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POSTER ABSTRACT BOOK

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WEDNESDAY

POSTER SESSION I ODD

PRE-CLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL CELLS

W1001

CLINICAL IMPROVEMENT IN MOTOR FUNCTION FOLLOWING INTRACRANIAL INJECTION OF MODIFIED BONE MARROW-DERIVED MESENCHYMAL STEM CELLS (SB623) IN ISCHEMIC STROKE PATIENTS WITH CHRONIC MOTOR DEFICITS

Bates, Damien[1], Steinberg, Gary[2], Kondziolka, Douglas[3], Wechsler, Lawrence[3], Lunsford, L. Dade[3], Kim, Anthony S.[4], Johnson, Jeremiah[2], McGrogan, Michael[1], Yankee, Ernest W.[1], Liu, Wenzhong Jerry[1] and Schwartz, Neil E.[2], [1]SanBio, Inc., Mountain View, CA, U.S., [2]Stanford School of Medicine, Stanford, CA, U.S., [3]University of Pittsburgh Medical Center, Pittsburgh, PA, U.S., [4]University of California, San Francisco, San Francisco, CA, U.S.

Chronic stroke is a major cause of disability for which there is a lack of clinically effective therapies. This is the preliminary 12-month data analysis of a single-ascending dose (2.5, 5, or 10 million cells), open label, Phase 1/2A clinical study (NCT01287936). Patients with stable chronic stroke (6-60 months post-stroke) were treated with stereotactic intracranial implantation of modified bone marrow-derived mesenchymal stem cells (SB623) and followed for up to 24 months. The study was approved by an institutional review board, and patients provided written informed consents. Preliminary pooled dose efficacy analyses on the intent-to-treat population (N=18) demonstrated statistically significant changes from baseline at Months 6 and 12 for the following scales: 1) European Stroke Scale (ESS) total score: +6.50 (M6, 95% CI: [2.6, 10.4]) / +6.88 (M12, 95% CI: [3.5, 10.3]), 2) National Institutes of Health Stroke Scale (NIHSS) total score: -1.69 (M6, [-2.8, -0.6]) / -2.00 (M12, [-2.7, -1.3]), 3) the Fugl-Meyer (F-M) total score: +19.19 (M6, [11.9, 26.5]) / +19.20 (M12, [11.4, 27.0]), and 4) the F-M motor function total score: +10.69 (M6, [4.3, 17.1]) / +11.40 (M12, [4.6, 18.2]). However, changes in modified Rankin Scale (mRS) were not statistically significant at Months 6 or 12: -0.13 (M6, 95% CI: [-0.3, 0.1]) / 0.00 (M12, 95% CI: [-0.2, 0.2]). Sustained clinical improvements in motor function following intracranial injection of SB623 cells in chronic stroke patients were observed at 6 and 12 months after the surgery.

Funding Source: Funding for this research was provided by SanBio, Inc.

W1003

ULTRASOUND-MEDIATED GENE TARGETING TO ENDOGENOUS MESENCHYMAL STEM CELLS: A NOVEL THERAPY FOR BONE REGENERATION

Pelled, Gadi[1,2], Bez, Maxim[1], Tawackoli, Wafa[2], Avalos, Pablo[2], Giaconi, Joseph[2], Sheyn, Dmitriy[2], Shapiro, Galina[1], Ben-David, Shiran[2], De Mel, Sandra[2], Ferrara, Katherine[3], Bae, Hyun[2], Gazit, Zulma[1,2] and Gazit, Dan[1,2], [1]The Hebrew University-Hadassah Faculty of Dental Medicine, Jerusalem, Israel, [2]Cedars-Sinai Medical Center, Los Angeles, CA, U.S., [3]University of California, Davis, Davis, CA, U.S.

2.2 million bone-grafting procedures for segmental bone defects are performed each year using autografts or allografts. Yet due to various disadvantages of both types of grafts there is a clear medical need for the development of new therapies for massive bone loss. We hypothesized that the attraction of endogenous MSCs to a bone defect site followed by ultrasound-mediated bone morphogenetic protein (BMP) gene delivery would induce efficient bone regeneration and defect repair in a clinically relevant large animal model. We first determined that implanting a biodegradable collagen scaffold in segmental bone defects created in the tibia bones of mini-pigs led to the attraction of endogenous MSCs to the fracture site, which peaked fourteen days post operation. Next, we injected a GFP plasmid mixed in microbubbles (MBs), under fluoroscopic guidance, to the defect site followed by transcutaneous ultrasound application to induce MBs cavitation and bursting. Flow cytometry analysis performed five days later, showed that 50% of the cells in the defect expressed GFP, and that 70-90% of the transfected cells co-expressed MSC markers (CD 90, 29, and 44). In the next step, twenty-seven mini-pigs were operated. Segmental defects were created in their tibia bones and implanted with collagen scaffolds. Two weeks later the animals were treated with ultrasound-mediated BMP-6 gene delivery, BMP-2 gene delivery, ultrasound only, plasmid only or left untreated. Six weeks post treatment the animals were sacrificed and the tibia bones analyzed. MicroCT analysis demonstrated significantly more bone volume and bone density in tibia defects treated with ultrasound and BMP-6, which healed completely, compared to all other groups. Biomechanical analysis of the treated bones revealed mechanical superiority of tibia bones treated with ultrasound and BMP-6. ELISA was used to demonstrate BMP-6 secretion at the defect site that peaked on Day 5 post gene delivery and was undetected

on Day 10. Plasmid biodistribution analysis did not detect DNA copies in internal organs. Collectively these results are a proof-of-concept for the feasibility of the proposed “graft-less” therapy for segmental bone defect repair. This is also the first report of ultrasound-mediated gene delivery to endogenous MSCs in a large animal model.

Funding Source: CIRM grant # TR4-06713

W1005

MICRORNA-MEDIATED DOWN-REGULATION OF ASK1 ATTENUATES APOPTOSIS OF HUMAN MSCS TRANSPLANTED INTO ISCHEMIA/ REPERFUSION INJURED HEART

Lee, Chang Youn[1], Lee, Jiyun[2], Seo, Hyang-Hee[3], Lim, Kyu Hee[4], Kim, Hye-Min[1], Shin, Sunhye[1], Kim, Sang woo[5,6], Choi, Eunhyun[5,6], Lim, Soyeon[5,6], Lee, Seahyoung[5,6] and Hwang, Ki-chul[5,6], [1]Department of Integrated Omics for Biomedical Sciences, Yonsei University, Seoul, Korea, [2]Brain Korea 21 PLUS project for Medical Science, Yonsei University, Seoul, Korea, South, [3]Brain Korea 21 PLUS project for Medical Science, Yonsei University, Seoul, Korea, [4]Department of Veterinary Physiology, Chonbuk National University, Jeonju, Korea, [5]Catholic Kwandong University International St. Mary’s Hospital, Incheon, Korea, [6]Institute for Bio-Medical Convergence, Catholic Kwandong University, Gangneung, Korea

Stem cell therapy using adult stem cells such as mesenchymal stem cell (MSCs) has produced some promising results in treating diseased heart demonstrating its therapeutic potential for the repair of myocardial injury. However, a low survival rate of MSCs after transplantation compromises the effectiveness of stem cell therapy. Therefore, developing strategy to support long-term survival of MSC after transplantation has clinical significance in terms of efficacy of stem cell therapy. In the damaged heart, oxidative stress due to production of reactive oxygen species (ROS) can cause death of transplanted MSCs. Apoptosis signal-regulating kinase 1 (ASK1) has been implicated in the development of oxidative stress-related pathologic conditions. Thus, we hypothesized that down-regulation of ASK1 in MSC may attenuate post-transplantation death of MSC. To test this hypothesis, we utilized miRNA to down-regulate endogenous ASK1 expression of MSCs. We first screened miRNAs that target ASK1 based on miR-target prediction databases and empirical data. Our data indicated that miR-301 most significantly suppressed ASK1 expression in MSCs. Apoptosis-related genes were downregulated significantly in miR-301 enriched MSCs exposed to hypoxia, and the number of annexin V/PI stained cells also decreased. Furthermore, when miR-301-enriched MSCs were transplanted into ischemia/reperfusion injured heart of rats, the number of MSCs survived after transplantation increased while the number of TUNEL-positive cells decreased. Additional analysis indicated that miR-301-enriched MSCs improved cardiac function compared to normal MSCs. Taken together, these data indicate that miR-mediated down-regulation of ASK1 may protect MSCs post-transplantation and increases the efficacy of MSCs-based cell therapy

W1007

FUNCTIONAL RECOVERY OF STROKE RATS INDUCED MICROVESICLES DERIVED FROM BRAIN EXTRACT-TREATED MESENCHYMAL STEM CELLS

Choi, Seong-Mi[1], Lee, Ji Yong[2], Jun, Eun Young[2] and Kim, Han-Soo[2,3], [1]Institute for Bio-Medical Convergence, Incheon, Korea, South, [2]Catholic Kwandong University International St. Mary’s Hospital, Incheon, Korea, South, [3]Catholic Kwandong University College of Medicine, Gangneung-si, Korea, South

The transplantation of mesenchymal stem cells (MSCs) has been shown to improve functional outcomes in a rat model of ischemic stroke. Subsequent studies using the same model have suggested that MSC microvesicles (MVs) can replace the beneficial effects of MSCs. In this study, we investigated the ability of normal and stroke rat brain extract-treated MSC-derived microvesicles (NBE-MSC-MVs and SBE-MSC-MVs, respectively) to attenuate ischemic brain injury induced by permanent middle cerebral artery occlusion (pMCAO) in rats. We found that the cytokine profiles of normal and stroke brain extracts were similar; the extracts contained a number of neurogenic and neurotrophic factors and cytokines that can significantly influence the quality and quantity of MSC-derived MVs. To examine the therapeutic benefits of MVs of brain extract-treated MSCs in an ischemic stroke model, intracarotid MV injections (0.2 mg/kg) were administered to Sprague-Dawley rats 2 days after pMCAO. Our results demonstrated that NBE-MSC-MVs and to a lesser extent SBE-MSC-MVs ameliorated ischemic brain injury with improved functional recovery. Immunohistochemical analyses showed that NBE-MSC-MVs reduced inflammation, enhanced angiogenesis with increased endogenous neurogenesis in rat brain. To obtain mechanistic insights into the therapeutic effect of these MVs, we performed an integrative mass spectrometry-based proteomics analysis and found that the NBE-MSC-MV proteome is highly enriched for vesicular proteins. Finally, using a systems biology approach, we reconstructed a network of NBE-MSC-MV therapeutic factors linked to anti-inflammation, angiogenesis, neurogenesis, and apoptosis; this network may represent a proteome system stimulated by brain extract. Our study demonstrated the treatment of ischemic rats with NBE-MSC-MVs promotes the functional recov-

ery of damaged stroke brain via modulation of anti-inflammation, angiogenesis and neurogenesis.

Funding Source: Study supported by the Bio & Medical Technology Development Program of the National ResearchFoundation (NRF) funded by the Ministry of Science, ICT and Future Planning, Korea, No.2012M3A9B4028639.

W1009

A SMALL MOLECULE INHIBITOR FOR CULTURE EXPANSION OF UNDIFFERENTIATED HUMAN MESENCHYMAL STEM/STROMAL CELLS

Gurung, Shanti[1], Deane, James[1], Werkmeister, Jerome[2] and Gargett, Caroline[1], [1]The Ritchie Centre,Hudson Institute of Medical Research, Monash University, Melbourne, Australia, [2]CSIRO, Melbourne, Australia

Mesenchymal stem/stromal cells (MSCs) are popular candidate cells for cell-therapy and regenerative medicine. Due to their rarity and the large numbers required for clinical use, extensive *ex vivo* expansion is required, but MSCs undergo spontaneous differentiation into fibroblasts and replicative senescence, decreasing their purity, survivability and efficacy for clinical applications. We showed that A83-01, a TGF-b receptor inhibitor promoted SUSD2$^+$ human endometrial MSC (eMSC) proliferation, and blocked senescence and apoptosis *in vitro*. The aims of this study were to determine if A83-01 1) has similar effects in MSCs derived from post-menopausal endometrium, bone marrow, adipose tissue, placenta and menstrual blood *in vitro* and 2) improves eMSC survivability *in vivo.* MSCs were isolated from dissociated tissues using SUSD2 magnetic beads and cultured in serum free medium (SFM) with bFGF/EGF in 5%O_2/5%CO_2. At passage 6 (P6), MSCs were incubated with or without A83-01 for 7 days, then analysed for MSC properties. A83-01 treatment promoted SUSD2$^+$ MSC proliferation, increasing the %SUSD2$^+$ post-menopausal endometrial, bone marrow, adipose tissue, placental, and menstrual blood MSC cells in P6 cultures. There was no change in expression of CD90, a standard MSC marker or CD140b, while CD146 was downregulated. P1 SUSD2$^+$ eMSC transduced with a mCherry vector and treated with/without A83-01 retained high expression of the fluorescent protein until P6, after which 5X10^5 were transplanted under kidney capsule of NSG mice until harvest at 7, 14, 30 days. The A83-01 treated eMSC survived under the kidney capsule for a longer duration and in greater number than the untreated eMSCs as detected by mCherry immunofluorescence, SUSD2 flowcytometry and Alu sequence PCR. Small molecules such as A83-01 that promotes proliferation of various MSC types in the undifferentiated state may provide an approach for the expansion of undifferentiated MSC for use in tissue engineering and cell-based therapies.

Funding Source: This study was funded by the NHMRC of Australia grant, Senior Research Fellowship, Monash University Medicine Faculty Postdoctoral fellowship Youanmi Foundation and Victorian Infrastructure Support Program.

W1011

PGE2 MAINTAINS SELF-RENEWAL OF HUMAN ADULT STEM CELLS VIA EP2-MEDIATED AUTOCRINE SIGNALING AND ITS PRODUCTION IS REGULATED BY CELL-TO-CELL CONTACT

Lee, Byung-Chul[1], Kim, Hyung-Sik[2], Shin, Tae-Hoon[3], Kang, Insung[4], Lee, Jin Young[5], Kim, Jae-Jun[3], Seo, Yoo Jin[6], Choi, Soon Won[3] and Kang, Kyung-Sun[2], [1]Seoul National University, Seoul, Korea, South, [2]Seoul National University, Seoul, Korea, South, [3]Adult Stem Cell Research Center, Seoul National University, Seoul, Korea, South, [4]Seoul National University, Seoul, Korea, South, [5]Seoul National University, Seoul, Korea, South, [6]Adult Stem Cell Research Center, Seoul, Korea, South

Mesenchymal stem cells (MSCs) possess unique immunomodulatory abilities. Therefore, clinical efficacy and underlying mechanisms of MSCs in immune disorders have been elucidated by a number of studies. Although several immunoregulatory factors including Prostaglandin E_2 (PGE_2) and their mechanism of action on immune cells have been revealed, their effects on MSC itself and the regulation of their production by culture environment have been less verified. Therefore, we sought to investigate the autocrine effect of PGE_2 on MSCs and the regulation of its production by cell-to-cell contact, followed by determination of immunomodulatory properties. MSCs treated with specific inhibitors to suppress PGE_2 secretion were determined for their proliferative phenotype. PGE_2 exerted autocrine regulatory function in MSCs via E-Prostanoid (EP) 2 receptor triggering and the inhibition of its production led to the growth arrest, whereas addition of MSC-derived PGE_2 restored the proliferation. The level of PGE_2 production from the equivalent number of MSCs was down-regulated via gap junctional intercellular communication. Cell contact-mediated diminish in PGE_2 secretion resulted in the decline of immunosuppressive effect. In conclusion, PGE_2 produced by MSCs contributes to the maintenance of self-renewal capacity through EP2 in an autocrine manner, and the PGE_2 secretion is down-regulated by cell-to-cell contact, attenuating immunomodulatory potency.

Funding Source: This work was supported by the Materials and Components Technology Development Program

of MOTIE/KEIT, Republic of Korea (10046508, Development of immunomodulatory cell therapeutics through the development of culture media s

W1013

LONG-TERM COMPARISON OF THERAPEUTIC EFFICACY OF AUTOLOGOUS AND ALLOGENEIC UMBILICAL CORD BLOOD THERAPY FOR CHILDREN WITH CEREBRAL PALSY RELATED TO PERIVENTRICULAR LEUKOMALACIA

Min, Kyunghoon[1], Cho, Kye Hee[2] and Kim, MinYoung[1], [1]Bundang CHA Medical Center, Seongnam-si, Korea, South, [2]CHA Bundang Medical Center, Seongnam, Korea

Cerebral palsy (CP) is the leading cause of disability in children. Brain damage is known to be persistent and such active damage process can be the target of cell therapy. Umbilical cord blood (UCB) has been highlighted as one of the promising cell sources given that safety, availability and immune tolerance for them. Autologous UCB therapy has been known to be feasible and efficient for children with CP. For those lacking autologous UCB, allogeneic UCB can be alternative. Even though accumulating studies on UCB therapy suggests its benefit, no study has compared the difference in long-term effects between the sources. This study aims to reveal the different efficacy of autologous and allogeneic UCB in children with CP with periventicular leukomalacia (PVL). Patients who received UCB therapy from January 2010 until September 2015 were reviewed retrospectively. Total 423 patients received UCB therapy. Among them, 170 patients were included with the criteria of (1) CP (2) PVL in brain MRI and (3) a single UCB therapy. The numbers of children treated with autologous and allogeneic UCB therapy were 16 and 154, respectively. Erythropoietin was used as an adjuvant to potentiate the effects of UCB therapy. The caregivers of children with the above inclusion criteria were surveyed through the telephone about the gross motor function, safety issues and the level of satisfaction for the UCB therapy. The average duration of telephone survey was about four years post UCB therapy. Autologous UCB group (0.75) showed significant better improvements in Gross Motor Function Classification System compared to allogeneic UCB group (0.13) (p=0.012). In terms of safety, there was no report of malignant tumor. Tolerability throughout the procedure was significantly higher in autologous UCB group. These results suggest that autoglous UCB therapy may be more effective cell source for children with CP. It might suggest that autologous UCB therapy is more therapeutic potential to ameliorate motor dysfunction in CP with PVL. Further studies with prospective, larger sample size are required to determine which UCB is better in term of immunogenicity.

W1015

HUMAN MESENCHYMAL STEM CELLS INDUCE M2 MACROPHAGES THROUGH THE DOWNREGULATION OF RAGE EXPRESSION IN COLLAGEN-INDUCED ARTHRITIS

Shin, Yong Dae, Division of Rheumatology, Department of Internal Medicine, Department of Medical, Seoul, Korea, South

Mesenchymal stem cells (MSCs) have profound immunomodulatory properties. Macrophages are key regulators of the innate immune system. Depending on the microenvironment, macrophages can acquire distinct functional phenotypes as classically activated macrophages (M1 macrophages) and alternatively activated macrophages (M2 macrophages). M1 macrophages are characterized by the expression of proinflammatory cytokines and promotion of Th1 response. In contrast, M2 macrophages have immunoregulatory functions. In this study, we investigated the immunomodulatory property of MSCs on macrophages in vitro and in vivo. Macrophages were isolated by lavage of the peritoneal cavity of DBA/1 mice. We co-cultured human adipose-derived MSCs (hAD-MSCs) and mouse peritoneal macrophages directly with 1:5 ratio for 24 hour. The effect of hAD-MSCs on macrophage phenotype polarization was assessed by flow cytometry, and supernatants for induced production of cytokines were also analyzed. Protein expressions were examined by western blot. In addition, we evaluated the effect of hAD-MSCs on macrophages of peritoneal cavity in collagen induced arthritis (CIA) mice. hAD-MSCs significantly induced M2 macrophages in co-culture condition. And, the levels of IL-10 and TGF-β1 were markedly elevated. The expressions of RAGE were decreased in 1:5 co-culture ratio at 24 hour. Moreover, hAD-MSCs treated mice significantly decreased clinical scores and induced M2 macrophage in peritoneal cavity. The relative protein expression of Arg-1 was increased, and those of RAGE and NF-κB were decreased in hAD-MSCs treated CIA mice. Our data suggest that the therapeutic effect of hAD-MSCs in CIA might be related to M2 macrophages induction by hAD-MSCs and showed that hAD-MSCs downregulate the RAGE expression in macrophages in both in vitro and in vivo.

Funding Source: This study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI13C1270 and HR14C0006) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2013R1A1A2058120)

W1017

USING AN INDUCED PLURIPOTENT STEM CELL MODEL TO UNDERSTAND AND TREAT IDIOPATHIC PULMONARY FIBROSIS

Vijayaraj, Preethi, Durra, Abdo, Mehrabi, Mehrsa, Chung, Kathy, Zhang, Kelvin, Darmawan, Kelly, Karumbayaram, Saravanan, Damoiseaux, Robert and Gomperts, Brigitte, University of California, Los Angeles, Los Angeles, CA, U.S.

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and invariably lethal interstitial lung disease of unknown etiology. IPF represents the most common cause of death from progressive lung disease with no effective therapy other than lung transplantation. This is in large part due to the fact that the pathogenesis of IPF remains unclear. It is thought to mostly be a complex disorder resulting from an underlying genetic predisposition together with an environmental trigger. There are currently no assay systems for drug discovery nor animal models for efficacy studies that truly recapitulate IPF. In an attempt to identify a novel therapy for IPF, we have generated and extensively characterized an induced pluripotent stem cell (iPSC) based in vitro disease model that closely phenocopies IPF in a dish. These cells scar spontaneously and progressively when cultured over several days. Live cell imaging, transcriptome analysis, atomic force microscopy, immunostaining, apoptosis assays and cytokine arrays were used to characterize these scars. The progressive scarring is driven by a continuous signaling loop involving damage associated molecular patterns and a robust pro-inflammatory response, leading to the observed pathology. The inflammatory response is modulated through the Wnt/β-catenin signaling pathway. Using the model, we developed a primary phenotypic high throughput assay to identify compounds that would target one or more of the phenotypic characteristics of our model, such as increasing apoptosis of hyper-proliferative fibroblasts, targeting extracellular matrix interactions, targeting the stiffness of the cells, inhibiting epithelial-to mesenchymal transition etc. We have identified a small molecule (AA5) that prevents the formation of fibrosis and resolves fibrosis that has already formed in the dish. Target deconvolution efforts are currently underway to identify the cellular targets and to identify the mode of action of AA5.

