2176
Rossello	Ricardo	F-1110
Rothe	Katharina	T-1111
Rowbotham	Samuel	W-1030
Ruan	Jia-Ling	F-2097
Rueda-Argumedo	Nelson	T-1009
Ruetz	Tyson	F-2151
Ruiz	Sergio	T-2107
Russell	Keith	F-3141
Ruzov	Alexey	W-2236
Ryu	Chung Heon	F-3142
Sabapathy	Vikram	W-2177
Sabelstrom	Hanna	F-3007
Sachewsky	Nadia	T-2085
Sackett	Sara	F-2022
Sadarangani	Anil	W-1109
Sadiq	Saud	F-3143
Saha	Krishanu	F-2152
Saiki	Norikazu	F-2213
Saito	Mikako	W-2055
Saitta	Biagio	F-2169
Sakano	Daisuke	T-1010
Salama	Sofie	F-2023
Salomonis	Nathan	F-2170
Samata	Bumpei	F-2024
Sampaio	Arthur	F-3091
Sánchez	Almudena	F-2021
Sang In	Park	F-3144
Santos	Mark	W-2178
Sarma	Sailendra	F-2025
Satarian	Leila	F-3039
Sathiyanathan	Padmapriya	F-3040
Sato	Yukio	F-3092
Sayed	Nazish	W-2157
Schaffer	Ashleigh	F-3008

Scheicher	Ruth	W-1077
Schlieve	Christopher	F-2214
Schmidt	Annette	F-3093
Schnabel	Lauren	W-2179
Schreiner	Petra	T-1011
Schulpen	Sjors	F-2026
Scopelliti	Alessandro	F-1026
Scott	Wilder	F-3080
Sebastiani	Guido	F-3094
Secreto	Frank	W-2180
Segeritz	Charis-P.	F-2215
Sekyrova	Petra	W-2056
Selvadurai	Hayden	T-1112
Semechkin	Ruslan	F-3009
Semi	Katsunori	W-2181
Senda	Sho	T-2109
Seok	Ji	F-1118
Seol	Hye Won	W-2047, T-2046
Serena	Elena	W-3054
Seung	Minji	F-3081
Sha	Hongying	F-2003
Shablii	Volodymyr	F-3082
Shahdadfar	Aboulghassem	W-1035
Shalom-Feuerstein	Ruby	F-1042
Shaw	Georgina	F-3083
Shaw	S.W. Steven	W-3126, T-3126
Sherman	Sean	T-2110
Shetty	Ashok	F-3145
Shim	Eun-Kyung	F-3095
Shin	Dong-Myung	W-2237
Shin	Ji-Hee	W-2140
Shin	Soojung	T-2111
Shineha	Ryuma	F-2242
Shintani	Takashi	W-2085
Shiota	Goshi	W-2158
Shoji	Emi	F-2216
Short	Michael	T-2112
Shtrichman	Ronit	T-2113
Shyu	Jia-Fwu	F-3146
Siddiquee	Khandaker	T-2114
Signer	Robert	W-1078
Silva	Patricia	W-1110
Sincennes	Marie-Claude	W-3055
Singer	Matthew	W-2159
Sirenko	Oksana	F-3010
Sirish	Padmini	T-3069
Skerjanc	Ilona	F-2027
Skinner	Amy	F-1111
Skirecki	Tomasz	W-1079
Smendziuk	Chris	T-2008
Smith	Julianne	W-1080
Sobol	Maria	F-3011
Soga	Minami	W-2183
Soh	Boon Seng	T-3070
Solanas	Guiomar	F-1043
Song	Jihwan	F-3147
Song	Yun Seob	F-3096
Soragni	Elisabetta	F-2217
Sorrells	Shawn	F-3012
Sougawa	Nagako	F-3069
Sperber	Henrik	W-2011
Squazzo	Sharon	F-2230
Sridharan	Rupa	F-2231
Steichen	Clara	F-2218
Steinbeck	Julius	F-3013
Stelzer	Yonatan	F-2219
Stummann	Tina Charlotte	F-2220
Suen	Colin	W-1052
Suga	Mika	T-2116
Sugimori	Michiya	F-3014
Sugiyama	Hayami	W-2057
Suh	Nayoung	F-3097
Suh	Wonhee	F-2028
Sultana	Naznin	F-3070
Sun	Meiyu	F-3098
Sun	Yongming	W-2184
Sun	Yuh-Man	F-3015
Sun Hwa	Kim	F-3099
Suter	David	T-2117
Sutherland	Margaret	W-2185
Suzuki	Naoya	F-3016
Suzuki	Shingo	T-2118
Svitina	Hanna	W-1036
Swaroop	Manju	F-3017
Sylakowski	Kyle	W-2186
Synnergren	Jane	F-2029
Sze	Christie	F-3018
Szkolnicka	Dagmara	T-1021
Tada	Masako	W-1013
Tadokoro	Susumu	W-2160
Taghiyar Renani	Leila	F-3100
Tagle	Jorge	F-2247
Takada	Hitomi	W-2058
Takada	Kei	T-2119
Takahashi	Tsuneo	T-2120
Takarada	Takeshi	F-3101
Takasato	Minoru	F-2030
Takase	Hinako	F-2004