W1019

IDENTIFICATION OF OSTEOPROTEGERIN AS ONE OF THE IMPORTANT PARACRINE FACTORS OF MSC THAT FACILITATE THE BENEFICIAL EFFECT OF MSC TRANSPLANTATION

Lee, Jiyun[1], Lee, Chang Youn[2], Seo, Hyang-Hee[3], Lim, Kyu Hee[4], Kim, Hye-Min[2], Shin, Sunhye[2], Kim, Sang woo[5,6], Choi, Eunhyun[5,6], Lim, Soyeon[5,6], Lee, Seahyoung[6,7], Park, Jong-Chul[3,8] and Hwang, Ki-chul[6,7], [1]Brain Korea 21 PLUS project for Medical Science, Yonsei University, Seoul, Korea, South, [2]Department of Integrated Omics for Biomedical Sciences, Yonsei University, Seoul, Korea, [3]Brain Korea 21 PLUS project for Medical Science, Yonsei University, Seoul, Korea, [4]Department of Veterinary Physiology, Chonbuk National University, Jeonju, Korea, [5]Institute for Bio-Medical Convergence, Catholic Kwandong University, Gangneung, Korea, [6]Catholic Kwandong University International St. Mary's Hospital, Incheon, Korea, [7]Institute for Bio-Medical Convergence, Catholic Kwandong University, Gangneung, Korea, South, [8]Department of Medical Engineering, Yonsei University, Seoul, Korea

Stem cell therapy using adult stem cells such as mesenchymal stem cells (MSCs) and adipose-derived stem cells (ADSCs) has been demonstrated to be a viable option for treating damaged heart. Although in situ differentiation and/or paracrine effect of stem cells are regarded as two major mechanisms through which stem cells exert beneficial effect after transplantation, evidence suggests that the paracrine effect of stem cells is more likely to be the major one. Even for now, thorough analysis of soluble factors released by stem cells has not been completed yet, and thus, elucidating the underlying mechanisms of how stem cell transplantation improves host tissue viability and identifying the soluble factors involved deserve own significance. Osteoprotegerin (OPG) can bind both TNF-related apoptosis-inducing ligand (TRAIL) and receptor activator of NF-kB ligand (RANKL) that are known to facilitate apoptosis and hypertrophy of cardiomyocytes, respectively. When OPG binds to these ligands, it functions as decoy for those ligands suppressing the activation of both TRAIL and RANKL-mediated signaling cascades. Thus, in the present study, we investigated the expression of OPG in MSCs under hypoxia and examined whether MSC-released OPG contributes to the reported beneficial effect of MSCs. Our data show that the expression and release of OPG in MSC increased under hypoxic condition. Furthermore, MSC-conditioned media treatment prevented apoptosis and hypertrophy of cardiomyocytes, and this was abrogated when MSCs were treated with siRNA specific to OPG prior to hypoxia. Our study demonstrates that hypoxic condition induces OPG expression in MSCs

and strongly suggest that the MSC-released OPG under hypoxic condition is one of the important factors that facilitate the reported cardiac protective effect of MSCs.

MESENCHYMAL STEM CELL DIFFERENTIATION

W1021

FGFR3 CONTROLS MESENCHYMAL STEM CELL PROLIFERATION, MIGRATION AND DIFFERENTIATION.

Carstairs, Alice, Quick, Sophie and Genever, Paul, University of York, York, U.K.

Signaling through fibroblast growth factor receptor 3 (FGFR3) is essential for bone elongation during skeletal development, acting as a negative regulator of bone growth. FGFR3 knockout (KO) mutations in mice results in severe skeletal dysplasia in which bones formed by endochondral ossification are dramatically elongated and hypertrophic chondrocyte zones increase in size. A similar phenotype is observed in humans with germline homozygous loss-of-function mutations. Less well known are the effects of FGFR3 on the mesenchymal cells from which skeletal elements arise and the differences in FGFR3 signaling during embryonic and post-natal development. We generated an FGFR3-KO in an immortalized, clonal adult human mesenchymal stem cell (MSC) line using CRISPR/Cas9 to introduce a single base insertion, causing a frameshift in the extracellular domain of FGFR3, truncating the protein. New single cell-derived MSC colonies were established by serial dilution and the insertion verified by sequencing. FGFR3-KO MSCs were viable but had altered morphologies compared to wild type (WT) controls, with elongated cell bodies, reorganized actin microfilaments and broad lamellipodia, consistent with a migratory phenotype, however cell size was not significantly different. In scratch wound assays, FGFR3-KO MSCs healed at a faster rate versus WT over 12h (50% and 18% closure respectively, $p<0.05$). In proliferation assays over 72h, 76.7% and 94.9% of WT MSCs and FGFR3-KO MSCs respectively incorporated EdU ($p<0.0001$) demonstrating increased cell cycling, supported by increased staining in an MTT assay at 96 hours ($p<0.05$). In differentiation assays, FGFR3-KO caused complete disruption of osteogenesis with significant decreases in Alizarin Red staining ($p<0.0001$) and alkaline phosphatase activity was 2-fold lower in FGFR3-KO MSCs ($p<0.0001$). Adipogenesis was severely delayed in FGFR3-KO MSCs with a significant decrease in lipid droplet accumulation compared to WT MSCs ($p<0.0001$). Mutating FGFR3 in this manner results in an altered behaviour of MSCs where proliferation and migration are favoured over differentiation. Our findings shed new light on the role of FGFR3 in development of skeletal dysplasias *in vivo* and identify an influential function for FGFR3 in post-natal MSC biology.

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W1023

BIOMECHANICAL REGULATION OF HUMAN MESENCHYMAL STEM CELL DIFFERENTIATION INTO VASCULAR PHENOTYPES

Lee, Jason, Deb, Chaarushena, Armenta-Ochoa, Miguel, Maceda, Pablo, Spencer, Adrianne, Yoon, Eun, Samarneh, Lara, Crosby, Cody, Sligar, Andrew and **Baker, Aaron**, University of Texas at Austin, Austin, TX, U.S.

Over the past decades, scientific advances and economic pressures have driven the need for improving drug discovery and screening technologies. Scientific studies examining mechanobiology using conventional technologies have been limited by the relative low throughput of the experimental systems. We have recently developed two novel devices for the high throughput investigation of stem cell mechanobiology, one that generates controlled shear stresses in a 96-well format and another that applies dynamic mechanical strain to cells in a 6 x 96-well format. Using these systems we investigated the role of mechanical forces in regulating the response of human mesenchymal stem cells (hMSCs) to biochemical stimulation with growth factors and a variety of differentiation conditions. Using the shear device, we examined the activation of the transcription factors FOXO, SRE, TCF/LEF, SMAD and HIF by the growth factors TGF-β1 and VEGF-A in combination with different shear conditions including static culture, steady flow with 1 or 5 dynes/cm^2 of shear stress or oscillatory shear stress. Our results indicate that moderate steady or oscillatory shear stress reduces FOXO activity and that oscillatory shear stress activates SRE, TCF/LEF and HIF in comparison to static culture. In addition, we found that oscillatory shear synergistically increased SMAD activation in combination with TGF-β1. We examined the differentiation of stem cells into vascular phenotypes using multiple treatments and found that differentiation toward the endothelial lineage only occurred in the presence of 5 dynes/cm^2 of shear stress. Further analysis demonstrated that this level of shear leads to an increase in VEGFR1, potentiating this response. Using the mechanical strain system, we explored a variety of mechanical conditions and found that Smad2/3 and TCF-LEF transcription factors were highly activated with low frequency dynamic strain (0.1 Hz) with a maximum strain magnitude of 7.5%. Mechanical strain regulated actin cytoskeletal remodel, focal adhesion formation, regulation of nuclear Yap/Taz levels and led to a decrease in markers for endothelial cells. Further investigations could unlock the optimal mechanical conditions that could be used to

drive hMSCs towards the desired differentiation pathway for therapeutic use.

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W1025

UNDERSTANDING THE ROLE OF TET ENZYMES IN HUMAN AND MOUSE OSTEOBLAST DIFFERENTIATION

Grandi, Fiorella Carla, Smeriglio, Piera and Bhutani, Nidhi, Stanford University, Stanford, CA, U.S.

Many important medical challenges, such as osteoporosis and osteoarthritis, occur in the musculoskeletal system. However, little is known about the epigenetic regulation of mesenchymal stem cells (MSCs) differentiation to bone and cartilage. These diseases will benefit from the potential of regenerative stem cell derived therapies; therefore, understanding the epigenetics pathways that govern their differentiation is crucial. DNA methylation is an important epigenetic mark, associated with gene silencing. The recently discovered oxidized derivative, 5hmC, is associated with gene activation and has been found to be a stable lineage mark in many tissues. 5hmC is deposited by the TET(1/2/3) family of enzymes. This work aims to understand how the TET enzymes, and DNA demethylation dynamics, affect the development and pathology of bone. Underscoring the crucial role of this mark in the differentiation of mesenchymal lineages, mouse ESCs with genetic deletion of all three TET enzymes fail to form either cartilage or bone. We find that TET enzymes are expressed in both mouse and human osteoblasts and that 5hmC levels remain stable throughout osteoblast differentiation. Using an *in vitro* model of osteoblasts differentiation, the MC3T3-E1 cell line, we show that TET2 knockdown impaired osteogenesis, including the expression of key lineage genes, and decreased the amount of mineralized matrix, suggesting a role for TET2 in controlling genes related to matrix mineralization. Supporting this hypothesis, we show that in pediatric osteosarcoma samples, a bone cancer hallmarked by the presence of undifferentiated osteoblast that fail to mineralize calcium, both levels of TET enzymes as well as 5hmC deposition, is low. Furthermore, gene expression studies of MSCs from women with osteoporosis, another disease where matrix mineralization is deficient, find a reduction of TET2 levels. Collectively, these findings underscore the critical role of TET2 in the proper differentiation of osteoblasts and overall bone health and regeneration.

W1027

TRANSCRIPTOME ANALYSIS OF HUMAN MESENCHYMAL STEM CELLS IN RESPONSE TO TLR4 LIGAND

Kim, Sun Hwa[1], Das, Amitabh[2], Chai, Jin Choul[2], Binas, Bert[2], Choi, Mi Ran[2], Park, Kyoung Sun[2], Lee, Young Seek[2], Jung, Kyoung Hwa[2] and Chai, Young Gyu[2], [1]Hanyang University, Ansan, Korea, South, [2]Hanyang University, Ansan, Korea

Due to their multipotentiality and immunomodulation, human mesenchymal stem cells (hMSCs) are widely studied for the treatment of degenerative and inflammatory diseases. Transplantation of hMSCs to damaged tissue is a promising approach for tissue regeneration. However, the physiological mechanisms and regulatory processes of MSC trafficking to injured tissue are largely unexplored. Here, we evaluated the gene expression profile and migratory potential of hMSCs upon stimulation with the TLR4 ligand lipopolysaccharide (LPS). Using RNA sequencing, we identified unique induction patterns of interferon stimulated genes, cytokines and chemokines involved in chemotaxis and migration. The -950 to +50 bp regions of many of these LPS-responsive genes were enriched with putative binding motifs for the transcription factors (TFs) interferon regulatory factor (IRF1) and nuclear factor kappa B (NF-κBIA), which were also induced by LPS along with other NF-κB TFs. In addition, IRF1 attenuation significantly down-regulated interferon stimulated genes as well as key cytokines. Furthermore, using pharmacological inhibitors, we showed that the NF-κB and phosphatidylinositol 3-kinase (PI3K) pathways regulate the migratory and cytokine/chemokine response to LPS. These unprecedented data suggest that IRF1 and NF-kBIA orchestrate the TLR4-primed immunomodulatory response of hMSCs.

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W1029

ISOLATION AND CHARACTERIZATION OF HUMAN DENTAL PULP STEM CELLS UNDER XENO-FREE CULTURE CONDITIONS FOR CELL-BASED THERAPEUTIC APPLICATIONS

Mochizuki, Mai and Nakahara, Taka, The Nippon Dental University School of Life Dentistry, Tokyo, Japan

We previously isolated and characterized several types of dental stem cells derived from extracted wisdom teeth using a fetal bovine serum (FBS)-containing culture medium. This study indicated that these stem cells exhibit

a multidifferentiation potential and higher proliferative activity than iliac bone-derived bone marrow stem cells. However, the use of FBS allows for potential pathogenic contamination and immunoreaction. In order to develop safer cell-based therapies, the present study aimed to isolate and characterize dental pulp stem cells (DPSCs) using a xenogenic serum-free culture medium. Dental pulp tissues were collected from the exfoliated deciduous teeth and wisdom teeth of healthy volunteers between the ages of 10 to 37 years, and were digested with a solution of collagenase-dispase for 1 h at 37 °C. The resulting cells were cultured using two types of culture media, PRIME-XV MSC Expansion XSFM (XFM) or DMEM/F12 with 15% FBS (SCM), and were subcultured at 5 × 10^4 cells in 60-mm dishes. The culture dishes for XFM cultivation were pre-coated with PRIME-XV Human Fibronectin. A proliferative assay, estimation of population doubling time, RT-PCR, flow cytometry, and multidifferentiation induction experiments (osteogenic and adipogenic lineages) were performed using cells at passage 3 or 4. Compared to SCM cells, XFM cells exhibited significantly greater growth and a shorter population doubling time. Both types of cells expressed CD44, CD90, CD105, and STRO-1 but not CD14 and CD34, as determined by flow cytometry analysis. RT-PCR analysis revealed the gene expression of osteogenic (Vimentin, Runx2, and Type I collagen), neurogenic (Nestin), and stem cell markers (Nanog, Oct3/4, and Sox2). XFM cells possessed a multidifferentiation potential, similar to SCM cells. XFM cells exhibited a higher proliferative activity compared to SCM cells, indicating that XFM cultivation can likely result in a sufficient number of cells in a short time. Moreover, XFM cells like SMC cells exhibit a multidifferentiation potential and the stem cell phenotype; these findings indicate the successful characterization of DPSCs. In conclusion, the XFM culture medium allows for 1) cell isolation from the harvested tissue of patients, 2) ex vivo expansion, and 3) the maintenance of stemness as DPSCs.

W1031

HUMAN iPSCs DIFFERENTIATE INTO FUNCTIONAL MSCs AND REPAIR BONE DEFECT

Sheyn, Dmitriy[1], Ben-David, Shiran[1], Shapiro, Galina[2], De Mel, Sandra[1], Bez, Maxim[2], Ornelas, Loren A.[1], Sahabian, Anais[1], Sareen, Dhruv[1], Da, Xiaoyu[1], Pelled, Gadi[2,3], Tawackoli, Wafa[1], Liu, Zhenqiu[1], Gazit, Dan[1,2] and Gazit, Zulma[1,2], [1]Cedars-Sinai Medical Center, Los Angeles, CA, U.S., [2]The Hebrew University-Hadassah Faculty of Dental Medicine, Jerusalem, Israel, [3]Cedars Sinai Medical Center, Los Angeles, CA, U.S.

Mesenchymal stem cells (MSCs) are currently the most established cells for skeletal tissue engineering and regeneration; however, their availability and capability of self-renewal are limited. Recent discoveries of somatic cell reprogramming may be used to overcome these challenges. We hypothesized that induced pluripotent stem cells (iPSCs) that were differentiated into MSCs can be used for bone regeneration. Short-term exposure of embryoid bodies to TGFβ was used to direct iPSCs towards MSC differentiation. During this process 2 types of iPSC-derived MSCs (iMSCs) were identified: early (aiMSCs) and late (tiMSCs) outgrowing cells. The transition of iPSCs toward MSCs was documented using MSC markers flow cytometry. Both types of iMSCs differentiated in vitro in response to osteogenic or adipogenic supplements. The results of quantitative assays showed that both cell types retain their multi-differentiational potential, although aiMSCs demonstrated a higher osteogenic potential than tiMSCs and bone marrow–derived MSCs (BM-MSCs). The soft agar assay was performed to test tumorigenicity and showed that BM-MSCs displayed significantly higher colony formation rate in soft agar than both iMSC types despite having a lower doubling rate in vitro. Transcriptomic analysis found significant differences in MSC-related genes, cell division and focal adhesion between the two iMSC types. Ectopic injections of bone morphogenetic protein-6 (BMP6) overexpressing tiMSCs produced no or little bone formation, whereas similar injections of BMP6-overexpressing aiMSCs resulted in substantial bone formation. However, orthotopic injections into radial defects, all three cell types regenerated bone and contributed to defect repair. In conclusion, MSCs derived from iPSCs exhibit self-renewal without tumorigenicity. Compared to BM-MSCs, aiMSCs acquire more of a stem cell phenotype that might be useful in applications that like avascular necrosis, where angiogenesis plays pivotal role in tissue regeneration. Whereas tiMSCs acquire more of a differentiated osteoblast phenotype, which aids bone regeneration, but does not allow the cells to induce ectopic bone formation, even when triggered by BMPs, unless in an orthotopic site of bone fracture. These properties are advantages for bone regeneration applications.

MESENCHYMAL CELL LINEAGE ANALYSIS

W1035

PERIVASCULAR CELL-SECRETED PERSEPHIN INFLUENCES THE GROWTH OF HUMAN ADENOCARCINOMA EPITHELIAL CELLS

Kim, Eunbi[1], Park, Jeong-ah[1], An, Borim[1], Heo, Hye-Ryeon[1], Yang, Se-Ran[2], Na, Sunghun[3] and Hong, Seok-Ho[1], [1]Kangwon National University, Chuncheon, Korea, South, [2]Kangwon National University, Chuncheon, Korea, [3]Kangwon National University, chuncheon, Korea

Understanding of the crosstalk between perivascular cells (PVCs) and cancer might be beneficial for the prevention

of cancer development and metastasis. In this study, we investigated the paracrine actions of PVCs in the proliferation of cancer cell lines using transwell co-culture system. We non-enzymatically isolated PVCs from human umbilical cords and co-cultured with human cancer cell lines including lung adenocarcinoma epithelial cells (A549) and erythroleukemia cells (TF-1a and K562) for 48 hrs. We found that PVCs promote proliferative activity of A549 cell lines without morphological changes. However, PVCs had no effect on the proliferation of TF-1a and K562 cell lines. This finding suggests that augmented proliferation of A549 cells is mediated by the paracrine factors of PVCs. To identify the factors secreted from PVCs, we analyzed the conditioned medium (CM) harvested from PVC cultures using antibody array. We identified a set of cytokines including Persephin (PSPN), which is known as a neurotrophic factor and key regulator of oral squamous cell carcinoma progression. We further examined the proliferative effect of PSPN (10, 20, and 40 ng/ml) on A549 cell lines in vitro. Supplementation of 40 ng/ml of PSPN significantly increased the proliferation of A549 cells. These results indicate that PVCs and their secreting factors might be therapeutic targets to prevent cancer development and metastasis.

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W1037

IDENTIFICATION OF A RICH POPULATION OF CD34 POSITIVE MESENCHYMAL STROMAL/ STEM CELLS IN HUMAN FETAL PAROTID, SUBLINGUAL AND SUBMANDIBULAR GLANDS

Togarrati, Padma Priya, Blood Systems Research Institute, San Francisco, CA, U.S.

Mesenchymal stromal/stem cells (MSCs) play crucial roles in maintaining tissue homeostasis during physiological turnovers and injuries. Very little is known about the in situ phenotype, distribution and molecular gene expression profile of MSCs in freshly isolated human fetal salivary glands (SGs) as most reports have focused on phenotypic and functional analyses of culture grown adherent MSCs. In this study we found that the cell adhesion molecule CD34 was widely expressed by the MSCs of human fetal parotid (PAG), sublingual (SLG) and submandibular (SMG) glands. $CD34^+$ SG cells, especially $CD34^+$ cells isolated from more branched glands such as SLG and SMG showed significantly high expressions of *FGF7, FGF10, BMP2* and *BMP7* as compared to $CD34^-$ cells. These growth factors have earlier been shown to be essential for branching morphogenesis and SG development. Additionally, higher expressions of *BMP6, EGF, EDAR* and *TGFβ1* were found in $CD34^+$ SMG cells as compared to $CD34^-$ cells, however differences were not statistically significant. Furthermore, F*GF10* and *BMP7* were also expressed at significantly high levels in $CD34^+$ PAG cells. Only $CD34^+$ SG cells retained fibroblast colony forming potential and it was completely absent in $CD34^-$ cells. Intraglandular transplantation of culture expanded $CD34^+$ cell derived SG-MSCs in immunodeficient mice showed that these cells were able to engraft in the stromal region surrounding the acini and ducts of injected as well as uninjected contralateral and ipsilateral murine glands. In summary, we show that CD34 marks a rich pool of MSCs in human fetal major SGs, which express significantly high levels important growth factors that are known to play key roles in gland development and regeneration. Moreover, $CD34^+$ SG cells had the ability to expand under culture conditions and upon xenotransplantation could engraft in the glandular stromal milieu. Hence, $CD34^+$ SG-MSCs could be a promising cell source for adoptive cell based SG-therapies, and in bioengineering artificial SGs.

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HEMATOPOIETIC CELLS

W1039

PARACRINE EFFECT OF HUMAN PERIVASCULAR CELLS ON THE MAINTENANCE AND HEMATOPOIETIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

An, Borim[1], Park, Jeong-ah[1], Kim, Eunbi[1], Heo, Hye-Ryeon[1], Yang, Se-Ran[2] and Hong, Seok-Ho[1], [1]Kangwon National University, Chuncheon, Korea, South, [2]Kangwon National University, ChunCheon, Korea

In recent years, perivascular stem cells (PVCs) have attracted increasing attention due to their greater proliferative and regenerative capacity compared to mesenchymal stem cells. PVCs also create a specialized microenvironment that regulates the self-renewal and differentiation of hematopoietic stem cells in the bone marrow. However, the interaction between PVCs and other stem cells has poorly investigated. In this study, we isolated PVCs from human umbilical cords and examined their paracrine effect on the maintenance and hematopoietic differentiation of human pluripotent stem cells (hPSCs). Human PSCs maintained on mitomycin-inactivated PVCs displayed typical features of hPSCs including undifferentiated proliferative ability with the expression of pluripotency markers, cell morphology and differentiation potential into three embryonic germ layers. We also found that commitment of hemogenic precursors into hematopoietic lineage was robustly promoted in hPSCs induced in the conditioned medium (CM) collected from PVC cultures.

These results suggest that the long-term maintenance and improved hematopoietic differentiation of hPSCs is mediated by PVC-secreted factors. In order to define the factors secreted from PVCs, we analyzed the PVC-CM using antibody array. Some extracellular matrix proteins were detected and might be involved in regulating function of hPSCs. These findings suggest that PVCs can be considered as critical niche component for the regulation of stem cell functions as well as an alternative feeder source to avoid xeno-contamination from animal feeders for the maintenance of hPSCs.