Takayama	Kazuo	W-1014
Takebe	Takanori	F-2098
Takehara	Toshiyuki	W-2059
Taketani	Takeshi	F-3148
Taketani	Tamara	W-1015
Talavera-Adame	Dodanim	W-1008
Talbot	Neil	W-2161
Talwar	Baldeep	F-2099
Tan	Jean	W-1037
Tanabe	Koji	F-2153
Tarunina	Marina	T-2121
Tatehara	Seiko	W-2090
Tatsumi	Rie	W-1053
Taylor-Weiner	Hermes	F-2031
TCW	Julia	F-2154
Tedesco	Francesco Saverio	W-3056
Teino	Indrek	F-2032
Teo	Adrian	W-2162
Terunuma	Hiroshi	T-2122
Tewary	Mukul	W-2091
Thakar	Nilay	W-2060
Thomaidou	Dimitra	F-3019
Thomas	Kelsey	F-3102
Thomas	Samantha	W-2187
Thooyamani	Abinaya Sundari	F-2078
Thu	Mya	F-2079
Tian	Xiaoyu	W-1054
Tieberg	Deborah	T-2123
Toguchida	Junya	T-3153
Tomizawa	Minoru	W-1017
Tompkins	Joshua	F-3071
Tong	Zhi-Bin	F-3020
Tonge	Peter	W-2188
Torper	Olof	F-2155
Toyoda	Taro	W-1009
Tran	David	F-3072
Tremblay	Jacques	W-2163
Trentin	Andrea	W-2092
Trujillo	Michael	T-3002, F-3002
Trunda	Miroslav	F-3149
Tsai	Ming-Song	W-2087, T-3127
Tsai	Pei-Jiun	F-3150
Tsao	Yeou-Ping	F-3041
Tschudy Seney	Benjamin	W-1018
Tseng	Huan-Chin	F-2005
Tsuchida	Hiroshi	W-2151
Tsui	Jonathan	W-2093
Tsui	Yat-Ping	F-3021
Tsurumi	Fumitoshi	F-2221
Uda	Junki	F-1044
Uenishi	Gene	W-1081
Ulrich	Saskia	F-2033
Umbach	Nadine	T-2124
Umebayashi	Daisuke	F-3022
Ungrin	Mark	T-2125
V	Sivakamasundari	F-2034
Vadodaria	Krishna	W-2164
Valadares	Marcos	F-3103
Valensisi	Cristina	F-3023
van de Kamp	Julia	W-2094
van den Berg	Cathelijne	T-3071
Vanhatupa	Sari	F-3104
Vankelecom	Hugo	W-1111
Varga	Balazs	W-2165
Velasco	Talia	W-1112
Velayudhan	Shaji	W-1070
Velletri	Tania	F-3105
Vemuri	Mohan	T-2158
Vestergaard	Maj	F-3073
Victor	Matheus	W-2152
Vidal	Jason	T-2126
Vijayaraj	Preethi	F-2222
Vulliet	Richard	F-3106
Wada	Masanori	W-2189
Wade	Staton	W-2238
Wagey	Ravenska	F-3151
Wagner	Daniel	F-2080
Wali	Gautam	F-2223
Wang	Allen	W-1010
Wang	Bo	W-2061
Wang	Li	T-3072
Wang	Mengyu	F-3107
Wang	Penglai	W-2095
Wang	Pin Yu	F-3084
Wang	Shufang	F-2007
Wang	Tian	F-2006
Wang	Tongguang	F-3024
Wang	Xiao Qi	T-1113
Wang	Xiaojie	F-2081
Wang	Yanling	F-3025
Wang	Ying	F-3085
Wang	Yuan	T-2016, T-2234
Wang	Yu-Chieh	W-2153
Wang	Yunfang	W-2154
Watanabe	Makoto	F-2224
Watters	Robin	T-2127
Watts	Tammara	F-1113
Wedeken	Lena	W-1011