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W1041

A DEVELOPMENTALLY RESTRICTED HEMATOPOIETIC STEM CELL THAT GIVES RISE TO INNATE-LIKE B- AND T-LYMPHOCYTES BUT DOES NOT PERSIST IN SITU

Beaudin, Anna E.[1], Boyer, Scott W.[1], Perez-Cunningham, Jessica[1], Hernandez, Gloria E.[1], Derderian, S. Chris[2], Mackenzie, Tippi C.[2] and Forsberg, E. Camilla[1], [1]University of California Santa Cruz, Santa Cruz, CA, U.S., [2]University of California San Francisco, San Francisco, CA, U.S.

The generation of innate-like immune cells mediating self-recognition and tolerance distinguishes fetal from adult hematopoiesis, but the mechanisms underlying differential cell production during prenatal hematopoietic development remain unknown. We utilized a Flk2/Flt3 lineage tracing mouse model wherein Flk2-driven expression of Cre recombinase results in the switching of a ubiquitous dual-color reporter from Tomato (Tom) to GFP expression. As the switch from Tom to GFP expression in this model involves an irreversible genetic excision of the Tom gene, a GFP+ cell can never give rise to Tom+ progeny. Importantly, we have shown that all adult hematopoietic stem cells (HSCs) in this model express Tom and that only Tom+ cells within the adult bone marrow (BM) possess long-term reconstituting potential. In contrast, both Tom+ and GFP+ fetal liver (FL) and neonatal BM cells support serial reconstitution upon transplantation into irradiated adult recipients. We have therefore identified a novel, developmentally restricted GFP+ HSC that gives rise to long-term multilineage reconstitution upon transplantation into irradiated adult recipients yet does not normally persist into adulthood. These developmentally restricted GFP+ HSCs display greater lymphoid potential, lymphoid lineage-priming, and greater capacity to generate innate-like B and T lymphocytes as compared to coexisting Tom+ FL and adult HSCs. In contrast to their demonstrated capacity to serially reconstitute irradiated adult recipients, GFP+ FL HSCs engrafted poorly when transplanted into an unconditioned, fetal environment, despite maintaining enhanced innate-like immune potential. Consistent with limited persistence into adulthood, GFP+ FL HSCs express lower levels of CD150, are more proliferative, and down-regulate self-renewal genes as compared to Tom+ FL HSCs and adult HSCs. Our results provide direct evidence for a phenotypically and functionally unique developmentally restricted HSC that gives rise to a layered immune system. As early lymphoid cells play essential roles in establishing self-recognition and tolerance, these findings are critical for understanding the development of autoimmune diseases, allergies, and tolerance induction upon organ transplantation.

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W1043

THE HISTONE METHYLTRANSFERASE ACTIVITY OF DOT1L IS CRITICAL FOR THE MAINTENANCE OF NORMAL HEMATOPOIETIC STEM AND PROGENITOR CELLS

Chase, Jennifer, Grigsby, Sierrah, Balbin Cuesta, Ginette, Waas, Bridget, Friedman, Ann, Muntean, Andrew, Nikolovska-Coleska, Zaneta and Maillard, Ivan, University of Michigan, Ann Arbor, MI, U.S.

Disruptor of telomeric silencing 1-like (DOT1L) encodes the only known mammalian H3K79 methyltransferase. DOT1L interacts with fusion partners of the MLL1 oncogene and its enzymatic activity is required for leukemic transformation by MLL1 fusion proteins. Thus, DOT1L is an attractive new therapeutic target in MLL1-driven leukemias. However, Dot1l is also essential for hematopoietic homeostasis. To date, the mechanisms of DOT1L action and the requirement for DOT1L's enzymatic activity in normal hematopoiesis have not been elucidated. To address these questions, we used genetic and pharmacological methods to inactivate Dot1l or disrupt its enzymatic activity in vitro and in vivo. In liquid culture, the DOT1L methyltransferase inhibitor EPZ5676 induced a dose-dependent decrease in hematopoietic progenitor expansion correlating with loss of H3K79 methylation. EPZ5676 markedly decreased accumulation of differentiated cells. In vivo, we induced Dot1l deletion with poly(I:C) in Mx1-Cre^{+}Dot1l$^{f/f}$ mice. We observed loss of hematopoietic progenitor populations starting seven days after poly(I:C) and first visible among subsets of Lin^{-}c-Kit^{+} progenitors, with profound depletion by day 10. Lin^{-}Sca-1^{+}c-Kit^{+} (LSK) progenitors and phenotypically defined long-term hematopoietic stem cells (LT-HSC: CD150^{+}CD48^{-}LSK) became depleted only after decreased numbers of downstream progenitors were detected. These findings suggest that LT-HSCs and downstream progenitors are affected by Dot1l loss, but that the latter are exquisitely sensitive. To assess if DOT1L's meth-

yltransferase activity is required in normal hematopoiesis, we transduced Mx1-Cre+Dot1l$^{f/f}$ progenitors with retroviruses expressing GFP vs. wild-type DOT1L or the methytransferase-defective mutant DOT1L-RCR. After reconstitution of lethally irradiated hosts, recipients were treated with poly(I:C) to inactivate endogenous Dot1l. Unlike wild-type DOT1L, DOT1L-RCR failed to rescue hematopoiesis, leading to lethal hematopoietic failure or reconstitution by residual host cells. Thus, DOT1L's methyltransferase activity is essential for normal hematopoietic stem and progenitor cell function. Ongoing studies focus on molecular mechanisms of DOT1L action in normal hematopoiesis, as compared to its effects in leukemic transformation.

W1045

THE IMPACT OF AGING OF HEMATOPOIETIC STEM AND PROGENITOR CELLS (HSPCs) IN NON-HUMAN PRIMATES AS INTERROGATED BY GENETIC BARCODE CLONAL TRACKING

Yu, Kyung-Rok[1], Wu, Chuanfeng[1], Espinoza, Diego[1], Yabe, Idalia[1], Panch, Sandhya[1], Hong, So Gun[2], Koelle, Samson[1], Lu, Rong[3], Bonifacino, Aylin[1], Krouse, Alan[1], Metzger, Mark[1], Donahue, Robert[1] and Dunbar, Cynthia[1], [1]National Institutes of Health, Bethesda, MD, U.S., [2]National Heart, Lung, Blood Institute/National Institutes of Health, Bethesda, MD, U.S., [3]University of Southern California, Los Angeles, CA, U.S.

Aging of the hematopoietic system is associated with a number of observations, including diminished regenerative potential, skewed lineage differentiation, increased incidence of anemia, and higher rates of neoplastic transformation. Despite advanced age being a strong poor prognostic factor, an increasing number of older patients are receiving hematopoietic stem and progenitor cell (HSPC) transplantation. To quantitatively elucidate the age-related changes that compromise hematopoietic function at a clonal level, we applied a genetic barcoding approach to quantitatively track the clonal behavior of HSPCs in young versus old rhesus macaques following autologous transplantation. We successfully transplanted barcoded HSPCs into 2 macaques aged 18 and 25 years, constituting "old" macaques based on an average lifespan in captivity of 20-30 years, and compared results to clonal patterns observed in 2 "young" macaques aged 3-5 years. There were marked differences in the patterns of clonal lineage relationships between young and old animals, as assessed via pairwise Pearson correlations of all contributing clones as well as clustering algorithms allowing interrogation of patterns of clonal behavior. We discovered a pattern of clonal reconstitution distinct from that in young animals, with persistent unilineage or highly-biased lineage progenitors in the aged animals. In old animals, clones contributing to Gr/Mo versus B or T lineages remained almost completely distinct or markedly biased up to 4m, and B and Gr/Mo became correlated from 5m, B/T/Mo/Gr multilineage clones appeared only by 6.5m, whereas B/T/Mo/Gr multilineage clones appeared by 3-4.5m in young animals. Furthermore, old animals showed the delayed reappearance of CD4+ and CD8+ naïve T cells in the blood, whereas CD4+ and CD8+ effector memory T cells showed rapid and abundant recovery compared to young animals. Longer follow-up will be required to determine if this biased pattern persists, and detailed analysis of clonal distribution of each naïve/memory T cell subsets will be presented. This approach should improve our understanding of the effects of aging on the basic properties of aging HSCs and provide insights important for improving therapies in elderly patients being treated for hematologic diseases.

W1047

DISSECT THE ENDOTHELIAL TO HEMATOPOIETIC TRANSITION THROUGH TARGETING AN EGFP REPORTER GENE TO GATA2 LOCUS IN HUMAN EMBRYONIC STEM CELLS

Huang, Ke[1], Gao, Jiao[2], Du, Juan[1], Ma, Ning[1], Zhu, Yanling[1], Wu, Pengfei[1], Wang, Wenqian[3], Li, Yuhang[1], Chen, Qianyu[1], Hutchins, Andrew[1], Yang, Zhongzhou[1], Zheng, Yi[1], Zhang, Jian[1], Shan, Yongli[1], Liao, Baojian[1], Liu, Jiajun[3], Wang, Jinyong[1], Liu, Bing[2] and Pan, Guangjin[1], [1]Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China, [2]307-Ivy Translational Medicine Center, Laboratory of Oncology, Affiliated Hospital of Academy of Military Medical Sciences, Beijing, China, [3]The Third Affiliated Hospital;Sun Yat-sen University, Guangzhou, China

Hematopoietic stem/progenitor cells (HS/PCs) are originated from hemogenic endothelial cells (HECs) through endothelial to hematopoietic transition (EHT). Understanding the regulatory network driving EHT and defining surface markers for HECs are critical for generating transplantable HS/PCs in vitro. Here, we generated a GATA2w/eGFP reporter in human embryonic stem cells (hESCs) to trace cells expressing GATA2, a critical gene for EHT. We show that hESC derived HECs are restricted in GATA2/eGFP+ population. Then, through transcriptome analysis by RNA-Seq, we generated a regulatory network for HEC determination by transcription factors enriched in GATA2/eGFP+ population. Significantly, we also identified surface markers which could define *bona fide* HECs in both hematopoietic differentiation of human pluripotent stem cells (hPSCs) and mouse embryo. Our data provide a valuable platform in understanding the mechanism of EHT.

W1049

HIGH RESOLUTION IMAGING AND COMPUTATIONAL ANALYSIS OF HEMATOPOIETIC CELL DYNAMICS IN VIVO

Koechlein, Claire Steeves[1], Harris, Jeffrey[2], Lee, Timothy[3], Weeks, Joi[4], Fox, Raymond[4], Zimdahl, Bryan[4], Blevins, Allen[4], Jung, Seung-Hye[2], Chute, John[2], Chourasia, Amit[1], Covert, Markus[3] and Reya, Tannishtha[4], [1]University of California, San Diego, La Jolla, CA, U.S., [2]Duke University Medical Center, Durham, NC, U.S., [3]Stanford University, Stanford, CA, U.S., [4]University of California San Diego School of Medicine, La Jolla, CA, U.S.

During the past few decades, much has been learned regarding the phenotype and function of hematopoietic stem and progenitor cells. However, a major challenge in the field has been mapping the dynamic behavior of these cells within their native microenvironment. This is primarily due to limitations in the ability to gather quantifiable dynamic information about hematopoietic stem and progenitor cells in living animals. Here we describe a strategy that uses backlighting of the microenvironment to image cells in vivo with a high degree of spatial and temporal resolution. Importantly, the resolution and time frame of the imaging allowed the development of a computational approach to track in a quantitative high throughput way the migration and interaction of hematopoietic cells within the niche. With this strategy, we found that hematopoietic cells cluster in three distinct zones around the vascular or endosteal domains, and are distributed across contact, proximal and distal positions. Further, immature hematopoietic cells display a high spatial affinity for being in contact with the vascular niche relative to the endosteal niche; this preference decreases as cells differentiate. At a temporal level, we found that in contrast to more differentiated progenitor cells, populations enriched for hematopoietic stem cells make more long term associations with the vascular niche and more short/transient associations with the endosteal niche, suggesting that differentiation status is a key determinant of the spatial and temporal behavior of hematopoietic cells, and that programs that confer the undifferentiated state dictate preferential exposure to signals from the vasculature. Importantly, we used a novel knock-in reporter of the fate determinant Msi2, that allowed us to track endogenous immature hematopoietic cells, and show that these cells also dominantly interact with vascular domains. These data collectively demonstrate that high-resolution imaging coupled with a high-throughput computational-based approach can provide new insight into the dynamic behavior of cells in context of the living microenvironment.

W1051

Grb2 REGULATES PROLIFERATION AND SURVIVAL SIGNALING OF LONG-TERM HEMATOPOIETIC STEM CELLS

Ofran, Yishai[1,2], Frelin, Catherine[3], Hayun, Michal[1,4], Derdikman, Yael[2,4], Iscove, Norman N.[5] and **Louria-Hayon, Igal**[1,6], [1]Rambam Health Care Campus, Haifa, Israel, [2]Bruce Rappaport Faculty of Medicine, Technion, Haifa, Israel, Haifa, Israel, [3]University Health Network, Ontario Cancer Institute, Toronto, ON, Canada, [4]The Hematology Research Center, Haifa, Israel, [5]Ontario Cancer Inst, University Health Network, Toronto, ON, Canada, [6]Rambam Health Care Campus, Haifa, Israel, Israel

Although HSC proliferation, survival and expansion have been shown to be supported by the cooperative action of different cytokines, little is known about the intracellular signaling pathways that are activated by cytokines upon binding to their receptors. Growth factor receptor-bound protein 2 (Grb2) is a ubiquitous adaptor protein known to be involved in signaling induced by a variety of growth factors and cytokines; however, its physiological role in HSC has never been characterized. Our study has shown that Grb2 mRNAs are highly expressed in HSC relative to more differentiated cells of the myeloid and erythroid lineages. Conditional deletion of Grb2 induced a progressive decline of long-term (LT-) HSC numbers and cytopenia with animal aging. While Grb2 deletion did not modify LT-HSC quiescence, it impaired HSC regenerative and self-renewing abilities in a cell-autonomous fashion. We revealed that Grb2 deletion impaired LT-HSC proliferative response to 5-FU treatment in vivo and to cytokine stimulation in vitro and that cytokine-induced ERK phosphorylation was dependent on both Sh2 and Sh3 domains of Grb2. Our findings position Grb2 as a key adaptor protein governs cytokines signaling in cycling LT-HSC and a master regulator of LT-HSC transplantation, proliferation and survival.

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W1053

EphrinB1 IS IMPORTANT FOR BONE MARROW MESENCHYMAL STEM/ STROMAL CELL SUPPORT OF HEMATOPOIESIS

Nguyen, Thao Minh[1], Arthur, Agnes[1], Purton, Louise[2], Paton, Sharon[1] and Gronthos, Stan[3], [1]University of Adelaide, Adelaide, Australia, [2]St. Vincent's Institute, Fitzroy, Australia, [3]University of Adelaide, Adelaide SA, Australia

Bone marrow stem/ stromal cells (BMSC) maintain bone homeostasis and are fundamental regulators of bone marrow hematopoiesis. The contact-dependent erythropoietin-producing hepatocellular (Eph) tyrosine kinase ligand, ephrinB1, contributes to skeletal development. Mutations of the ephrinB1 gene cause Craniofrontonasal Syndrome in human. Deficiency of ephrin-B1 in osteoblasts results in skull defect, reduced body size, bone mineral density and trabecular bone volume in mouse. We have previously demonstrated that ephrinB1, expressed by human BMSC, plays an important role in human BMSC attachment, migration and osteochrondral differentiation. However, the role of ephrinB1 in BMSC support of hematopoiesis remains unknown. Here we showed that EphB2, the highest affinity binding partner of ephrinB1, is exclusively expressed by human primitive hematopoietic stem cells ($CD34^+CD38^-$ HSC), but not by lineage committed hematopoietic cell populations such as $CD3^+$ T-cells, $CD19^+$ B-cells, $CD14^+$ monocytes, $CD15^+$ granulocytes or Gly-A^+ erythroid cells. Functional studies showed that ephrinB1-Fc molecules significantly promoted the frequency of human $CD34^+$ HSC derived colony forming cells (CFC) compared with human IgG-treated controls. Parallel studies indicated that blocking ephrinB1/EphB2 interactions significantly inhibited BMSC capacity to support HSC derived colony formation in long-term culture initiating cell assays, using EphB2 inhibitor binding peptides. Moreover, ephrinB1 conditional knockout mice (ephrinB1KO), under the transcriptional regulation of the Osterix (Osx) promoter, a marker of osteoblast precursor cells, exhibited lower frequencies of HSC and progenitor cell populations correlating with a reduced output of CFCs compared with the Osx-Cre control mice. Interestingly, we found that the frequencies of B-cells ($B220^+$) and myeloid cells ($CD11b^+$) were increased in the bone marrow, blood and spleen of ephrinB1KO mice compared with Osx-Cre control mice. However, the frequency of T-cells ($CD3^+$) were lower in the blood, spleen and thymus of ephrinB1KO mice compared with Osx-Cre controls. Taken together, these observations indicate that ephrinB1 expressed by BMSC plays an important role in mediating BMSC support of hematopoiesis.

W1055

COMPARISON OF INTEGRASE-DEFECTIVE LENTIVIRAL VECTOR DONOR TEMPLATES FOR GENE CORRECTION OF SICKLE CELL DISEASE

Parma, Marie[1], Romero, Zulema[2], Kuo, Caroline Y.[2], Lumaquin, Dianne[2], Koziol, Colin[2], Hollis, Roger P.[2] and Kohn, Donald B.[2], [1]California State University Northridge, Granada Hills, CA, U.S., [2]University of California, Los Angeles, Los Angeles, CA, U.S.

A single substitution mutation (A→T) at the sixth codon of the β-globin gene locus gives rise to the hemoglobin sickle (HbS) phenotype and causes the monogenic disorder sickle cell disease (SCD). HbS tetramers polymerize under low oxygen conditions and form fiber aggregates that disrupt the architecture of red blood cells, leading to the occlusion of capillaries and tissue ischemia. The only cure available for SCD is allogeneic hematopoietic stem cell (HSC) transplant from bone marrow; however, the procedure is still limited by donor availability and immune complications. Gene therapy offers an alternative to potentially treat SCD and restore normal β-globin function by ex vivo manipulation of a patient's own HSCs. This manipulation can be achieved by the co-delivery of programmable endonucleases that produce site-specific double-strand DNA breaks and a homologous DNA donor template with the normal base pair change. Current data produced in our laboratory shows successful cleavage of the target β-globin gene locus in cord blood (CB) CD34+ cells by in vitro transcribed mRNA encoding target-specific zinc finger nucleases, as well as high rates of gene modification in the presence of either an integrase defective lentiviral vector (IDLV) encoding a β-globin donor template of 1.1kb in length or a 101 bp oligonucleotide (oligo) donor. It was observed that the oligo donor provided higher and more consistent rates of gene modification, but also resulted in higher toxicity to the cells than the IDLV donor. We evaluate if a shorter IDLV donor (141 bp) will provide gene modification rates comparable to the oligo donor while maintaining the lower toxicity observed in the IDLV-treated samples. We conduct the initial IDLV donor comparison in K562 erythro-leukemia cells and a more exhaustive comparison will be performed in CB CD34+ cells.

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W1057

TRACING THE EMERGENCE OF HEMATOPOIETIC STEM CELLS FROM THE EXTRA-EMBRYONIC MESODERM.

Shukla, Ankita, University of California, Irvine, Irvine, CA, U.S.

Hematopoietic stem cells (HSCs) have great therapeutic potential to treat nearly all blood diseases as they can give rise to all cells of the immune system. HSC transplants, or bone marrow transplants, often result in graft rejection, graft failure, or in the worst cases, graft versus host disease, and are only performed in the most severe cases. Generation of HSCs from patient-derived induced pluripotent stem cells (iPSCs) will circumvent these issues. However, this remains challenging because much is unknown about HSC emergence during development, including which tissues produce the first HSCs in the embryo. We have previously identified putative embryonic precursors to adult HSCs (or pre-HSCs), defined as CD43+ ckit+ Sca1+ CD144+ CD11a-, which are able to give rise to all hematopoietic lineages in vitro. Additionally, these multipotent pre-HSCs are predominantly found in the yolk sac and the placenta, regions which comprise the extra-embryonic mesoderm (EEM). To trace the emergence of pre-HSCs *in vivo,* we used a transgenic HoxB6 promoter to drive tamoxifen-dependent Cre recombinase (HoxB6-CreER) to induce GFP expression in EEM. HoxB6 is homeobox gene that is transiently expressed in all mesoderm. HoxB6 expression in the EEM precedes that in the embryo proper, allowing EEM-specific labeling of pre-HSCs. Tamoxifen and 4-Hydroxytamoxifen (4OHT) have short half-lives in vivo (~24 hours), and thus define a precise window for Cre induction after injection. The first hematopoietic cells arise in the yolk sac around embryonic day 7.5 (e7.5), and injection of 4OHT at e6.5 allows reporter labeling exclusively in the EEM, with minimal labeling of mesoderm in the embryo proper. This system allows us to trace the origin of pre-HSCs in HoxB6-CreER embryos as well as the cells they give rise to in adults. Flow cytometry analysis of adult blood revealed that at least 30% of all adult blood lineages are labeled with GFP. Approximately 25% of adult HSCs were also GFP+. Therefore, our results indicate that extra-embryonic tissues like the yolk sac and placenta, which are not present after birth, substantially contribute to adult hematopoiesis. Resolving the early molecular events that occur in the EEM may allow us to recapitulate them in vitro to advance the efforts of generating HSCs from patient-derived iPSCs.

W1061

INTEGRATED BIOPHYSICAL AND BIOCHEMICAL SIGNALS AUGMENT MEGAKARYOPOIESIS AND THROMBOPOIESIS IN A THREE-DIMENSIONAL ROTARY CULTURE SYSTEM

Zhou, Jiaxi, Insitite of Hematology, Chinese Academy of Medical Sciences, Tianjin, China

Platelet transfusion has been widely used in patients undergoing chemotherapy or radiotherapy; however, the shortage of the platelet supply limits the care of patients. Although derivation of clinical-scale platelets in vitro could provide a new source for transfusion, the devices and procedures for deriving scalable platelets for clinical applications have not been established. In the present study, we found that a rotary cell culture system (RCCS) can potentiate megakaryopoiesis and significantly improve the efficiency of platelet generation. When used with chemical compounds and growth factors identified via small-scale screening, the RCCS improved platelet generation efficiency by as much as~3.7-fold compared with static conditions. Shear force, simulated microgravity, and better diffusion of nutrients and oxygen from the RCCS, altogether, might account for the improved efficient platelet generation. The cost-effective and highly controllable strategy and methodology represent an important step toward large-scale platelet production for future biomedical and clinical applications.

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CARDIAC CELLS

W1065

HUMAN DISEASE-CAUSING GATA4 MUTATION REDISTRIBUTES TRANSCRIPTION FACTOR LOCALIZATION IN IPS-DERIVED CARDIOMYOCYTES

Ang, Yen-Sin[1], Rivas, Renee[1], Ribeiro, Alexandre[2], Rivera, Janell[1], Srivas, Rohith[2], Pratt, Karishma[1], Fu, Ji-Dong[1], Mohamed, Tamer M A[1], Rand, Timothy[1], Yamanaka, Shinya[1], Hood, Leroy[3], Pruitt, Beth[2], Snyder, Michael[2] and Srivastava, Deepak[1], [1]Gladstone Institute, San Francisco, CA, U.S., [2]Stanford University, Stanford, CA, U.S., [3]Institute for Systems Biology, Seattle, WA, U.S.

Proper lineage commitment and tissue function require accurate transcription factor dosage and localization of transcriptional complexes, and dysregulation of this process can cause human disease. Heterozygous mutations in

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GATA4, a master cardiogenic transcription factor, cause congenital heart defects and cardiomyopathy through unknown mechanisms. Here, we used systems-level approaches on iPS–derived cardiomyocytes from patients with a GATA4 G296S mutation—which affects interaction with T-box protein, TBX5—to understand GATA4 gene regulatory mechanism in homeostasis vs. disease. GATA4 mutant cardiac progenitors and myocytes had attenuated cardiac gene program and aberrant up-regulated endothelial/endocardial gene program, as well as impaired contractility, calcium handling and metabolic activity. ChIP-seq showed GATA4 and TBX5 co-occupancy at thousands of loci, and enrichment at H3K27ac-marked enhancers and 213 Mediator-bound cardiac super-enhancers. GATA4 mutation resulted in diminished recruitment of TBX5 to cardiac super-enhancers, while the two proteins were mislocalized in endothelial topologically associating domains, consistent with activation of an anomalous endothelial gene program. ATAC-seq revealed increased open chromatin at endothelial promoters with enriched motifs for ETS family of endothelial regulators. Computational prediction of the GATA4-TBX5 controlled network revealed "hubs" centered on hyperactive PI3K signaling and modulation of this pathway in mutant cardiomyocytes confirmed the significance of this dysregulation on cardiomyocyte physiology. These results reveal that GATA4, TBX5 and Mediator complex cooperatively establish transcriptional-epigenetic identities for cardiac gene program and suggest novel regulatory nodes for restoring disease-related phenotypes.

W1067

MANUFACTURED LASER MICRO-GROOVED CULTURE PLATES IMPROVE THE MATURATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Dariolli, Rafael, Moyano, Juan José Augusto, Zezell, Denise Maria, Rossi, Wagner and Krieger, José Eduardo, University of Sao Paulo, Sao Paulo, Brazil

Heart muscle is a functional and organized interstitial tissue. Human cardiomyocytes derived from pluripotent stem cells (hiPSC-CMs) are promising source of contracting cells and may benefit applications such as disease modeling, drug screening, toxicity testing and therapeutic regenerative strategies. The immature phenotypes acquired by current differentiation protocols may limit some of this potential and requires further refinement. Here, we want to test the hypothesis that laser micro-grooved cell culture plates improve maturation of hiPSC-CMs. We differentiated human induced pluripotent stem cells from skin fibroblasts to cardiac phenotype by standard methods using small molecules to modulate Wnt pathway (GSK-3 inhibitor and IWP-2/IWP-4). Beating cells (day 7-10) were seeded in laser micro-grooved or standard culture plates. Morphological assessments and RNA extraction were performed 30 days after cell seeding on both conditions. Immunofluorescence analysis to phalloidin and TNNI3 structural markers revealed cellular elongation and sarcomere organization respectively only for hiPSC-CMs seeded in laser micro-grooved plates. Atrial Light Chain-2 (MYL7) gene expression increased versus standard controls while the gene expression of a cardiac progenitor marker NKX2.5 decreased. Gene expression of structural protein such as PLN, MYH7, troponin T and I, and channels proteins as HCN4, CACNA1C and CACNB2 remained unchanged in both conditions. Altogether, these results provide morphologic and genetic evidence that hiPSC-CMs cultivated in laser micro-grooved plates are more mature than cells cultured in standard conditions within a 30-day period.

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W1069

REACTIVE OXYGEN SPECIES AND OXIDATIVE DNA DAMAGE REGULATE MURINE ADULT BMI1+ CARDIAC PROGENITOR CELL FATE DECISIONS

Herrero, Diego[1], Tomé, María[2], Cañón, Susana[1,2] and Bernad, Antonio[1], [1]Spanish National Center for Biotechnology, Madrid, Spain, [2]Spanish National Cardiovascular Research Center, Madrid, Spain

The adult mouse heart is a highly oxidative organ with no notable regenerative capacity compared with embryonic mouse or lower vertebrate heart, although it has very limited cardiomyocyte proliferation and cardiac progenitor cell differentiation. Reactive oxygen species (ROS) and oxidative DNA damage are important effectors in various pathologies including several cardiac diseases. In a physiological context, ROS are responsible for the loss of cardiomyocyte proliferation capacity after post-natal stage P7 and are necessary for optimal in vitrocardiomyocyte differentiation from embryonic stem cells. The role of ROS in the adult heart has nonetheless been very little explored. A low redox balance is characteristic of adult stem cells in several organs, where it is associated with hypoxic niches, side population dye-efflux phenotype and subsequent enhanced anaerobic glycolysis. The cells most resistant to oxidative stress and DNA damage might therefore constitute the limited source of cardiac turnover in adult mouse heart. We focused on the Polycomb transcription factor BMI1, previously linked to multipotent cell populations, including those in the heart. The $Bmi1^+$ cardiac population corresponds mainly to a $Sca1^+$non-myocyte subpopulation, which suggests a non-myocyte adult cardiac progenitor identity. BMI1 also regulates mitochondrial function and is recruited to sites of DNA damage. Using Bmi-1-green fluorescent protein (GFP)-knock-in mice and

the Bmi1-Cre/LoxP-based conditional transgenic mouse model, we demonstrate that cardiac Bmi1+ progenitors are a population with low redox state, able to respond to distinct genotoxic stresses. After persistent oxidative stress, Bmi1+cardiac progenitors significantly increase their contribution to the three main cardiac lineages. In conclusion, deep ROS analysis in post-natal heart suggests a direct relationship between ROS levels and differentiation status in cardiac progenitor cells. These results reinforce the relationship between low redox state and adult cardiac progenitors in mouse heart, and suggest ROS as a key regulator of in vivo cardiac turnover.

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W1071

PROTEIN QUALITY CONTROL, CARDIOMYOPATHY, AND CARDIOTOXICITY IN ISOGENIC IPSCS

Judge, Luke M.[1,2], Truong, Annie[2], Yoo, Jennie[2], Jensen, Christina[2], Lizarraga, Paweena[2], Huebsch, Nathaniel[2], Perez-Bermejo, Juan[2], Mandegar, Mohammad A.[2], So, Po-Lin[2] and Conklin, Bruce[1,2], [1]University of California, San Francisco, San Francisco, CA, U.S., [2]Gladstone Institutes, San Francisco, CA, U.S.

Dysregulation of protein quality control, due to either genetic or acquired causes, is increasingly recognized as an important mechanism contributing to various forms of cardiac disease. Recent biochemical and genetic studies suggest a central role for BAG3 in coordinating protein quality control in various cell types, as well as involvement in genetic forms of dilated cardiomyopathy (DCM). We employed human iPS-derived cardiomyocytes as a model system to study BAG3 function and protein quality control in the heart, using isogenic disease lines and expression of transgenic reporters. BAG3 protein was expressed at high levels with distinct localization pattern at the sarcomeric Z-disk in cardiomyocytes, suggesting that it may regulate folding and/or degradation of Z-disk client proteins. Consistent with its proposed function as a stress-induced co-chaperone, BAG3 was induced along with HSP70 and specific HSPB family proteins, by conditions of increased proteotoxic stress. Cardiomyocytes with targeted mutations in BAG3 had increased propensity for myofibrillar disarray, with characteristic disorganization and fragmentation of Z-disks. In order to monitor Z-disk structure and processing in live cells, we generated fluorescent alpha-actinin fusion proteins that were used for single-cell time-lapse imaging and measurement of protein turnover. Transgenic expression of actinin reporters demonstrated that proteasome inhibition by the chemotherapy drug bortezomib led to progressive sarcomeric disruption, which was exacerbated in BAG3 mutant cardiomyocytes. Video microscopy to measure contractile motion confirmed that BAG3 mutant cardiomyocytes had enhanced sensitivity to the cardiotoxic effects of proteasome inhibition by bortezomib. BAG3 has been shown to regulate autophagy in other cell types; to determine whether defects in autophagy contribute to the phenotype of BAG3 mutant cardiomyocytes we expressed a photo-convertible LC3 fluorescent reporter to measure autophagy flux. Our results highlight the critical role of BAG3 in regulating the proteome in contractile cardiomyocytes, which is disrupted in DCM and may contribute to bortezomib cardiotoxicity. Our iPS-cardiomyocyte system serves as a platform for future mechanistic studies and development of therapeutic strategies.

W1073

HUMAN HEART RNAs INDUCE DIFFERENTIATION OF STEM CELLS AND FIBROBLASTS INTO CARDIOMYOCYTES IN CULTURE

Lemanski, Larry F., Kochegarov, Andrei, Neal, Michael, Davis, Allyson, Mitchell, Lena, Shahankary, Kaitlyn, Fetters, Hallie, Scarcelli, Nikolas and Vaughn, Greg, Texas A&M University-Commerce, Commerce, TX, U.S.

We have developed two human-derived functional homologs of an RNA that promotes heart development in cardiac non-function mutant salamanders, Ambystoma mexicanum. These RNAs are termed Cardiac-Inducing RNAs (CIR). One of the RNAs is associated with the mitochondrial cytochrome c oxidase gene and a second with the caspace recruitment gene. We have found that both are capable of inducing myofibril formation in mutant axolotl myocardial cells which ordinarily do not form myofibrils. We have found further that human iPSCs, mouse ESCs and mouse embryonic fibroblasts transfected with the human CIRs significantly increase the expression of cardiac specific proteins after only one week incubation demonstrating that these RNAs have the ability to induce nonmuscle cells, including fibroblasts, to form into cardiac myoblast cell lineages. This approach may offer a novel way to treat myocardial infarctions by reprogramming an individual's fibroblasts to form into functioning cardiomyocytes. Stem cells or fibroblasts transfected with the human CIRs form into cells characteristic of early developing cardiomyocytes, and express cardiac protein markers including cardiac specific cardiac troponin-T, tropomyosin and α-actinin as detected by immunohistochemical staining. Furthermore, these contractile proteins organize into sarcomeric myofibrils characteristic of striated cardiac muscle cells. Computer analyses of the CIR secondary structures reveal significant similarities to the myofibril-inducing RNA (MIR) secondary structure described in salamander, that also promotes nonmuscle cells to differ-

entiate into cardiac muscle. Thus, the axolotl and human RNAs appear to have evolutionarily conserved secondary structures suggesting that both may play major roles in vertebrate heart development and in the differentiation of cardiomyocytes from non-muscle cells. Our current goal is to generate a myocardial infarction model in mice by coronary artery ligation and use CIR-treated stem-cell-derived cardiomyocytes to regenerate the damaged areas of the infarcted mouse hearts in vivo. We hope in the future that this type of approach can be used to treat myocardial infarctions in human patients such that heart attack victims could return to normal pre-heart-attack activity levels.

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W1075

THE ROLE OF MICRORNAS IN REGULATING STEM CELL DERIVED CARDIOMYOCYTE MATURATION

Miklas, Jason Wayne[1], WANG, Yuliang[2], Fischer, Karin[1], Macadangdang, Jesse[1], Leonard, Andrea[1], Madan, Anup[3], Sniadecki, Nathan[1], Kim, Deok-Ho[1] and Ruohola-Baker, Hannele[1], [1]The University of Washington, Seattle, WA, U.S., [2]The Chinese University of Hong Kong, Shatin, Hong Kong, [3]Labcorp, Seattle, WA, U.S.

The ability to generate human cardiomyocytes (CMs) from human pluripotent stem cells (hPSC) provides an unprecedented opportunity to establish human in vitro cardiac models for the testing of novel cardiac therapeutics. Yet, modeling human cardiac disease in vitro using hPSC-CMs, has been questioned since differentiated CMs do not recapitulate the adult cardiac phenotype. To address this, we have previously shown that overexpressing one microRNA (miR), Let7, can lead to a robust, albeit incomplete, maturation of hPSC-CMs. We are now validating novel miRNAs and generating a cocktail of these miRs with Let7 to rapidly shift the immature hPSC-CM gene expression profile and functional parameters to one of an adult CM. To elucidate impactful miRs for validation, we performed pathway analysis on differentially regulated miRs from our 1-year matured hESC-CM microRNA sequencing. The highly up-regulated miRs connected to a strong mRNA network were -378e, -208b and -452. To determine functional improvements of each of these new miRs, we overexpressed miR-378e, -208b or -452 and utilized micro-electrode arrays (MEA) to examine cardiomyocyte electrophysiology, Seahorse to examine mitochondrial capacity and a micropost assay to determine single cell force of contraction. MEA showed that miR-378e brought a robust increase in sodium spike amplitude and slope (0.79mV, -1.53V/s) as compared to empty vector (EV) hPSC-CMs (0.24mV, -0.46V/s). This increase in sodium spike amplitude in miR-378e samples correlates with an increase in voltage gated sodium channel SCN3a levels, an indication of cardiomyocyte maturation which was not found in Let7 hPSC-CMs. While miR-378e, -208b and -452 do not show significant beneficial effects in mitochondrial oxygen consumption rates, single cell force of contraction showed that hPSC-CMs treated with miR-208b and -452 were able to generate more power (21.8fW, 25.2fW) compared to EV (13.9fW). We have shown that miR-378e is able to mature electrophysiology while miR-208b and -452 are able to mature cell contractile machinery. These data show each novel miR elicits a certain aspect of CM maturation suggesting the need for a miR cocktail to mature hPSC-CMs. We will now combine these miRs with Let7 to study their combinatorial actions in maturation.

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W1077

IDENTIFICATION OF KEY REGULATORS IN THE DIFFERENTIATION OF CARDIOMYOCYTES

Pappoe, Herman A., California State University of Northridge, Northridge, CA, U.S.

Hematopoietic and cardiovascular systems are major targets for cell-based therapies due to high mortality rates associated with blood and heart diseases. Generating cells in vitro that can be transplanted has not proven successful so far, demonstrating, the undeniable need to better understand the differentiation process of these lineages in multipotent cardiovascular mesoderm and to clarify how cell identity is appointed. Studying the transcriptome and chromatin signatures in cardiomyocyte differentiation, can result in the identification of key transcription factors, novel transcripts and regulatory networks associated with the process. A thirty-day directed differentiation system for lineage determination in cardiomyocytes was employed to generate RNA-seq data collected at five time course samples specific to the developmental process. Computational approaches focusing on epigenetic transcriptional changes in relation to gene expression, histone modifications and chromatin marks across the differentiation, as well as analysis of highly induced LincRNA help to define behaviors of stage-specific regulators and relationships between transcription factors and induced target genes during heart development.

W1079

MODELING CARDIAC DEFECTS IN SPINAL MUSCULAR ATROPHY

Yu, Hongbing[1], Rodriguez Muela, Natalia[2], Sun, Chicheng[3], Ng, Shi-Yan[1] and **Soh, Boon-Seng**[1], [1]Institute of Molecular and Cell Biology, Singapore, Singapore, [2]Harvard Stem Cells and Regenerative Biology Department, Cambridge, MA, U.S., [3]Harvard University, Cambridge, MA, U.S.

SMN deficiency is known to cause spinal muscular atrophy (SMA), which is the leading genetic cause of infant mortality. Traditionally, SMA has been described as a motor neuron disease that is characterized by motor neuron loss and skeletal muscle atrophy. However, in recent years, a growing body of evidence suggests that SMN deficiency also leads to cardiac defects in SMA patients. Coupled with our recent findings that SMN deficiency resulted in elevated levels of ER stress in diseased motor neurons, we attempted to investigate the effects of ER stress on cardiac progenitors and cardiomyocytes derived from both wild-type and SMA iPSCs. Our results demonstrated that both cardiac progenitors and cardiomyocytes derived from SMA iPSCs expressed elevated ER stress markers and undergo apoptosis. In addition, SMN-deficient cardiomyocytes also exhibited an arrhythmic phenotype. Moving forward, we are exploring the possibility of alleviating the condition by inhibiting ER stress in the affected cells.

W1081

RISK ALLELE AT 9p21 UNIQUELY REGULATES CONNEXINS TO INDUCE ARRHYTHMIC PHENOTYPES IN PATIENT-DERIVED iPSCs

Kumar, Aditya[1], **Wong, Kirsten Chiyen**[1], Thomas, Stephanie[1], Tenerelli, Kevin[1], Lo Sardo, Valentina[2], Ferguson, William Corbett[2], Topol, MD, Eric[2], Baldwin, Kristin K.[2] and Engler, Adam[1], [1]University of California, San Diego, La Jolla, CA, U.S., [2]The Scripps Research Institute, La Jolla, CA, U.S.

Genome-wide association studies identifying single nucleotide polymorphisms (SNPs) at non-coding locus are difficult to interpret, especially when the locus and flanking regions do not occur in or yield phenotypes in animal models. To understand mechanism between the non-coding 9p21 locus and its increased risk of cardiovascular disease, induced pluripotent stem cell-derived cardiomyocytes (CMs) were produced from patients homozygous for (R/R) and without 9p21 SNPs (N/N). To future mimic disease, CMs were cultured within a "heart attack-in-a-dish" bioreactor that mirrors changes from normal to disease, e.g. stiffening like a fibrotic scar. While CMs beat synchronously in normal niche conditions independent of genotype, R/R CMs exhibit asynchronous contractions compared to N/N CMs when cultured on the remodeled niche. The dynamic shift in conditions downregulated connexin 43 expression and gap junction assembly in R/R CMs but not in N/N CMs. Isogenic deletion of the locus from R/R CMs restored synchronous contraction, even in the remodeled niche, demonstrating that niche changes can differentially affect cell function depending on the presence of a non-coding but risk associated locus. It further suggests that this "heart attack-in-a-dish" model could be used throughout biology to understand disease phenotypes in vitro that require disease-like niche only after initially appearing normal, e.g. cancer.

W1083

A UNIQUE COMBINATION OF SMARTR BIOINFORMATICS AND CARDIOMYOCYTE SCREENING TECHNOLOGY ALLOWS FOR DEVELOPMENT OF NOVEL CARDIO-PROTECTIVE DRUGS

Park, Eunhye[1], Pham, Lisa[2], Cadag, Eithon[2], Lum, Pek[2], **Pike, Nirupama K.**[2] and Armstrong, Chris[1], [1]Stem Cell Theranostics, Redwood City, CA, U.S., [2]CapellaBio, Palo Alto, CA, U.S.

Anthracyclines are a class of highly effective chemotherapy drugs used in the treatment of many cancers, including leukemias, lymphomas, breast, uterine, ovarian, bladder and lung cancers. Approximately 50% of childhood cancer treatment regimens include anthracyclines. Dose dependent cardiotoxicity is a major side effect of anthracyclines. Cancer survivors receiving anthracycline therapy are 15 times more likely to develop heart failure and 8 times more likely to die of cardiovascular disease. Dexrazoxane is the only clinically approved cardio-protectant that has been shown to reduce the risk of cardiotoxicity. However, it is only indicated for metastatic breast cancer and has been linked to secondary malignancies. It is, therefore, critical to develop safer and more effective cardio-protectant drugs to address this major unmet medical need. The recent advancement in generation of human cardiomyocytes from induced pluripotent stem cells (hiPSC-CMs) enables a biologically relevant setting for compound screening because hiPSC-CMs possess many similar functional characteristics as primary human cardiomyocytes and can be generated in significant quantities. We have established a platform to screen small compounds and natural product for efficacy and safety across multiple donor lines using hiPSC-CMs. Compounds to be screened were selected in collaboration with Capella Biosciences who used their SMarTR computational analysis platform to identify potential candidates for further screening in hiPSC-CMs. Using this novel drug discovery engine that combines bioinformatics-driven in-silico screening and hiPSC-CM-based compound screening platforms, we discovered four drugs that protect hiPSC-CMs from doxorubicin-induced cardiotoxicity. We used two cancer cell lines to confirm that these candi-

dates do not interfere with doxorubicin's anti-cancer activity. We also demonstrated that these candidates do not show acute cardiotoxicity mediated through ion channels. Thus, a unique platform combining SMarTR computational approaches and hiPSC-CMs screening technology allows for rapid identification of highly effective cardio-protective drugs.

MUSCLE CELLS

W1085

MECHANISMS OF ALTERNATIVE POLYADENYLATION IN MUSCLE STEM CELLS

de Morree, Antoine[1], Gan, Qiang[1] and Rando, Thomas A.[2], [1]Stanford University, Stanford, CA, U.S., [2]Stanford University School of Medicine, Stanford, CA, U.S.

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, muscle stem cells in diaphragm maintain the expression of Pax3 transcript in the presence of 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). Using morpholinos that block these binding sites we could modify the 3'UTR length of reporter genes bearing the Pax3 3'UTR in cultured cells and of endogenous transcripts in vivo. Our results hint at a novel mechanism for post transcriptional control of gene expression in muscle stem cells.

Funding Source: Muscular Dystrophy Association, FSH Society

W1087

SKELETAL MUSCLE MODELLING OF MYOTONIC DYSTROPHY FROM HUMAN PLURIPOTENT STEM CELLS

McKernan, Robert[1], **Main, Heather Marie**[1], Dumevska, Biljana[2], Petersen, Suzanne[1], Caron, Leslie[2], Schaft, Julia[1] and Schmidt, Uli[3], [1]Genea Biocells, La Jolla, CA, U.S., [2]Genea Biocells, Sydney, Australia, [3]Genea Biocells SD, San Diego, CA, U.S.