Wee	Shimei	W-1113
Wegrzyn Woltosz	Joanna	W-1082
Wei	Lisa	W-1083
Wei	Wei	W-1038
Weinacht	Katja	W-2166
Weiss	Mark	T-2115
Weissbein	Uri	F-3026
Weltner	Jere	W-2190
White	Adam	T-2128
Wilcox	Jared	F-3027
Williams	Kaylyn	W-2191
Williams	Luis	F-2036
Wilson	Patricia	W-2192
Wilson	Val	W-2012
Wobus	Manja	F-3108
Wolvetang	Ernst	W-2193
Woodsworth	Daniel	W-2155
Wörsdörfer	Philipp	F-2035
Wrighton	Paul	F-2037
Wu	Han-Chung	W-1114
Wu	Jiaqian	F-3028
Wu	Meng-Hsueh	F-3109
Wu	Mian	W-2064
Wu	Qiang	W-2063
Wu	Wen-shu	W-2062
Wu	Yaojiong	F-3110
Wu	Zhao	F-1120
Wylot	Bartosz	W-1084
Xiang	Jinyi	F-1027
Xie	Wen	F-2226
Xing	Yalan	W-2008
Xu	Hao	F-2038
Xu	Jun	F-3111
Xu	Ren-He	F-1121
Xu	Wenjun	F-3030
Xu	Zhiheng	F-3029
Yaegaki	Ken	F-3112
Yakubov	Eduard	F-2082
Yamada	Daisuke	W-2194
Yamada	Takatsugu	W-2167
Yamahara	Kenichi	F-3086
Yamamizu	Kohei	F-2039
Yamamoto	Yusuke	W-1039
Yamane	Junko	T-2129
Yamauchi	Kaori	F-3074
Yan	Kenneth	T-1114
Yan	Liying	W-2065
Yanagida	Aya	F-3042
Yanagihara	Kana	W-2195
Yang	Daniel	T-3073
Yang	Dapeng	T-2043
Yang	Fan	W-2196
Yang	Han-Mo	W-2197
Yang	Jay (Jiwei)	F-2039
Yang	Jiao	W-2066
Yang	Jinok	F-3113
Yang	Liang-Tung	F-1045
Yang	Tsunglin	W-2096
Yang	Wenli	W-2198
Yang	Xian-Jie	F-3043
Yao	Chao-Ling	T-1055
Yao	Chao-Ling	W-1022
Yap	Lynn	F-2040
Yaqubi	Moein	F-3031
Yasuda	Shinya	T-2130
Yen	Men-Luh	F-3114
Yi	Sang Hoon	F-2041
Yin	Dezhong	F-3115
Yokoyama	Kazushige	W-2199
Yoo	Tai June	T-2073
Yoo	Tai June	F-3126
Yoon	Kyung-Ae	W-1085
Yoshida	Junko	W-2067
Yoshikawa	Masahide	F-2042
Yu	Jason	F-2043
Yu	Kyung-Rok	W-2140
Yu	Pengzhi (Palmer)	T-3074
Yu	Vionnie	T-1056
Yu	Vionnie	F-1057
Yu	Yin	W-2097
Yuan	Changyong	W-2098
Yuan	Guo-Cheng	T-2238
Yuan	Jie	W-1019
Yuan	Ye	W-2068
Yun	Ina	F-3087
Yun	Ji-Yeon	W-1086
Yung	Jasmine	F-2227
Zakinova	Angela	W-2200
Zaltsman	Yefim	F-2044
Zanni	Giulia	F-3032
Zarzeczny	Amy	T-2246
Zemke	Martina	T-3036
Zhang	Ben	W-2100
Zhang	Chengfei	W-2099
Zhang	Jianhua	W-2169
Zhang	Jin	W-2201
Zhang	Ranran	W-2168
Zhang	Regan-Heng	W-3057

Zhang	Rong	T-2131
Zhang	Xiuyan	W-1087
Zhang	yi	F-3116
Zhang	Zhiyong	F-2083
Zhao	Chang	W-2202
Zhao	Meng	W-1088
Zheng	Chunxing	F-3117
Zheng	Hui	F-2232
Zheng	Jie	W-3001
Zheng	Jie	T-2088
Zheng	Yunwen	W-1020
Zhikang	Liu	W-2069
Zhou	Qiao	F-3033
Zhu	Jianhong	F-3044
Zhu	Lifang	T-2084
Zhu	Lili	W-2203
Zhu	Saiyong	W-1012
Zi	Yin	F-3118
Zimmerlin	Ludovic	W-2070
Zimmermann	Tina	F-3034
Zito	Giovanni	F-1046
Zou	Chunlin	W-2204
Zuba-Surma	Ewa	W-2071
Zuba-Surma	Ewa	F-2101
Zuo	Wei	W-1031
Zusso	Morena	F-3035
Zutshi	Massarat	F-3152
Zweigerdt	Robert	T-2132

NOTES