Myotonic dystrophy (DM) is the most common form of muscular dystrophy in adults. There are two types, Myotonic Dystrophy 1 (DM1), caused by a heterozygous CTG repeat expansion (50-4,000 copies) in in the 3' untranslated region of the DMPK gene, and Myotonic Dystrophy 2 (DM2), caused by heterozygous CCTG repeat expansion (75-11,000 repeats) in intron 1 of the ZNF9 gene. Both diseases lead to nuclear ribonucleoprotein accumulation, deregulated splicing and display clinical dystonia. We have previously developed a protocol for efficient differentiation of human pluripotent stem cells to skeletal muscle (SkM). Briefly, stem cells are induced to differentiate to myogenic progenitors (Pax3/7+) and intermediate myoblast (MyoD+) stages to form terminally differentiated (MF20+) skeletal muscle (SkM) myotubes in 26 days using 3 defined culture media. This method provides a virtually unlimited renewable source of muscle cells for high-throughput, high-content screening and cell-based therapy development. In order to develop a scaleable human model for DM we have derived and characterised male DM affected hESC lines, Genea066 (DM2) and Genea067 (DM1). We show here that these lines are genetically normal by CGH karyotyping and determined their repeat length expansion. We further show that they are pluripotent by immuofluoresence and PluriTest. We then differentiated the pluripotent cells to skeletal muscle myotubes and analysed differentiation efficiency, nuclear ribonucleoprotein foci at multiple developmental stages and myotube morphology compared to normal control cell lines. Our results demonstrate the validity of using these, and other muscular dystrophy-affected pluripotent cells for disease modelling, therapeutic drug screening and potentially for future clinical cell therapy applications.

W1089

A PLURIPOTENT CELL BASED DUX4 REPORTER FOR FSHD MODELING

Cabral-da-Silva, Mauricio Castro[1], Rickard, Amanda Marie[2], de la Garza-Rodea, Anabel[2], Caron, Leslie[1], Arjomand, Jamshid[2], Miller, Daniel[3] and **Schmidt, Uli**[2], [1]Genea Biocells, Sydney, Australia, [2]Genea Biocells, San Diego, CA, U.S., [3]University of Washington, Seattle, WA, U.S.

Facioscapulohumeral Muscular Dystrophy (FSHD) is one of the most common forms of muscular dystrophy and is characterized by variable, progressive weakness in skeletal muscle that typically starts in the face, shoulder girdle and arms, then progresses to the trunk and lower extremities. Though the pathogenesis of FSHD is not entirely understood, the disease is thought to be caused by aberrant activation of the normally epigenetically repressed gene Double Homeobox Protein 4 (DUX4). Variegated and rare bursts of DUX4 protein can be detected in differentiated FSHD patient biopsy-derived muscle cells (1), though due to their restricted proliferative capacity these cells offer limited potential for therapeutics screening or cell therapy strategies. We have previously developed a protocol for efficient differentiation of human pluripotent stem cells through myogenic progenitor (Pax3/7+) and intermediate myoblast (MyoD+) stages to terminally differentiated (MF20+) skeletal muscle (SkM) myotubes in 26 days (2). This provides a virtually unlimited renewable source of muscle cells for high-throughput, high-content screening and cell-based therapy development. We have combined our FSHD affected (Genea049/050/096) and control (Genea016) human embryonic stem cell (hESC) lines with a previously characterized fluorescent, nuclear DUX4-activated turboGFP (tGFP) lentiviral reporter (3) in order to identify DUX4+ cells in FSHD affected muscle development. We report that while DUX4-activated reporter fluorescence is undetectable in control lines the reporter is readily detectable in later stages of SkM derived from FSHD-affected hESCs. Maximal DUX4 expression was observed in FSHD myotubes. Using our optimized protocol, we have documented DUX4 expression in FSHD-affected myogenesis. Our DUX4-reporting FSHD cell lines will be invaluable as a screening platform for assorted FSHD therapeutics to silence DUX4 without inhibiting myogenesis.

Funding Source: Friends of FSH Research

PANCREATIC, LIVER, LUNG, OR INTESTINAL/GUT CELLS

W1093

IMPROVEMENT OF HUMAN IPS CELL-DERIVED HEPATOCYTE FUNCTIONALITY USING 3D CULTURE SYSTEMS

Lin, Zachary Yu Ching[1], Akahira, Rina[1], **Annand, Robert R.**[2], Yoshida, Shunsuke[1] and Hayashi, Yasayuki[1], [1]ReproCELL, Yokohama, Japan, [2]Stemgent, Lexington, MA, U.S.

Human primary hepatocytes are utilized for high-throughput screening in early-stage drug discovery in order to evaluate thousands of potential compounds. Yet, human primary hepatocytes have the disadvantage of a limited supply from a single donor as well as high donor-to-donor variability. To overcome these obstacles, functional human induced pluripotent stem (iPS) cell-derived hepatocytes are highly desirable, as they are available in unlimited quantities from the same donor. However, immaturity and donor-to-donor variability are common drawbacks of iPS cell-derived hepatocytes. To address hepatocyte maturation, we evaluated multiple methods using 3D cultivation for maturing iPS cell-derived hepatocytes. We compared different 3D culture systems with traditional 2D cultures by analyzing the expression levels of specific cytochrome P450 (CYP) enzymes, which play an important role in drug-metabolism. By culturing human iPS cell-derived hepatocytes using a 3D sphere culture system, the CYP3A4 basal level increased, and CYP3A4 fold induction doubled when compared to 2D cultivation. Further, when we applied a 3D culture system during the hepatocyte differentiation process, CYP3A4 basal expression was similar to primary hepatocytes, and CYP3A4 expression was induced five-fold by a typical inducer. Additionally, basal levels of CYP1A2 and CYP2B6 expression increased approximately 20-fold after 3D maturation, compared to 2D hepatic differentiation. In conclusion, 3D cell culture promotes increased maturation of human iPS cell-derived hepatocytes, potentially facilitating the creation of a human iPS cell-derived hepatocyte panel, which will enable assessment of donor-to-donor variability in iPS cell-derived hepatocyte function.

W1095

ROLE OF Lrig1-EXPRESSING CELLS AS GASTRIC EPITHELIAL PROGENITORS IN MOUSE STOMACHS

Choi, Eunyoung[1], Vlacich, Gregory[1], Coffey, Robert[1], Goldenring, James[1] and Powell, Anne[2], [1]Vanderbilt University Medical Center, Nashville, TN, U.S., [2]University of Oregon, Eugene, OR, U.S.

Lrig1, a pan-ErbB-negative regulator, is a marker of proliferative and quiescent stem cells in the skin, small intestine and colon. In this study, we investigated Lrig1 expression in the mouse stomachs, using Lrig1$^{CreERT2/+}$;R26R$^{YFP/+}$ mouse-based lineage tracing and Lrig1 antibody detection. We examined Lrig1-lineaged cells from Lrig1$^{CreERT2/+}$;R26R$^{YFP/+}$ mouse stomachs at 10 days after a single 2 mg dose of tamoxifen. Consistent with expression pattern of Lrig1 in the small and colon, Lrig1(+) cells were observed in the progenitor compartments of both the gastric fundus and antrum. Lrig1 lineage-labeling also persists for at least one-year post-injection. This lineage tracing indicates the Lrig1(+) progenitor cells can give rise to differentiated cells in both the gastric fundus and antrum, in contrast to published results for Lgr5(+) cells, where Lgr5(+) only marks progenitor cells within the gastric antrum. We have also investigated the gastric progenitor cell activity of *Lrig1*(+) cells during recovery phase from acute oxyntic atrophy in the gastric fundus induced by treatment with DMP-777, a parietal cell protonophore. Interestingly, the Lrig1(+) progenitor cells gave rise to all of the differentiated gastric epithelial lineage cells within the gastric fundus during recovery phase after damage. We next examined endogenous Lrig1 in the gastric fundus. Immunoreactivity with Lrig1 in the fundus was detected not only in the proliferating cells, but also in differentiated cell types, such as acid-secreting parietal cells and neuroendocrine cells (enterochromaffin-like cells). Moreover, expression of pEGFR was increased in Lrig1$^{-/-}$ mouse stomachs, suggesting that negative regulation of ErbB signaling by Lrig1 may be functionally important for proper regulation of Egfr, and possibly other ErbB receptors, in both progenitor and differentiated cells in the gastric oxyntic mucosa. In summary, Lrig1 is a marker of gastric epithelial progenitor cells and the Lrig1(+) progenitors can recover the damaged gastric oxyntic glands by differentiating into normal gastric lineage cells. Also, Lrig1 may have physiological roles on both progenitor cell differentiation and acid secretion.

W1097

THE ROLE OF EZH2 IN THE DIFFERENTIATION OF HEPATOCYTE-LIKE CELLS

Helsen, Nicky, Pistoni, Mariaelena, Vanhove, Jolien, Boon, Ruben, Ordovas Vidal, Laura, Xu, Zhuofei and Verfaillie, Catherine, SCIL, Leuven, Belgium

Currently, for drug toxicity and metabolisation studies, the pharmaceutical industry still relies on the use of hepatoma cell lines and human primary hepatocytes which both have conceivable limitations. Human embryonic (hESC) and induced pluripotent stem cell (hiPSC) - derived hepatocyte-like cells are an alternative source that can overcome these limitations. hESC and iPSC have numerous advantages compared to primary cells such as the ability to indefinitely self-renew, the potential to differentiate into any given cell type, the possibility to generate patient-specific disease models and the feasibility to be genetically modified. However, the efficiency of differentiation of pluripotent stem cells into mature hepatocytes is still relatively low. Polycomb Group (PcG) of proteins are implicated in controlling dynamics and plasticity of gene regulation. It was demonstrated that enchancer of zeste homolog 2 (EZH2), a functional enzymatic component of the polycomb repressive complex 2 (PRC2), which catalyzes tri-methylation of lysine 27 of histone H3 (H3K27me3), plays a role in the regulation of hepatocyte development. We here incorporated an inducible EZH2 cassette in the AAVS1 locus. EZH2 expression was induced during hPSC hepatocyte differentiation from day 0 onwards. We observed a significant improvement in definitive endoderm formation on day 4, which also resulted in a significantly improved hepatocyte-like cell generation on d20. Surprisingly, despite continuous EZH2 overexpression, transcript and protein levels of EZH2 decreased from day 4 onwards. We hypothesized that specific microRNAs might inhibit EZH2 expression during hepatocyte differentiation. We demonstrated that miR101, miR26a, miR124, miR214 and miR138, known to be able to bind to the EZH2 transcript, were increased during hepatocyte differentiation. We are currently assessing which of these miRNA(s) might be responsible for the post-transcriptional modification of EZH2 expression. In conclusion, our studies demonstrate that EZH2 expression appears to be tightly post-transcriptionally regulated during hepatocyte development, and that overexpression of EZH2 during endoderm specification significantly enhances the differentiation efficiency of pluripotent stem cells to hepatocyte-like cells.

co-sponsored by:
CIRM

W1099

AMNIOTIC FLUID STEM CELLS REPROGRAM THE INTESTINAL EPITHELIA DURING NEONATAL NECROTIZING ENTEROCOLITIS

Li, Bo, Zani, Augusto, Lee, Carol, Zani-Ruttenstock, Elke, Koike, Yuhki, Chen, Shigang, Martin, Zechariah, Li, Xinpei and Pierro, Agostino, Hospital for Sick Children, Toronto, ON, Canada

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency and a major cause of death in preterm infants. Despite recent advancements in neonatal care, mortality from NEC still remains high at 30-50%. The outcomes of NEC remain poor, demonstrating the need for innovative treatment. Intestinal epithelial stem cells (IESC) play a crucial role in intestinal epithelial homeostasis and renewal. It has been shown that in experimental NEC, amniotic fluid stem (AFS) cells administration improves survival, clinical status, gut structure and function. However, the underlying mechanism of this activity is not clear. Our aim is to investigate whether AFS cell administration prevent the intestinal damage that occurs in NEC by reprogramming the intestinal epithelia thus restoring intestinal epithelial homeostasis and renewal of the IESCs. Experimental NEC was induced in 5-day old neonatal C57BL/6 mice using gavage feeding of hyperosmolar formula, hypoxia and lipopolysaccharide. On days 6 and 7, mice received an intraperitoneal injection of PBS or $2x10^6$ AFS cells. Breastfed mice served as control. After sacrifice on day 9, distal ileum was harvested and analyzed. Groups were compared for IESC expression, enterocyte proliferation and differentiation, apoptosis, gut barrier function, mucosal damage and inflammation. Mitotically active Lgr5+ IESC were impaired in NEC and were activated by AFS cells to promote intestinal proliferation at the bottom of crypts and differentiation into intestinal epithelial cells. Conversely, quiescent Bmi1+ IESC were not affected by experimental NEC or AFS cells. NEC triggered endoplasmic reticulum stress and induced mucosal apoptosis, effects which were inhibited by AFS cells. In addition, AFS cells restored intestinal permeability by releasing mucin and improving tight junctions. NEC induced epithelial damage and bowel inflammation, which were improved after treatment with AFS cells. In summary, AFS cells administration reduces the intestinal damage due to experimental NEC. This is achieved by reprogramming the intestinal epithelia through cross talking with IESC and by restoring intestinal epithelial homeostasis and renewal. This study highlights the potential of stem cell therapy as a novel treatment for NEC.

W1101

THE TRANSCRIPTION FACTORS-MEDIATED MURINE LIVER TO PANCREAS TRANSDIFFERENTIATION IN SERUM-FREE, THREE-DIMENSIONAL CULTURE SYSTEM

Motoyama, Hiroaki, Kobayaashi, Akira, Yokohama, Takahide, Shimizu, Akira, Sakai, Hiroshi, Notake, Tsuyoshi, Kitagawa, Noriyuki, Fukushima, Kentaro, Shirota, Tomoki, Masco, Hitoshi and Miyazawa, Shinichi, Shinshu University, Matsumoto, Japan

Pancreatic lineage-specific transcription factors (TFs) display instructive roles in converting adult cells to endocrine pancreatic cells, in a process known as transdifferentiation. However, little is known about whether the culture system, namely culture format and supplemented small molecules, influences the efficiency of such transdifferentiation. The present study analyses the hypothesis that three-dimensional culture system utilizing defined small molecules optimizes the liver to pancreas transdifferentiation. Using ectopic adenoviral expression of three pancreatic TFs in adult liver-derived cells, we demonstrate that c-peptide expression in these transfected cells was manifested by 50-fold increase in aforementioned serum-free, three-dimensional culture system compared to those in conventional serum-contained monolayer culture. Furthermore, these cells not only expressed several pancreas-related genes but also secreted insulin in a glucose-dependent manner. Transplanting these differentiated cells into streptozotocin-induced immunodeficient diabetic mice led to the amelioration of hyperglycemia. These results suggest that three-dimensional culture with appropriate small molecules is crucial for the TFs-mediated liver to pancreas transdifferentiation. Our findings could facilitate the development of cell replacement therapy modalities for several degenerative diseases including diabetes.

W1103

HBV-INFECTED HUMAN HEPATOCYTE-LIKE CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS ALLOWED LONG-TERM VIRAL PROPAGATION AND WAS SPECIFICALLY TARGETED BY CYTOKINE-INDUCED KILLER CELLS

Sa-ngiamsuntorn, Khanit, Department of Biochemistry, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand, Wongkajornsilp, Adisak, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkoknoi, Thailand, Kongsomboonchoke, Pattida, Faculty of Science, Silpakorn University, Nakhon Pathom, Thailand, Thongsri, Piyanoot, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand and Hongeng, Suradej, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Hepatitis B virus (HBV) infects induces chronic liver inflammation which could eventually develop into hepatocellular carcinoma. The study of HBV biology and virus-host cell interaction have been hindered by the limitation of the available host cells. The long-term cultured functional hepatocyte that supports a complete HBV life cycle for a long term is essential for this task. In this study, hepatocyte-like cells (HLC) derived from human iPSCs were examined for HBV associated receptors, infectivity with HBV isolated from patient serum. The HBV receptors for example; HSPG, NTCP and clathrin were highly expressed in mature HLC as evaluated by immunofluorescent and real-time qPCR. HLCs sustained viral covalently closed circular DNA (cccDNA) replication and produced HBV particles after challenging with HBV from patient serum. The generation of HBV virions from HLCs outperformed those from HepaRG cell. Infection of HLCs with HBV from patient serum resulted in the expression of HBcAg, HBeAg and HBV cccDNA that was consistent with higher HBV protein synthesis and replication. The conditioned medium from infected HLCs was able to infect naïve HLCs and HepaRG cells. The HLCs upregulated the expression of IFN-α, TNF-α and IL-6 after the HBV infection. The co-culture of HBV-infected HLCs with cytokine-induced killer (CIK) cell exhibited 90% infected cell lysis whereas the non-infected HLCs exhibited 0% lysis. In summary, HLC could serve as an effective host for the study of HBV life cycle, virus-host interaction with a potential to develop hepatocellular carcinoma.

Funding Source: This study was funded by the research grants of Mahidol University and the Thailand Research Fund (TRF).

W1105

HUMAN INDUCED PLURIPOTENT STEM CELLS SURVIVED AND INITIATED HEPATOCYTE DIFFERENTIATION IN WILLIAM'S E MEDIUM FOLLOWED BY HEPATOCYTE DIFFERENTIATION INDUCER MEDIUM

Tomizawa, Minoru, National Hospital Organization Shimoshizu Hospital, Yotsukaido City, Japan

If hepatocytes are produced from iPS cells of patients with liver insufficiency, these hepatocytes can be transplanted into those patients as a curative treatment. Glucose and arginine are essential for cell survival. Hepatocytes produce glucose from galactose and arginine from ornithine through gluconeogenesis and the urea cycle, respectively. Hepatocytes can survive in a medium without glucose or arginine and with added galactose and ornithine. Hepatocyte differentiation inducer medium (HDI) does not contain glucose or arginine but contains galactose, ornithine, and oncostatin M. HDI initiates the differentiation of iPS cells into hepatocytes, as demonstrated by increased alpha-feto protein (AFP) expression. A major problem concerning HDI is that most cells cultured in HDI die within three days and no cells survive beyond 7 days. To increase the number of the cells obtained, iPS cells were cultured in conventional media after being cultured for 48 h in HDI. To determine the most appropriate media for use following HDI incubation, iPS cells were cultured in 13 different conventional media for 7 days, and AFP expression levels in these media were analyzed. The highest AFP expression in iPS cells was observed in William's E medium (WE) and Dulbecco's Modified Eagle's Medium/Nutrient F-12 Ham (DF12). The AFP expression levels were evaluated in iPS cells cultured in HDI for 48 h and then cultured in WE or DF12 for 5 days. The number of cells decreased to 5.4% of that on day 0 after 48-h culture in HDI and recovered to 60% of that on day 0 after 48-h culture in HDI followed by 5-day culture in WE. The AFP expression was higher in iPS cells cultured in HDI for 48 h and WE for 5 days than in iPS cells cultured in HDI only . These results suggested that culture in HDI for 48 h followed by culture in WE for 5 days was suitable to promote the differentiation of iPS cells into a hepatocyte lineage. To determine the culture period, iPS cells were cultured in HDI for 0, 3, 6, 12, 24, or 48 h and were then cultured in WE for 7 days, following which their AFP and albumin expression levels were analyzed. The AFP and albumin expression levels were highest in iPS cells cultured in HDI for 48 h followed by culture in WE for 5 days. Taken together, culture in HDI for 48 h followed by culture in WE for 7 days was suitable for the differentiation of iPS cells into a hepatocyte lineage.

ENDOTHELIAL CELLS/ HEMANGIOBLASTS

W1111

PATIENT-SPECIFIC IPSC DERIVED ENDOTHELIAL CELLS UNCOVER MECHANISMS CAUSING REDUCED PENETRANCE OF THE BMPR2 MUTATION IN PULMONARY ARTERIAL HYPERTENSION

Gu, Mingxia[1], Shao, Ningyi[1], Sa, Silin[1,2], Li, Dan[1], Grubert, Fabian[3], Cao, Aiqin[1], Ameen, Mohamed[2], Wu, Joseph C.[2], Snyder, Michael[2] and Rabinovitch, Marlene[4], [1]Stanford School of Medicine, Stanford, CA, U.S., [2]Stanford University, Stanford, CA, U.S., [3]Stanford, Stanford, CA, U.S., [4]Stanford University School of Medicine, Stanford, CA, U.S.

Pulmonary arterial hypertension (PAH) in its familial form (FPAH) is a heritable autosomal dominant disorder that results in progressive right heart failure and death within five years of diagnosis. Loss of function mutations in bone morphogenetic protein receptor (BMPR)2 occur in over 70% of FPAH patients. Intriguingly, only 20% of the mutation carriers develop clinical symptoms, indicating that the mutation is necessary but not sufficient for the disease. Thus, uncovering the molecular mechanisms underlying the protective phenotype in those BMPR2 mutation carriers without FPAH (Mut. Carriers) could lead to new therapeutic approaches for all PAH patients. A cohort of eight FPAH patients and Mut. Carriers from three families carrying different BMPR2 mutations and three gender matched controls were recruited for the study. IPSC-ECs from the Mut. Carriers showed a compensatory p-p38 signaling pathway, not observed in FPAH patients. This compensatory signaling accounted for preserved cell adhesion to six different extracellular matrices and reduced vulnerability to apoptosis. We related compensatory p-p38 signaling to an increase in the BMPR2 activators LRP1 and caveolin1 and a decrease in BMPR2 repressors gremlin 1 and FKBP12 in Mut. Carriers vs. FPAH iPSC-ECs. Gene expression profiling by RNA-Seq uncovered potential downstream targets such as matrix metalloproteinase-3 (MMP3) and BIRC3 that could explain the compensatory phenotype in the Mut. Carriers. To determine to what extent the BMPR2 mutation is required for the abnormal FPAH iPSC-EC function, the BMPR2 mutation in family 1 was corrected using CRISPR-Cas9 technology. FPAH iPSC-ECs after BMPR2 correction show significantly improved cell adhesion as well as reduced apoptosis in response to either serum withdrawal overnight or hypoxia- reoxygenation. These results suggest that in this FPAH family member, the BMPR2 mutation is required for the impaired iPSC-EC function. This is the first study to use iPSC-ECs to show functional and signaling differences related to BMPR2 that distinguish PAH patients from unaffected BMPR2 Mut. Carriers. Since reduced function and expression of BMPR2 is also seen in patients with idiopathic and associated forms of PAH, these studies reveal compensatory mechanisms that might be targeted in developing novel therapies.

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W1113

EXOSOMES DERIVED FROM HUMAN ENDOTHELIAL PROGENITOR CELLS ACCELERATE CUTANEOUS WOUND HEALING BY PROMOTING ANGIOGENESIS THROUGH Erk1/2 SIGNALING

Li, Qing[1], Zhang, Jieyuan[2], Niu, Xin[2], Hu, Bin[2] and Wang, Yang[2], [1]Institute of Microsurgery on Extremities, Shanghai, China, [2]Institute of Microsurgery on Extremities, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China

Chronic skin wounds represent one of the most common and disabling complications of diabetes. Endothelial progenitor cells (EPCs) are precursors of endothelial cells and can enhance diabetic wound repair by facilitating neovascularization. Recent studies indicate that the transplanted cells exert therapeutic effects primarily via a paracrine mechanism and exosomes are an important paracrine factor that can be directly used as therapeutic agents for regenerative medicine. However, to date, there are few reports regarding the application of exosomes in diabetic wound repair. In this study, we demonstrated that the exosomes derived from human umbilical cord blood-derived EPCs (EPC-Exos) possessed robust pro-angiogenic and wound healing effects in streptozotocin (STZ)-induced diabetic rats. By a series of in vitro functional assays, we found that EPC-Exos could be incorporated into endothelial cells and significantly enhance endothelial cells' proliferation, migration, and angiogenic tubule formation. Moreover, gene expression profiling demonstrated that exosomes treatment significantly altered the expression of a class of genes involved in Erk1/2 signaling pathway. Inhibiting Erk1/2 signaling abolished the pro-angiogenic effect induced by EPC-Exos, indicating that this signaling was the critical mediator of exosomes-induced angiogenic responses of endothelial cells. In conclusion, EPC-Exos are able to stimulate angiogenic activity of endothelial cells by activating Erk1/2 signaling pathway, which finally facilitates cutaneous wound repair and regeneration.

Funding Source: This study was financially supported by the National Natural Science Foundation of China (Grant No.:81472152, 81572120 and 81572223).

W1115

RNA SEQUENCING ANALYSIS OF PULMONARY ARTERIAL HYPERTENSION NATIVE ENDOTHELIAL CELLS AND INDUCED PLURIPOTENT STEM CELL DERIVED ENDOTHELIAL CELLS REVEALS NOVEL PATHOGENIC GENES

Sa, Silin[1], Gu, Mingxia[1], Chappel, James[2], Shao, Ningyi[1], Li, Dan[1], Ameen, Mohamed[2], Grubert, Fabian[3], Wu, Joseph[2], Snyder, Michael[2] and Rabinovitch, Marlene[1], [1]Stanford School of Medicine, Stanford, CA, U.S., [2]Stanford University, Stanford, CA, U.S., [3]Stanford, Stanford, CA, U.S.

Pulmonary arterial hypertension (PAH), a potentially lethal disease leading to right-sided heart failure, is characterized by loss and obliteration of the vasculature. Induced pluripotent stem cells (iPSC) provide an opportunity to study the cell-specific pathology, and to test and develop new generic or personalized treatments for PAH. We have shown that pulmonary artery endothelial cells (PAEC) and iPSC differentiated EC (iPSC-EC) from the same PAH patients have comparable functional impairment and respond to potential PAH therapies in a patient-specific manner. To identify differentially expressed genes related to the PAH phenotype we analyzed RNA Seq datasets in PAEC and iPSC-EC from 6 control subjects and 6 PAH patients. We found 6 down-regulated and 10 up-regulated genes in PAH PAEC and iPSC-EC vs. controls. We next investigate the relevance of the differentially expressed transcripts to impaired PAH PAEC function. siRNA was used to reduce the level of KISS1, a transcript increased in both PAH PAEC and iPSC-EC. We observed that PAH PAEC and iPSC-EC transfected with KISS1 siRNA versus control siRNA, showed accelerated closure of 'wound' areas, suggesting that the impaired migration in PAH cells is related to elevated KISS1, an anti-angiogenic factor. We also reduced CES1 in control PAEC and iPSC-EC, and this resulted in increased susceptibility to apoptosis, a feature of PAH cells related to reduced CES1. The PAEC and iPSC PAH lines, were matched in their responsiveness to potential PAH drugs as assessed by angiogenesis assays (tube formation in matrigel). RNA Seq identified a set of 3 up-regulated and 8 down-regulated genes that distinguished drug non-responders from responders in both PAEC and iPSC-EC. Reducing gene expression level of Silt3 an anti-migratory factor, which is up-regulated in non-responders, resulted in improved migration of these cells. No single transcript manipulation could, however, reverse all the functional abnormalities that are necessary for improved angiogenesis. This suggests that in different PAH patients, deregulation of various genes may be involved in a similar functional abnormality or drug responsivity and iPSC-ECs are valuable in developing personalized gene expression signatures, identifying pathogenic mechanisms and optimizing pharmacotherapy.

Funding Source: NIH-NHLBI 5UO1 HL10739302

EPITHELIAL CELLS (NOT SKIN)

W1117

DEFINING PROGENITOR CELLS AND THEIR REGULATORS IN THE LACRIMAL GLAND

Finley, Jennifer K[1], Cruz-Pacheco, Noel[2] and Knox, Sarah[2], [1]University of California San Francisco, San Francisco, CA, U.S., [2]Program in Craniofacial Biology, University of California San Francisco, San Francisco, CA, U.S.

Tear secretion is essential for maintaining ocular acuity and vision. An aqueous deficient dry eye due to dysfunction of the tear producing lacrimal glands is one of two forms of the disorder and affects millions of people worldwide. With no cure and limited short-term treatment options available regenerative therapy offers a promising approach for restoring tear production. However, the identity of the progenitor cells that reconsitute the gland after injury and the mechanisms controlling these cells are not known. Here we demonstrate that cells marked by KRT14 are progenitors in lacrimal gland that are positively regulated by parasympathetic nerve derived factor neuropeptide Y. Using lineage tracing, we show that KRT14+ cells are multipotent progenitors in the developing gland that contribute to each of the 3 major epithelial lineages (acinar, ductal and myoepithelial). We further show that ablation of NPY receptors in KRT14+ cells reduced epithelial morphogenesis (production of acini and ducts), caused aberrant localization KRT14+ cells basal epithelium and disrupted myoepithelial cell formation. Finally, we demonstrate that KRT14+ cells are also progenitors in the adult tissue and that NPY serves a regenerative function by driving repopulation of the myoepithelial compartment by KRT14+ cells after radiation-induced injury. Thus, parasympathetic nerves positively regulate lacrimal gland progenitors and thus development and regeneration through an NPY dependent mechanism. We believe these studies will enable us to better understand what maintains the lacrimal gland and provide tools to find effective treatment options for patients suffering from dry eye disease.

Funding Source: This research is supported by the Kirschstein-NRSA grant awarded to Jennifer Finley from National Eye Institute. (F32EY025139).

W1119

CROSS-TALK BETWEEN HUMAN AIRWAY EPITHELIAL STEM CELLS AND 3T3-J2 FEEDER CELLS IN CO-CULTURE

Hynds, Robert E.[1], Gowers, Kate[2], Butler, Colin R.[1], Prêle, Cecilia M.[3] and Janes, Sam M.[2], [1]University College London, London, U.K., [2]Lungs for Living Research Centre, London, U.K., [3]University of Western Australia, Perth, Australia

Human airway basal epithelial cells are a stem/progenitor population that regenerates the mucosal surface following injury. In airway mucosecretory diseases, the epithelium is implicated in chronic inflammation and overproduction of mucous but the cellular mechanisms underlying these changes remain incompletely understood due to the complexity of signaling between epithelial, stromal and immune cell types. We describe a human airway epithelial cell culture system involving epithelial cell expansion from small endobronchial biopsy samples and subsequent co-culture with mitotically inactive 3T3-J2 fibroblasts in the presence of the small molecule Rho-associated protein kinase (ROCK) inhibitor Y-27632. This system rapidly expands cells expressing basal epithelial stem/progenitor cell markers. The molecular interactions between stromal cells and epithelial cells were investigated. HGF secretion increased following mitotic inactivation of feeder cells and incompletely activated the human MET receptor on epithelial cells, meaning that it cannot explain the increased epithelial proliferation caused by feeder cells. Contrary to previous reports that murine HGF is inactive on human cells, other downstream signaling pathways were nevertheless activated by murine HGF. The transcription factor STAT6, classically activated by interleukin 4 (IL-4) and IL-13, was activated in cells stimulated with HGF and altered the production of epithelial cytokines associated with neutrophil recruitment. These results suggest that stromal cells, which produce HGF in response to airway injury and disease in vivo, might alter the innate immune function of basal epithelial cells during airway regeneration.

Funding Source: British Biotechnology and Research Council (BBSRC), UHU Seed Funding Initiative

W1121

POU5F1 POSITIVE MOUSE AMNIOTIC CELLS CONTRIBUTE TO ALL THREE GERM LAYERS IN CHIMERAS

Miki, Toshio, Garcia, Irving and Nguyen, Samantha, University of Southern California, Los Angeles, CA, U.S.

Amniotic epithelial cells represent multipotent cells that arise from epiblasts before gastrulation. The differentiation capabilities of amniotic epithelial cells have been shown in vitro and in vivo. However, pluripotency of the single cells has not been demonstrated due to the lack of the ability to form teratomas. The aim of this study was to investigate the developmental potential of amniotic epithelial cells in a rodent platform that would allow generating chimeras and demonstrating the integration of pluripotent stem cell marker expressing mouse amniotic stem cells (mASCs) into the embryo. Using pou5f1 promoter driven green fluorescent protein (GFP) transgenic (Oct4-GFP) mouse, we confirmed that GFP positive cells were scattered in the mouse amnion. Transcriptional analysis revealed that the GFP positive cells maintained stem cell marker genes expression compared to the negative cells. In order to obtain traceable mASCs, Oct4-GFP homozygous mice and Rosa26/LacZ homozygous mice were crossed and GFP positive mASCs were isolated from the F1 fetal placenta at embryonic day (ED) 18.5. Ten mASCs were microinjected into each donor blastocyst, which were surgically transferred to pseudopregnant recipient females. Chimeric fetuses were analyzed at ED 10.5~13.5 to visualize the LacZ positive cells. Five of 58 fetuses demonstrated LacZ positive cell chimerism in various organs including the brain (ectoderm), heart (mesoderm), and liver (endoderm). Similarly, postnatal mice derived from injected embryos displayed LacZ positive cells in multiple tissues. However, the contribution level of mASCs found in the tissues was lower than that of mouse pluripotent stem cells. The results indicate that some of the stem cell marker positive mASCs can integrate into the embryo and contribute to all three germ layers with limited developmental potential.

W1123

3D IN VITRO CULTURE SYSTEM FOR DIFFERENTIATION OF SENSORY HAIR CELLS FROM MURINE Lgr5+ SOMATIC COCHLEAR STEM CELLS AND PLURIPOTENT STEM CELLS FOR OTO-TOXIC AND OTO-REGENERATIVE COMPOUNDS SCREENING

Roccio, Marta[1,2], Perny, Michael[1,3], Ting, Ching-Chia[1,2] and Senn, Pascal[2,4], [1]Department of Clinical Research University of Bern, Bern, Switzerland, [2]University Hospital, Insel, Bern, Switzerland, [3]University of Bern, Bern, Switzerland, [4]University Hospital Geneva (HUG), Geneve, Switzerland

Sensory hair cells located in the inner ear mediate sound perception and balance. Widely used antibiotics, chemotherapeutics, diuretics and anti-malaria drugs result in the death or damage of hair cells. Due to the lack of regenerative capacity of the sensory epithelium, this causes permanent hearing loss. In vitro screening methods to identify oto-toxic, pro-regenerative or protective compounds have not yet been developed. To this end, we are making use of state-of-the-art organoid technology for in vitro differentiation of murine Embryonic Stem Cells

(mESCs) or Lgr5+ somatic cochlear hair cell progenitors into hair cells for screening purposes.

mESCs are differentiated using previously described 3D organoid culture methods. Briefly, following definitive ectoderm induction, non-neural ectoderm is specified by addition of BMP-4, and concomitant inhibition of TGFb signaling. Subsequently, pre-placodal ectoderm is induced by addition of FGF2 and inhibition of BMP signaling. Self-guided organogenesis is then exploited to drive otic vesicles formation and eventually hair cell differentiation. Lineage differentiation is monitored by making use of an Atoh1-GFP engineered mESC line, in combination with immunostainings, flow cytometry and gene expression analysis. We show here that activation of Wnt signaling and inhibition of Notch by small molecule compounds are effective novel strategies to further increment hair cell generation. In parallel, we are tesing the efficacy of 3D organoid cultures to improve hair cell differentiation efficiency of somatic Lgr5+ supporting cells resident in the murine cochlea. These cells have been shown by genetic means to act as hair cell progenitors *in vivo*. Lgr5-GFP sorted cells are cultured using 3D methods. Similarly to the differentiation of mESCs, activation of Wnt signaling and inhibition of Notch in Lgr5+ progenitors results in increased hair cell numbers. Optimization of in vitro differentiation protocols to obtain high number of mature hair cells is ongoing and feasibility to upscale this approach for HTP screen is being evaluated.

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W1125

SOX10-POSITIVE CELLS EMERGE IN THE RAT PITUITARY GLAND FROM THE LATE EMBRYONIC STAGE AND SETTLE IN THE POSTNATAL PITUITARY AS STEM/PROGENITOR CELLS

Ueharu, Hiroki[1], Yoshida, Saishu[1,2], Kanno, Naoko[1], Nishimura, Naoto[1], Kato, Takako[2,3] and Kato, Yukio[1,4], [1]Division of Life Science, Graduate School of Agriculture, Meiji University, Kawasaki-Shi, Japan, [2]Organization for the Strategic Coordination of Research and Intellectual Property, Meiji University, Kawasaki-shi, Japan, [3]Institute of Reproduction and Endocrinology, Meiji University, Kawasaki-shi, Japan, [4]Department of Life Science, School of Agriculture, Meiji University, Kawasaki-shi, Japan

The pituitary gland is composed of the anterior, intermediate and posterior lobes. The adult stem/progenitor cells are essential for maintenance of biological function of the pituitary by supplying the endocrine cells. Our resent analysis of the rat pituitary had demonstrated that qualitative transition in the pituitary stem/progenitor cell niche occurs during the postnatal growth wave. The marked alteration was emergence of $S100b^+$ cells. On the other hand, analysis of quail-chick chimeras had revealed that neural crest cells invade the pituitary. Hitherto, we have interested in whether $S100b^+$ cells derive from neural crest cells, and analyzed by immunohistochemistry using anti-SOX10 antibody, although *Sox10* is expressed not only in the neural crest derived tissues but also in the oligodendrocytes and the embryonic otic vesicles. In addition, immunostaining for S100b, SOX2 (a stem/progenitor cell marker) and PROP1 (a pituitary specific factor) was performed. The preliminary data had been reported in ISSCR 2015. In the present study, further experiments were performed to improve the previous report. Results showed that $SOX10^+$ cells emerge in the rostral side of the late embryonic posterior lobe and then in the caudal side on postnatal day (P) 3. In the intermediate lobe by P15, they localized in the margin of the lobule structures and the marginal cell layer (MCL), a pituitary stem/progenitor cell niche. Some of them showed elongated shape possessing phalloidin-positive signals in leading end, revealing migration toward the MCL. In the postnatal anterior lobe, only a few SOX10-postiive cells were found. Further immunostaining showed that all $SOX10^+$ cells in the pituitary were positive for SOX2 but negative for S100b when they first detected. The number of $SOX10/S100b^+$ cells increased to reach 16% of $SOX10^+$ cells by P60. In the intermediate lobe, about 50% of $SOX10^+$ cells were $S100b^+$ on P15 and the proportion reached to 100% by P60. Remarkably, some of them were positive for PROP1, indicating that they acquired characteristics of the pituitary stem/progenitor cell. In conclusion, this study demonstrated that, after emergence in the posterior lobe, $SOX10^+$ cells alter to positive for $S100b^+$ and spread into the intermediate and anterior lobes taking part in adult pituitary stem/progenitor cells.

W1127

MIR205 CONTROLS MOUSE MAMMARY GLAND DEVELOPMENT THROUGH REGULATION OF WNT AND YAP STEM CELL SELF-RENEWAL SIGNALING

Lu, Yang[1], McManus, Michael[2] and Rosen, Jeffrey M.[1], [1]Baylor College of Medicine, Houston, TX, U.S., [2]University of California San Francisco, San Francisco, CA, U.S.

Mammary gland development is fueled by stem cell self-renewal and differentiation. However, the molecular mechanisms that regulate mammary stem cell behavior remain incomplete. Here we identify a miR205 regulatory network required for mammary gland morphogenesis and stem cell maintenance. MiR205 is the only miRNA reported to date whose deletion causes embryonic lethality. As a direct ΔNp63 target gene, miR205 is predominant-

ly expressed in the basal/stem cell enriched population of the postnatal mammary gland. Conditional deletion of miR205 in mammary epithelial cells severely impaired stem cell self-renewal and mammary repopulating potential both in the in vitro mammosphere formation assay and *in vivo* cell/tissue transplantation assay. miR205 null glands displayed significant decrease in the $K5^+$ basal population, discontinuous basement membrane and loss of collagen deposition. Furthermore, three unique basal cell subpopulations representing stem ($\Delta Np63^+/K5^-/\alpha SMA^-$), progenitor ($\Delta Np63^-/K5^+/\alpha SMA^-$) and differentiated states ($\Delta Np63^-/K5^-/\alpha SMA^+$) were first identified following miR205 deletion. Mechanistically, NKD1, PP2A-B56 and AMOT, which inhibit the Wnt stem cell self-renewal signaling pathway and the YAP oncoprotein, respectively, were identified as miR205 targets. The elevated expression of NKD1, PP2A-B56 and AMOT upon miR205 deletion results in the loss of nuclear YAP expression as well as the activation of non-canonical Wnt signaling mediated by alternative Ror2 receptor to inhibit canonical Wnt signaling mediated by β-catenin. Collectively these findings reveal an essential role of miR205 in balancing mammary stem cell self-renewal and differentiation.

Funding Source: NIH Grant CA16303-38

EPIDERMAL CELLS

W2001

A 3D IN VITRO MODEL OF WOUND HEALING RE-EPITHELIALIZATION PHASE: A TOOL TO STUDY HUMAN EPIDERMAL PRECURSORS AND CUTANEOUS REGENERATION

Deshayes, Nathalie, Bloas, Fabienne and **Paris, Maryline**, L'Oreal R&I, Aulnay-sous-Bois, France

Keratinocytes of the basal layer (the niche of epidermal stem cells and precursor cells) are involved in cutaneous regeneration process. Their function is to maintain tissue homeostasis in physiological condition and to fulfill skin repair in response to an external aggression such as a wound. In humans, clinical observations of the skin indicate that regenerative potential, preservation of tissue homeostasis and more widely skin functions are altered with ageing. In wound-healing, during re-epithelialization phase, epithelial precursor cells are highly requested and migrate gradually from the edges of the wound. The process is stimulated and controlled by growth factors produced by fibroblasts or by keratinocytes. The 3D epidermal reconstruction model called “standard model”, allows to study the vertical cutaneous regeneration process (proliferation/ differentiation), and appreciate keratinocyte function in preserving cutaneous homeostasis. In this study, we developed and characterized a “migration model” which introduces a step of keratinocyte migration such as the one observed in the phase of re-epithelialization in wound-healing process. We validated the added value of this model by demonstrating a pro-epithelializing effect of several compounds: an unsaturated fatty acid (Punicic Acid), a plant polyphenol (Ellagic Acid), an essential oil of flower and Ascorbic Acid. No effect of these compounds has been shown on standard model. The new 3D model allows the role of keratinocytes in different biological, biomechanical and environmental requests to be better understood, and brings a new tool to assess the impact of alteration of epidermal precursors on mechanisms involved in cutaneous regeneration.

W2003

ROLE OF EPIDERMAL BASEMENT MEMBRANE IN MAINTAINING EPIDERMAL STEM/ PROGENITOR CELLS

Iriyama, Shunsuke, Nishikawa, Saori, Hosoi, Junichi and Amano, Satoshi, Shiseido Global Innovation Center, Yokohama, Japan

Epidermal basement membrane (BM) plays important roles in epithelial proliferation and differentiation, contributing to epidermal homeostasis. However, the role of epidermal BM in maintaining epidermal stem/progenitor cells remains unclear. In this study, we investigated the age-dependent changes of epidermal stem/progenitor cells in sun-protected and sun-exposed human skin. We selected melanoma chondroitin sulfate proteoglycan (MCSP) and beta1 integrin as markers of stem/progenitor cells and analyzed MCSP and beta1 integrin-positive cells in epidermal basal layer by immunestaining. We found that MCSP and beta1 integrin-positive cells decreased earlier in sun-exposed skin than in sun-protected skin. A significant decrease in MCSP and beta1 integrin-positive cells was also observed during culture of human abdominal skin. Since we have previously reported that MMPs and heparanase are involved in deterioration of the epidermal BM in sun-exposed skin, we examined MMPs and heparanase in epidermis. MMPs and heparanase were markedly increased and the staining intensity of basement membrane components, such as laminin 332 or type VII collagen, was reduced with increasing culture time. Treatment with both N-hydroxy-2(R)-[[(4-methoxyphenyl)sulfonyl](3-picolyl)-amino]-3-methylbutanamide hydrochloride (CGS27023A; a MMP inhibitor) and 1-[4-(1H-Benzoimidazol-2-yl)phenyl]-3-[4-(1H-benzoimidazol-2-yl) phenyl] urea (BIPBIPU; a heparanase inhibitor) protected the BM structure and, intriguingly, also protected MCSP-positive cells in epidermis, suggesting a role of the BM in maintaining epidermal stem/progenitor cells. Overall, our data suggest that improving the integrity of BM structure by controlling enzymatic degradation may be an effective way of maintaining stem/progenitor cells.

W2005

OVEREXPRESSION OF sPLA2 IIA IN MICE EPIDERMIS DISRUPTS EPIDERMAL HOMEOSTASIS AND HAIR FOLLICLE STEM CELL DEPLETION WITH INCREASED DIFFERENTIATION

Sarate, Rahul MARUTI, Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Navi Mumbai, India

Epidermis is maintained throughout the adult life by stem cells that self-renew and generate progeny that undergo terminal differentiation. Secretory phospholipase A2 Group-IIA (sPLA2-IIA), a growth factor modulator, is involved in lipid biogenesis and deregulated in various cancers; however, its role in epidermal homeostasis and hair follicle stem cell regulation is yet to be explored. In the transgenic mice overexpressing sPLA2 IIA (K14-sPLA2 IIA mice), we investigated the hair follicle cycle at various postnatal day (PD) ages by performing histological analysis, tail whole mount and immunofluorescence staining by using the stem cells, proliferation and differentiation markers. Here we show a transgenic mice overexpressing $sPLA_2$-IIA (K14-$sPLA_2$-IIA) showed epidermal hyperplasia, increased differentiation, loss of ortho-parakeratotic organization and enlargement of sebaceous gland, infundibulum and junctional zone. Flow cytometry and immunofluorescence analysis showed depletion of hair follicle stem cell pool with the age. In addition, the BrdU pulse-chase was performed to investigate the slow-cycling Label Retaining Cells (LRCs) in the hair follicle stem cell niche which showed loss of long-term BrdU label retaining cells. Functional characterization showed reduction in colony forming efficiency in transgenic keratinocytes. Our study for the first time uncovered the role of sPLA2 IIA in epidermal stem cell regulation and homeostasis.

Funding Source: Indian Council Of Mesical Research,India

W2007

PEDF SHORT PEPTIDES FACILITATE FULL THICKNESS CUTANEOUS WOUND HEALING BY PROMOTING EPITHELIAL BASAL CELL AND HAIR FOLLICLE STEM CELL PROLIFERATION

Tsao, Yeou-Ping, Mackay Memorial Hospital, Taipei, Taiwan

A 44 amino acid pigment epithelium-derived factor (PEDF) derived short peptide was shown to facilitate corneal epithelial wound healing. In this study, the 44 amino acid peptide was shortened further into 20 amino acid and 29 amino acid short peptide, and we investigated their promoting effect on the healing of full thickness skin wounds. Peptides were delivered periodically by topical application to punch wounds. The wound healing speed was evaluated by measuring the reduction of wound areas at 4 and 7 days after injury. Histological analysis with Masson's trichrome staining was used to confirm epithelialization and dermal collagen deposition. Proliferation of epithelial basal cells was documented by BrdU incorporation. Hair follicle stem cells were identified by Lgr6 immunostaining. The results indicated that both the 20 amino acid and 29 amino acid short peptides significantly reduce the time needed for wound healing compared to the vehicle group. Histological analysis confirmed faster epithelial cell coverage of open wounds. PEDF peptide treatment also contributed to granulation tissue formation by increasing the fibroblast population and enhancing collagen deposition in dermis. Wound treated with PEDF peptides contained more proliferated basal cells in the epithelium. Moreover, hair follicle stem cells were also stimulated to proliferate by peptide exposure. In conclusion, we report the identification of two short peptides that can enhance the healing of full thickness skin wounds following topical application. The underlying mechanisms may involve activation of basal cell proliferation and mobilization of hair follicle stem cells.

EYE OR RETINAL CELLS

W2009

SUBRETINAL TRANSPLANTATION OF PHOTORECEPTOR PRECURSORS AND RETINAL PIGMENT EPITHELIUM DERIVED FROM HUMAN EMBRYONIC STEM CELLS IN RETINAL DEGENERATION RATS

Park, Un Chul[1], Park, Jung Hyun[2], Shin, Joo Young[1], Cho, In Hwan[1], Cho, Myung Soo[3] and Yu, Hyeong Gon[1], [1]Seoul National University College of Medicine, Seoul, Korea, South, [2]Seoul Paik Hospital, Seoul, Korea, South, [3]Jeil Pharmaceutical Co Ltd, Seoul, Korea, South

Degeneration and loss of photoreceptor or retinal pigment epithelium (RPE) is the major pathologic change in retinal degenerative diseases such as retinitis pigmentosa and age-related macular degeneration. We developed a defined, effective method to differentiate photoreceptor precursors and RPEs from human embryonic stem cells (hESCs) with a relatively high efficiency and short incubation time via the formation of cell clumps with neural structures, spherical neural masses (SNMs). SNMs are able to expand for long periods without loss of differentiation capability, to be stored easily and do not need for feeder cells. The differentiated photoreceptor precursors and RPEs were characterized with immunocytochemistry and reverse transcription-polymerase chain reaction. To investigate therapeutic potential of these differentiated RPE and photoreceptor precursors, we transplanted RPE or photoreceptor precursors into subretinal space of Roy-

al College Surgeon rats which were divided into 3 groups; a photoreceptor group (n=25) transplanted with photoreceptor precursors, an RPE group (n=25) transplanted with RPE cells, and a control group (n=26) transplanted only with culture media. After subretinal transplantation of photoreceptor precursors or RPEs, the transplanted cells were integrated into the retina and there was no evidence of severe inflammation or tumor formation until 24 weeks of observation. The thickness of outer nuclear layer was higher in the photoreceptor and the RPE groups compared with the control group both at 4 and 24 weeks after transplantation. The amplitudes of b-wave in the photoreceptor and RPE groups were higher compared with the control group at 4, 12, and 24 weeks after transplantation. These results suggest that subretinal transplantation of photoreceptor precursors or RPE was well tolerated and delayed retinal degeneration and the differentiated RPE and photoreceptor precursors from hESCs have potential to preserve visual function in the retinal degenerative rats.

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W2011

INDUCTION OF CORNEAL EPITHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS BY A METHOD BASED ON SPONTANEOUS OCULAR CELL DIFFERENTIATION

Hayashi, Ryuhei[1], Ishikawa, Yuki[1], Sasamoto, Yuzuru[1], Katori, Ryosuke[1], Ichikawa, Tatsuya[1], Nomura, Naoki[1], Soma, Takeshi[1], Kawasaki, Satoshi[1], Quantock, Andrew[2], Sekiguchi, Kiyotoshi[3], Tsujikawa, Motokazu[1] and Nishida, Kohji[1], [1]Osaka University Graduate School of Medicine, Suita, Japan, [2]Cardiff University, Cardiff, U.K., [3]Institute for Protein Research, Osaka University, Osaka, Japan

Human induced-pluripotent stem cell (hiPSC) has a great potential to treat severe ocular surface disorders such as corneal limbal stem cell deficiency caused by alkaline burn or Stevens-Johnson's syndrome. However, the method for the generation of functional corneal epithelial cells hasn't been established. Here, we demonstrate the generation of corneal epithelial cells and the isolation of functional corneal epithelial stem/progenitor cells from hiPSCs. hiPSCs were maintained in StemFit™ medium on Laminin-511 E8 fragment coated culture plates. The differentiation was initiated by changing the medium to 10% KSR containing differentiation medium (DM) and the cell were cultivated for 4 weeks at 37°C in 5% CO_2. After DM culture, the hiPSCs were cultivated in corneal differentiation medium (CDM) for 4 weeks. After CDM culture, the hiPSCs were cultivated in corneal epithelium maintenance medium (CEM) for 2 to 8 weeks (10-16 weeks in total). Then, the cells were harvested and subjected to FACS for isolating corneal epithelial progenitor cells. After 3-4 weeks differentiation, hiPSCs spontaneously formed colonies with multi-cellular zones. Each zone showed distinct ocular cell phenotypes including neuronal cells, retinal cells, neural crest cells and ocular surface epithelial cells co-expressing PAX6 and p63. By cell sorting, corneal epithelial progenitor cells were isolated as SSEA-4$^+$/ITGB4$^+$/TRA-1-60$^-$ cells. The hiPSC-derived corneal epithelial progenitor cells formed PAX6$^+$ colonies on 3T3 feeder layer. After stratification culture, the reconstructed corneal epithelial cells expressed corneal specific markers K12, PAX6 and MUC16. In conclusion, we have successfully induced corneal epithelial progenitor cells from hiPSCs and fabricated corneal epithelial cell sheets.

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W2013

RAPID AND EFFICIENT GENERATION OF hESC/HIPSC-DERIVED RETINAL PIGMENTED EPITHELIUM FOR THE STUDY OF RARE CAU.S.TIVE ALLELES IN AGE-RELATED MACULAR DEGENERATION

Michelet, Fabio, Genome Institute of Singapore, Singapore, Singapore

Age-related macular degeneration (AMD) is a common cause of irreversible vision loss among individuals over 65 years old in the developed world. Advanced AMD is estimated to affect 10 million patients world-wide, with the number of affected individuals reaching more than 150 million for earlier stages. AMD is characterized by damage to a tissue complex composed of the retinal pigment epithelium (RPE), Bruch's membrane and choriocapillaris. There are limited prevention options and no treatments available for the majority of AMD patients. Retinal pigment epithelium translocation surgeries have provided evidence that healthy RPE can support photoreceptor survival and visual function in human patients. Thus, understanding the pathophysiology of RPE degeneration will be crucial to prevent or cure AMD. Here we use hES/hIPSC derived RPE cells, obtained through a modified published protocol, to study the role of rare highly penetrant variants, with particular focus on the alternative complement pathway and the CFH protein. CFH is one of the main inhibitory factors of the alternative complement pathway and it is expressed by RPE cells where it regulates inflammatory processes caused by RPE senescence during ageing. Many rare CFH variants has been proved to be involved in AMD onset, suggesting a major role for inflammation in AMD. To shed more light on CFH activ-

ity in AMD, hES or hIPS cells will be genome edited via CRISPR/Cas9 to present homozygous alleles carrying different AMD-causative or -protective CFH variants. Each mutated cell line will be tested for the ability to enhance or weaken the alternative complement pathway response to pro-inflammatory stimuli.

W2015

MODELING AGE-RELATED MACULAR DEGENERATON USING PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS: NICOTINAMIDE REDUCES AMD-RELATED PROTEIN PRODUCTION

Saini, Janmeet S[1,2], Corneo, Barbara[3], Miller, Justine[2], Boles, Nathan[2], Kiehl, Thomas R[2], Blenkinsop, Timothy A[4], Stern, Jeffrey[2] and Temple, Sally[2], [1]University at Albany, Albany, NY, U.S., [2]Neural Stem Cell Institute, Rensselaer, NY, U.S., [3]Columbia University Medical Center, New York, NY, U.S., [4]Icahn School of Medicine at Mount Sinai, New York, NY, U.S.

Age-related Macular Degeneration (AMD) is the leading cause of visual impairment in the elderly, affecting the retinal pigment epithelium (RPE), a monolayer of cells essential for photoreceptor survival. AMD manifests in two forms: dry AMD is characterized by extracellular sub-retinal deposits termed drusen in the macular region of the retina, and wet AMD by the invasion of choroidal blood vessels into the central retina, accompanied by rapid vision loss. There are no disease-altering therapies for dry AMD nor that check its progression. Drusen are rich in components of the complement cascade, which contributes to the inflammatory environment and the upregulation of VEGF-A, promoting the transition from dry to wet AMD. The mechanism of drusen formation, including the cell types involved, is not fully understood, although studies have implicated RPE cells as these can produce several drusen proteins. In addition to environmental risk factors such as smoking, multiple genome-wide association studies for AMD have identified a significant association of a risk allele in the ARMS2 gene (SNP rs10490924) and a linked SNP (rs11200638) in the promoter region of the nearby HTRA1 gene. We tested whether hiPSC-derived RPE (hiPSC-RPE) from AMD and control patients could model early AMD pathogenesis and investigated the effect of the ARMS2/HTRA1 risk genotype on the production of AMD-associated proteins. We report higher expression of complement/inflammatory proteins in AMD hiPSC-RPE, more significant in the ARMS2/HTRA1 subset compared to control hiPSC-RPE, corroborating involvement of the complement cascade and inflammation in AMD pathogenesis. Using a panel of AMD biomarkers and candidate drug screens, we discovered that Nicotinamide (NAM) could significantly inhibit the production of drusen-associated proteins, repress inflammatory pathways and VEGF-A secretion. We found that NAM treatment of hiPSC-RPE markedly affects complement and extracellular matrix pathways, and significantly increases expression of Sirtuin-1, an NAD-dependent protein deacetylase implicated in chronic inflammation and aging. In conclusion, we created an in vitro model of AMD, using patient-specific/genotype-specific hiPSC-RPE, which sheds light on the mechanism of increased AMD risk, and identified NAM as a potential drug for AMD.

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W2017

GENERATION OF CORNEAL ENDOTHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Thakore-Shah, Kaushali, University of California Los Angeles, Los Angeles, CA, U.S.

The cornea is the transparent tissue in front of the eye. The pump and barrier function of the corneal endothelium (CE), the most posterior corneal layer, is crucial for maintaining corneal thickness and transparency. However, corneal endothelial cells (CECs) are severely limited in their proliferative ability. Decreased CEC density can result in edema, loss of corneal clarity, and ultimately, blindness. Developing an approach for in vitro generation of CECs would significantly increase the number of individuals whose vision could be restored. Thus, we set out to develop a protocol for derivation of CECs from human pluripotent stem cells (hPSC). Differentiation of hPSCs to CECs was carried out in two steps: 1) derivation of neural crest cells (NCC) from hPSCs, and 2) derivation of CECs from NCCs. The derivation of NCCs was performed using a published protocol, with the modification that hPSCs were cultured on either Matrigel or human recombinant Laminin-521 matrix. Efficiency of derivation was measured by flow cytometry for P75NTR, a marker of NCCs as well as proliferative CECs. P75NTR expressing NCCs as well as unsorted NCCs were then seeded onto Laminin-521 coated wells and induced to differentiate towards CEC by addition of growth factors. Expression of P75NTR and three additional CEC markers was measured by qPCR. We found that NCCs derived on Matrigel (Mat-NCCs) had a 1.86 fold increase in the number of P75NTR expressing cells compared to NCCs derived on Laminin521 (Lam-NCCs). P75NTR expression was higher in CECs derived from Lam-NCCs compared to Mat-NCCs. As expected, P75NTR expression was also higher in sorted compared to unsorted cells. The three additional CEC markers tested were expressed at higher levels in CECs derived from Mat-NCCs compared to Lam-NCCs. These markers were also expressed at higher levels in unsorted cells compared to P75NTR sorted cells. These results demonstrate that it is feasible to derive CEC like cells from hPSCs. Future

experiments will test protein expression of CEC markers and barrier function of the CEC monolayer.

NEURAL CELLS

W2019

LUNATIC FRINGE IS A SELECTIVE MARKER OF HIPPOCAMPAL NEURAL STEM CELLS, NECESSARY FOR THEIR MAINTENANCE

Semerci, Fatih[1,2], Choi, William Tin-Shing[1,2], Bajic, Aleksandar[2,3], Thakkar, Aarohi[2,3], Encinas, Juan Manuel[2,4], Depreux, Frederic[5], Segil, Neil[6], Groves, Andrew Kelton[1,7] and Maletic-Savatic, Mirjana[1,3], [1]Graduate Program in Developmental Biology Baylor College of Medicine, Houston, TX, U.S., [2]Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, TX, U.S., [3]Department of Pediatrics Baylor College of Medicine, Houston, TX, U.S., [4]The Basque Foundation for Science, Bizkaia, Spain, [5]Rosalind Franklin University of Medicine and Science, Chicago, IL, U.S., [6]Broad CIRM Center, Keck School of Medicine, University of Southern California, Los Angeles, CA, U.S., [7]Department of Neuroscience Baylor College of Medicine, Houston, TX, U.S.

Adult hippocampal neurogenic niche is confined to a spatially restricted area where stem cells cannot propagate indefinitely. However, the mechanisms that control the amount of neural stem cell (NSC) proliferation are not known. The optimization of the NSC proliferation could be accomplished if there is a feedback communication from the progeny. A known mechanism for such cell-cell communication involves the Notch pathway. Thus, we hypothesized that amplifying neuroprogenitors (ANPs), the first progeny of NSC in direct contact with the primary NSCs, may participate in regulating the NSC quiescence vs. active proliferation. Indeed, the key components of the Notch pathway are expressed in a cell-type specific manner in the neurogenic niche: Notch receptor is expressed in the NSCs, while Notch ligands, Jag1and Delta1, are expressed in ANPs and granule cells, respectively. Moreover, utilizing a comprehensive bioinformatics query, we discovered that Lunatic Fringe (Lfng), a key modifier of Notch receptor, is selectively expressed in NSCs. Further characterization of the Lfng-eGFP expressing cells confirmed that they are NSCs, as they gave rise to neurogenic progeny, decreased in number with increased age, and responded to the neurogenic stimuli known to increase NSC proliferation. Using this mouse, we were able to gain first insight into the stimulus-dependent molecular responses of NSCs to running and electroconvulsive shock. Functionally, removal of Lfng resulted in increased NSC proliferation followed by a decrease in NSC number and neuron production, suggesting the necessity of Lfng for proper NSC function and maintenance. On the other hand, constitutive heterozygote deletion of Notch ligand Jag1 resulted in increased NSC and ANP cell cycle duration with no change in their absolute numbers. These results strongly implicate Notch signaling in the control of NSCs and their progeny, and suggest a potential communication and feedback mechanism between the NSCs and their progeny to fine tune adult neurogenesis. Overall, we present Lfng-eGFP and Lfng-CreERT2 as new mouse models that permit comprehensive and specific studies of NSC properties and propose a novel mechanism for preservation of NSCs that may operate in the adult hippocampal neurogenic niche.

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W2023

IN VIVO RIBOTAG ANALYSIS REVEALS TRANSLATIONAL CONTROL DURING NEURAL STEM CELL DIFFERENTIATION

Baser, Avni, Llorens Bobadilla, Enric, Fischer, Bernd and Martin-Villalba, Ana, German Cancer Research Center (DKFZ), Heidelberg, Germany

It is well known that epigenetic modifications as well as regulation of transcription factors are closely associated to cellular identity, highly distinguishing between the pluripotent and committed status of cells. However, little is known about the importance of changes at the posttranscriptional level. Recent studies on hematopoietic stem cells emphasize that protein translation is tightly controlled in the stem cell compartment and changes in both directions severely deregulate the system and deplete the stem cell pool. Highly polarized cells including neurons are characterized by a significant degree of post-transcriptional regulation. Here we look at the gradual changes of the translatome while differentiation of subventricular zone neural stem cells (NSCs) to olfactory bulb interneurons. Using Ribotag mouse lines which express a HA-tagged variant of a ribosomal protein at different stages along the differentiation path of NSCs, we profiled both, the total mRNA as well as the ribosome-bound mRNA in vivo. By this approach, we show that total mRNA is an inaccurate measure for protein levels for a significant part of the genome. Several genes including key transcription factors show comparable levels in their mRNA expression over the investigated cell populations, their action however is limited to certain stages by actual translation into proteins. The degree of this uncoupling increases with differentiation and maturation of the cells, which is in line with the literature describing local and temporal protein synthesis in mature neurons. However, the fact that trans-

lation is already specifically regulated in NSCs indicates that these mechanisms already exist very early in cells which will later become neurons. This study adds a novel layer of complexity to the process of neuronal differentiation. Identification of upstream regulators as well as associated mechanisms will be key to further complete this picture.

W2025

THE ROLE OF BREAST CARCINOMA AMPLIFIED SEQUENCE 2 (BCAS2) IN ADULT NEUROGENESIS

Chen, Show-Li, National Taiwan University, Taipei, Taiwan

Adult neurogenesis occurs in the dentate gyrus (DG) throughout life; the disruption of neurogenesis would cause neurodegenerative diseases. Breast carcinoma amplified sequence 2 (BCAS2) is a lethal gene in prenatal. Now we generated conditional knock out mice to deplete BCAS2 expression (cKO) in hippocampus and cortex to investigate BCAS2 role in adult neurogenesis. BCAS2 cKO mice showed microcephaly-like phenotype with the reduced volume of DG and the decreased thickness of cortex those resulted in low learning and fear memory by Morris water maze analysis and passive avoidance respectively. The Golgi staining revealed the less dendrite length, dendrite order, the short distance from soma, the less spine density of distal region; those could be further confirmed the disordered MAP2-positive dendrites by confocal analysis. Moreover, the cKO displayed short dendrite length by dendrite marker-DCX; and slow dendrite growth by BrdU incorporation in postnatal-born neurons. To further examine the mechanism of BCAS2 role in adult neurogenesis, exon array showed the b-catenin be a target of BCAS2 splicing regulation. b-catenin is well documented to regulate dendrite growth and morphology. In primary neuron culture showed that b-catenin could restore the dendrite growth in the absence of BCAS2 that further supported from N2A cells by fiolopodia assay. In summary, we provide the BCAS2 cKO mice revealing the impairment dendrite growth with the defect cognition that can be a microcephaly animal model for future therapeutic target.

W2027

TRANSPLANTATION OF CONTACTIN2+ MIDBRAIN DOPAMINERGIC PROGENITOR CELLS DERIVED FROM HUMAN EMBRYONIC STEM CELLS INTO THE RAT MODEL OF PARKINSON'S DISEASE

Dolatyar, Banafsheh[1,2], Fathi, Ali[2], Sahraei, Seyedeh Saeideh[2], Hosseini Salekdeh, Ghasem[3,4], Baharvand, Hossein[1,2] and Javan, Mohammad[2,5], [1]Department of Developmental Biology, University of Science and Culture, ACECR,Tehran,Iran., Tehran, Iran, [2]Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran., Tehran, Iran, [3]Department of Systems Biology, Agricultural Biotechnology Research Institute of Iran,Karaj, Iran., Karaj, Iran, [4]Department of Molecular Systems Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR,Tehran.Iran., Tehran, Iran, [5]Department of Physiology, Faculty of Medical Sciences, Tarbiat Modarres University,Tehran, Iran., Tehran, Iran

Parkinson's disease is a progressive neurological disorder which results from degeneration of dopaminergic neurons in the substantia nigra. To overcome disease symptoms there are custom medications for balancing dopamine neurotransmitter in the striatum and using deep brain stimulation for relaxing the muscle rigidity. The only cure for these types of movement disorders is cell replacement therapy by fetal midbrain cells or pluripotent sources of cells. In the present study we used a defined method to generate human dopaminergic (DA) progenitor cells from human embryonic stem cells that is based on dual SMAD inhibition and embryoid body formation. We confirmed the expression of DA specific marker genes in the mRNA and protein level. In our protocol differentiated DA progenitor cells purified by fluorescence-activated cell sorter using novel cell surface proteins based on our unpublished proteomics data and characterized with their differentiation capacity to mature dopaminergic neurons and transplantation to animal model of the Parkinson's disease. The results showed that human DA progenitor cells expressed midbrain DA progenitor markers and can be successfully differentiate into tyrosine hydroxylase positive mature neurons. Flow cytometry analysis of these cells indicated that DA progenitor cells on day 12 of differentiation expressed contactin2, as a novel surface protein. After transplantation of contactin2^{+} cells and unsorted human DA progenitors into the 6-OHDA-lesioned rats the histological results and behavioral results of motor performance tests showed that sorted cells can survive and significantly improve motor behavior in comparison to the control cells. Our method is favorable in terms

of efficiency and indicates promise for development of cell-based therapies in Parkinson's disease.

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W2029

NANOFIBROUS SCAFFOLD-MEDIATED REST KNOCKDOWN INFLUENCE ON DIFFERENTIATION OF HUMAN FETAL NEURAL STEM CELLS

Fan, Yiping[1], Diao, DJ[2], Lee, Jh[2], Choolani, Mahesh[3], Chew, Sing Yian[2] and Chan, Jerry[1], [1]KK Women's and Children's Hospital, Singapore, Singapore, [2]NTU, Singapore, Singapore, [3]National University of Singapore, Singapore, Singapore

Controlled differentiation of neural stem cells into relevant cells for therapy represents a promising treatment strategy for neurodegenerative disorders. However, directed differentiation remains a challenge and further understanding of the developmental differentiation pathways is necessary. REST is downregulated during neurogenic differentiation in development. Here, the influence of REST knock down (kd) on differentiation of human fetal neural stem cells (hfNSCs) was examined. hfNSC were derived in serum-free media from early mid-trimester clinical waste after informed consent (15-21 wks gestation). Tissues were minced and dissociated enzymatically (n=3). Neurospheres at passage 1 were subcultured and placed into one of 3 different media (DM0 containing B27, bFGF, hEGF and LIF, DM1 containing IL-1β and DM2 containing BDNF, dopamine, forskolin and retinoic acid) over nanofibrous meshes loaded with REST siRNA or scrambled siRNA (neg control). RNA was extracted on D5 to determine RESTkd and ICC for MAP2ab, tyrosine hydroxylase (TH), nestin, PDGFRα, GFAP were performed on D7.

REST kd was observed at D5 in all 3 media. Scaffold mediated RESTkd resulted in lower MAP2ab & TH in DM0 and DM1 and a higher expression in DM2 at D5. Under the condition of DM0, RESTkd resulted in a upregulation of GFAP (81.5±3.8 vs 50.2±6.0, $p=0.01$), PDGFRα (83.8±5.0 vs 0.8±0.8, $p<0.001$) and a downregulation of nestin (0±0 vs 9.6±2, $p<0.01$) by ICC. There is minimal differences for MAP2ab (13.0±2.7 vs 15.8±1.5, $p=0.44$) and TH (81.0±3.8 vs 73.0±9.1, $p=0.47$). In DM1, RESTkd did not affect differentiation, although a trend was observed for MAP2ab (1.1±0.7 vs 3.1±1.1) and TH (65.3±16.3 vs 39.1±3.6) after kd. In DM2, REST kd resulted in a reduced expression of PDGFRα (22.7±4.0 vs 55.6±7.8, $p=0.01$) and TH (65.1±6.4 vs 86.0±3.2, $p=0.09$). We demonstrated that REST kd affects the differentiation of hfNSCs, particularly into oligodendrocytes. More needs to be done to further determine the effect of REST silencing in combination with the nanofiber topography. These comparisons can potentially shed more light on the differentiating potential of hfNSCs, allowing a well-informed choice of cell source for directed differentiation aimed for cellular therapeutics and drug testing.

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W2031

MGE-DERIVED NSC TRANSPLANTATION INTO THE HIPPOCAMPUS INHIBITS POST-STATUS EPILEPTICUS SPONTANEOUS RECURRENT SEIZURES AND MEMORY DYSFUNCTION ON A LONG-TERM BASIS

Hattiangady, Bharathi[1,2], Upadhya, Dinesh[1,2], Shuai, Bing[1,2] and Shetty, Ashok K.[1,2], [1]Institute for Regenerative Medicine, Texas A&M HSC College of Medicine, Temple, TX, U.S., [2]Olin E. Teague Veterans' Medical Center, Central Texas VA Health Care System, Temple, TX, U.S.

An episode of status epilepticus (SE) can lead to temporal lobe epilepsy (TLE) in a sizeable fraction of SE patients. The attributes of TLE comprise spontaneous recurrent seizures (SRS) and memory dysfunction, which are linked with substantial loss of principal and GABA-ergic interneurons and altered neurogenesis in the hippocampus. We tested whether grafting of chlorodeoxyuridine (CldU) labeled neural stem cells (NSCs) derived from the embryonic (E13.5) medial ganglionic eminence (MGE) into the hippocampus early after SE would modulate the evolution of SE into TLE. The choice of MGE-NSCs is based on their ability to generate GABA-ergic interneurons and astrocytes secreting GDNF. We induced SE in young adult F344 rats via graded intraperitoneal injections of kainic acid, terminated acute seizures two-hours after SE by an injection of diazepam, and grafted MGE-NSCs into the hippocampus a week later (3 grafts/hippocampus, 100,000 live cells/graft, SE-grafted animals). Another group of rats that underwent SE was maintained as controls (SE-alone animals). Evaluation of spatial learning and memory retrieval function in the 6th month after SE through a water maze test revealed spatial memory retrieval dysfunction in SE-alone animals but not in SE-grafted animals. Analyses of SRS through continuous video-electroencephalographic recordings taken in the 8th month after SE revealed that, in comparison to SE-alone animals, SE-grafted animals display dramatic reductions in the frequency and intensity of SRS. The reductions were 87% for the frequency of SRS, 30% for the duration of individual SRS, and 90% for the percentage of time spent in seizure activity. Graft-derived cells survived well and displayed pervasive migration in the host hippocampus. A significant fraction of graft-derived cells also migrated into the neurogenic subgranular zone. The hippocampus of SE-grafted animals in addition displayed greater levels of normal neurogenesis, higher numbers of host neuropeptide Y and parvalbumin expressing interneurons and

reduced aberrant neurogenesis than the hippocampus of SE-alone animals. Phenotypic analyses of graft-derived cells are in progress. Thus, MGE-NSC grafting intervention into the hippocampus early after SE is effective for greatly restraining SRS and memory dysfunction on a long-term basis.

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W2033

USING MESC-DERIVED NEURONS GROWN ON MULTI-ELECTRODE ARRAYS AS AN IN-VITRO BIOASSAY FOR THE DETECTION OF CLOSTRIDIUM BOTULINUM NEUROTOXINS

Jenkinson, Stephen P.[1,2], Avondet, Marc-André[2], Grandgirard, Denis[1], Heidemann, Martina[3], Tscherter, Anne[3] and Leib, Stephen L.[1], [1]Neuroinfectiology Laboratory, Institute for Infectious Diseases (IFIK), University of Bern, Bern, Switzerland, [2]Biology Division, The Spiez Laboratory, Swiss Federal Office for Civil Protection, Spiez, Switzerland, [3]Department of Physiology, University of Bern, Bern, Switzerland

Clostridium botulinum neurotoxins (BoNTs) are the most poisonous naturally occurring protein toxins known to mankind, the toxicity range in humans starting as low as 0.3 ng/kg when administered intravenously. All BoNT serotypes (A-G) consist of two subunits, a ≈ 100-kDa heavy chain (HC) and a ≈ 50-kDa light chain (LC), which are linked by a disulphide bond. Upon binding to specific membrane receptors on the neurolemma of neurons, the HC translocates the LC over the membrane where it exerts its cleaving activity against SNARE proteins, thus inhibiting neurotransmitter release. Currently, the potency of biologically active BoNT is monitored using the murine LD_{50}-assay. Using differentiated neurons from mouse embryonic stem cells (mESC), we intend to develop an in-vitro assay capable of detecting BoNT activity using electrophysiological recording techniques. mESC are differentiated towards neurons by embryoid body (EB) formation. After 7 days the EBs are dissociated and the cells are cultured for 3 weeks on multi-electrode arrays (MEAs) allowing the extracellular recording of spontaneous neuronal activity. The cultures are then treated with different concentrations of BoNT serotype A (BoNT/A) and spontaneous network bursts, evoked through synaptic transmission, as well as total network activity are recorded 6 hours after exposure to the toxin. 48 cultures from 6 independent experiments were assessed. 23 cultures were used to quantify the effect of the toxin on synapses and treated with either 25ng/ml or 2.5ng/ml BoNT/A. 25 cultures served as control and did not receive any treatment. Exposure to BoNT/A for 6 hours resulted in a significant decrease of the burst rate for toxin concentrations of 25ng/ml (49.5 ± 27.2; $p<0.0005$; n=7) and 2.5ng/ml (66.7 ± 42.5; $p<0.005$; n=16) compared to untreated cultures (100 ± 20.3; n=25). A significant decrease in the total network activity was observed for toxin concentrations of 25ng/ml (45.5 ± 48.0; $p<0.005$; n=7) and 2.5ng/ml (78.0 ± 70.9; $p<0.05$; n=16) compared to untreated cultures (100 ± 31.9; n=25) The present assay detects toxin activity of BoNT/A. Thus proof of principle has been achieved. Given sufficient robustness and a further increase in sensitivity to detect BoNT/A, this assay may replace the murine LD50-assay in e.g. the batch control of pharmaceutical products.

W2035

SINGLE CELL RNA SEQUENCING RESOLVES DISTINCT LMX1A EXPRESSING NEURAL LINEAGES IN THE DEVELOPING VENTRAL MIDBRAIN

Kee, Nigel[1], Dahl, Lina[1], Volakakis, Nickolaos[1], Kirby, Agnete[2], Nolbrant, Sara[3], Joomardi, Eliza[1], Gillberg, Linda[1], Bjöklund, Åsa[4], Storvall, Helena[1], Sandberg, Rickard[1], Parmar, Malin[3] and Perlmann, Thomas[5], [1]LICR, Stockholm, Sweden, [2]Wallenberg Neuroscience Center, Lund, Sweden, [3]Lund University, Lund, Sweden, [4]SciLIfe Laboratories, Stockholm, Sweden, [5]Department of Cell and Molecular Biology (CMB), Karolinska Institutet, Stockholm, Sweden

Neural stem cells, in response to developmental cues, form a stringently patterned neural tube that in time generates the immense neural diversity and anatomical intricacy found in the adult CNS. Dopaminergic (DA) neurons arising in the ventral midbrain (vMB) have been extensively studied, in part due to the prospect of engineering stem cells into dopamine neurons for cell replacement in Parkinson's disease. However, classical approaches used to interrogate vMB neuron development and diversity are hamstrung by the mixtures of multiple neural lineages and maturation stages found in whole tissue dissections. To circumvent this challenge, we have utilized single-cell RNA-sequencing to reconstruct the differentiation of neuronal lineages in the mouse vMB between embryonic day 10.5 and 13.5. This comprehensive and unbiased approach provided a robust genome-wide reconstruction of how neural stem cells expressing the transcription factor Lmx1a transition into postmitotic differentiating neurons. In vivo validation of one distinct gene signature has uncovered an unexpected but considerable similarity between developing DA neurons, and more rostrally developing glutamatergic neurons of the Subthalamic Nucleus. Importantly, this similarity was preserved also in the developing human vMB, highlighting the significant challenge of generating pure cultures of DA cells for use in transplantation settings. Thus, single-cell RNA-seq can successfully interrogate fine lineage diversity, revealing developmental insights pertinent to stem cell engineering.

W2037

REGION-SPECIFIC NEURAL STEM CELL LINEAGES REVEALED BY SINGLE-CELL RNA-SEQ FROM HUMAN EMBRYONIC STEM CELLS

Levi, Boaz[1], Krostag, Anne-Rachel[1], Ku, Sherman[1], Martinez, Refugio[1], Menon, Vilas[1], Mich, John Kenneth[1], Yao, Zizhen[1], Mulholland, Heather[1], Bort, Susan[1], Fuqua, Maggie[1], Furchtgott, Leon[2], Gregor, Ben W.[1], Hodge, Rebecca D.[1], Jayabalu, Anu[1], May, Ryan C.[1], Nelson, Angelique[1], Ngo, N. Kiet[1], Shapovalova, Nadiya V.[1], Shehata, Soraya I.[1], Tait, Leah J.[1], Thomsen, Elliot[1], Ye, Chaoyang[1], Glass, Ian[3], Kaykas, Ajamete[1], Yao, Shuyuan[1], Phillips, John W.[1], Thompson, Carol[1], Grimley, Joshua S.[1], Wang, Yanling[1] and Ramanathan, Sharad[2], [1]Allen Institute for Brain Science, Seattle, WA, U.S., [2]Harvard University, Cambridge, MA, U.S., [3]University of Washington, Seattle Children's Hospital, Seattle, WA, U.S.

The human brain is a profoundly complex organ composed of billions of neurons, representing tens to hundreds of interconnected cell types, and elegantly assembled into an organ capable of consciousness, reason, and personality. The functions of human brain are orchestrated through highly inter- or intra-connected regions that are developmentally defined as the forebrain, the midbrain and the hindbrain. Here, we present a human brain cell-type lineage tree through analysis of differentiated human embryonic stem cells (hESCs), and comparison with primary *in vivo* samples. Progenitors and neurons were transcriptionally profiled at single-cell resolution throughout the differentiation time-course. The regional identities of the stem cell-derived cells – including developing cortex, ganglia eminences, and mid/hindbrain at early to mid-gestation – were validated by mapping the gene expression data to existing atlases and primary human fetal tissue. An inferred lineage tree was derived from the single-cell gene expression data and was consistent with clonal fate-mapping analyses. This work highlights a forebrain and mid/hindbrain lineage branch and the earliest molecular steps in establishing region-specific neural lineages. In summary, through comprehensive single-cell transcriptomic profiling, we present a hESC-derived lineage tree of multiple brain regions and demonstrate its similarity to progenitors and neurons found in primary tissues.

W2039

MUTATED Nox3 CAUSES EXCESSIVE CEREBELLAR GRANULE CELLS PROGENITORS PROLIFERATION

Mazzonetto, Patricia Camacho[1], Ariza, Carolina Batista[2], Sousa, Tiago Antonio[3], Ko, Gui Mi[4], Galindo, Layla Testa[1], Massironi, Silvia Maria Gomes[3] and Porcionatto, Marimélia[4], [1]Universidade Federal de São Paulo, São Paulo, Brazil, [2]Universidade Estadual de Londrina, Londrina, Brazil, [3]Universidade de São Paulo, São Paulo, Brazil, [4]Universidade Federal de São Paulo, São Paulo, Brazil

The cerebellum is the region in the central nervous system responsible for motor coordination and balance. It is an excellent model to study central nervous system development as it gathers important cellular events such as proliferation, migration and differentiation of neuronal precursors. A mouse lineage presenting lack of motor coordination was selected in an ENU mutagenesis project and was named equilibrio (eqlb; balance, in Portuguese). These mice have increased proliferation of cerebellar granule neuron progenitors during early postnatal development (up to 15 days after birth), as well as disorganized Purkinje cell layer. Genetic mapping by polymorphic microsatellite analysis and Next Generation Sequencing, identified the mutation as an A>T transversion at position 190 (transcribed sequence) in Nox3 gene, located on mouse chromosome 17. The mutation causes the substitution of Asp by Tyr at position 64 of the protein, generating a putative phosphorylation site inexistent in wild type NOX3. Furthermore, eqlb mice (renamed $Nox3^{N64Y}$) has higher expression of Nox1, indicating a possible compensation caused by the expression of mutated Nox3. NOX3 is a NADPH oxidase belonging to a family of transmembrane proteins which main function is to reduce molecular oxygen to form reactive oxygen species (ROS). ROS produced by NOX can modulate cell signaling in various physiological processes including proliferation. Because SHH (Sonic Hedgehog), secreted by Purkinje cells, is the main mitogen for neuronal cell precursors in the developing cerebellum, our goal was study the role of NOX and ROS in the control of proliferation of granule cell progenitors and cerebellar neural stem cells by SHH signaling pathway. Nox3N64Y mice cerebellar neural stem cell and neuronal progenitors produce high levels of ROS after 7 days in culture. Moreover, Nox3N64Y cells show increased expression of Gli1, Gli2, and Rb1 (Retinoblastoma 1), and decreased expression of Cdkn2A (cyclin-dependent kinase inhibitor 2A). Our hypothesis is that SHH is the main pathway affected by mutated Nox3 and ROS has an important role for the increased proliferation in Nox3N64Y cerebellar neural stem cell and neuronal progenitors.

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W2041

HUMAN LEUKOCYTE ANTIGEN (HLA)-MATCHED CELL TRANSPLANTATION FOR PARKINSON'S DISEASE WITH INDUCED PLURIPOTENT STEM CELLS

Morizane, Asuka[1], Kikuchi, Tetsuhiro[1], Doi, Daisuke[2], Okita, Keisuke[1], Mizuma, Hiroshi[3], Takara, Sayuki[3], Onoe, Hirotaka[3], Hayashi, Takuya[3], Shiina, Takashi[4], Ishigaki, Hirohito[5], Ogasawara, Kazumasa[5], Yamanaka, Shinya[1] and Takahashi, Jun[1], [1]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [2]Center for IPS Cell Res & Application, Kyoto city, Japan, [3]RIKEN Center for Life Science Technologies, Kobe, Japan, [4]Tokai University, Kanagawa, Japan, [5]Shiga University of Medical Science, Shiga, Japan

One of the advantages of induced pluripotent stem cells (iPSCs) for regenerative medicine is the possibility of autologous cell transplantation. The autologous strategy, however, need long time and high cost to prepare the donor cells for each patient. Human leukocyte antigen (HLA)-matched allogeneic transplantation would be more practical in the clinical situation. For this purpose, iPSCs from healthy volunteers that have HLA homozygotes (homo-iPSCs) will be stocked in our iPSC stock project. To confirm the efficacy of this strategy, we investigated the monkeys that received the major histocompatibility complex (MHC)-matched allogeneic dopamine neural cell grafts differentiated from the MHC-homo monkey iPSCs. The dopamine neural cells were differentiated through a protocol that included the dual SMAD inhibition strategy. The differentiated dopamine neurons were transplanted to the brains of both MHC-matched and –mismatched animals. After transplantation, positron emission tomography (PET) images of (S)-^{11}C-KTP-Me and ^{11}C-PK11195 were taken periodically for the estimation of the host brain's inflammation. Finally histological observations were performed. The MHC-matched allogeneic grafts showed less inflammatory response and better survival of the grafted dopamine neurons than the MHC-mismatched grafts. In some MHC-mismatched animals we observed accumulation of lymphocytes and activated microglia in the graft. Those animals with post-operative inflammation had shown higher reactivity in the pre-operative mixed lymphocyte reaction between the donor and the hosts. The PET scan also detected the post-operative inflammation in the live animals. The brain has been considered as immunologically privileged site. However, increasing evidence has shown the brain has an immune response after cell transplantation. Our results showed this response is less in MHC-matched transplantation than –mismatched one. Although immune suppression was not performed in this study at all, in the clinical situation, immune suppressive drugs might work for controlling the immune response. Preoperative MLR and postoperative PET imaging study will be helpful to monitor the immune response and to adjust the immunosuppressive strategies.

W2043

RESCUE OF MYELINATION DEFECTS IN PELIZAEUS MERZBACHER DISEASE PATIENT-DERIVED IPS CELLS BY IRON-CHELATION THERAPY

Nobuta, Hiroko[1], Yang, Nan[2], Ng, Yi Han[2], Marro, Samuele[2], Zhao, Chao[3], Franklin, Robin[3], Kriegstein, Arnold R.[1,4], Rowitch, David[1,5] and Wernig, Marius[2,6], [1]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California San Francisco, San Francisco, CA, U.S., [2]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, U.S., [3]Wellcome Trust-MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, U.K., [4]Department of Neurology, University of California, San Francisco, San Francisco, CA, U.S., [5]Department of Pediatrics, University of California, San Francisco, San Francisco, CA, U.S., [6]Stanford University, Stanford, CA, U.S.

Pelizaeus Merzbacher Disease (PMD) is a pediatric onset, progressive central nervous system disorder affecting oligodendrocytes, the myelinating glial cells. Causative mutations in PLP1 gene identified in patients afford us to study mechanisms underlying the disease. We demonstrate using patient-derived iPS cells, targeted gene correction, pharmacological intervention, human brain slice culture model, and mouse in vivo transplantation model that phenotype of PLP1 mutations causing severe, early-onset PMD can be rescued by controlling iron-mediated toxicity. In iPS cell-based disease modeling, the patient iPS cells produced reduced number of oligodendrocytes associated with increased apoptotic cell death, maturational arrest, and morphological abnormality, all of which were rescued in the gene-corrected isogenic wildtype cells. The gene-corrected cells were capable of forming compact myelin sheaths *in vivo* in transplanted mouse brains. When cells were plated on human brain slice culture, they differentiated to mature oligodendrocytes with similar morphology to endogenous human oligodendrocytes. Interventional screenings inferred from rodent PMD models identified overt iron-mediated toxicity as a potential disease mediator. When the iron levels were reduced with iron-binding protein apo-transferrin or iron-chelating drug deferoxamine, the patient-derived cells regained capacity to differentiate into mature oligodendrocytes. The rescued disease phenotype was associated with reduced oxidative stress and apoptotic cell death. Mechanistically, the mutant cells showed hypersensitivity to iron and accumulated lipid specific reactive oxygen species. Counteracting lipid oxidative stress with lipophilic antioxidants

but not hydrophilic antioxidants was effective in rescuing the mutant phenotype similar to iron chelation. Thus, PLP1 mutation renders intolerable iron-mediated lipid oxidative stress in oligodendrocytes, and iron chelation or lipophilic antioxidants are potential therapeutic interventions that necessitate further validations.

W2045

REGULATION OF MAMMALIAN HINDBRAIN NEURAL STEM/PROGENITOR POOL MAINTENANCE BY B-CATENIN AND SUPPRESSOR OF FUSED (SUFU)

Pan, Wenqi[1], Hor, Catherine Hong Huan[2], Hui, Chi-chung[3] and Sham, Mai Har[2], [1]the University of Hong Kong, Hong Kong, Hong Kong, [2]The University of Hong Kong, Hong Kong, Hong Kong, [3]SickKids, The Hospital for Sick Children Research Institute, Toronto, ON, Canada

Wnt/β-catenin signaling is well characterized for the proliferation and differentiation control of stem and progenitor cells in various systems. However the potential regulators and molecular mechanisms for the pathway during embryonic central nervous system development is not very clear. In this study, we have found that β-catenin is playing an essential role in the maintenance of developing hindbrain neural progenitor pool using β-catenin mutant mouse models. Conditional knock-out of β-catenin in mouse developing rhombomere 4 (b-cat-r4LoF) leads to an expansion of Tuj1+ population at the expense of Sox2+ neural progenitors. Moreover, Tuj1+ neurons are identified at the ventricular side of the neuroepithelium in b-cat-r4CKO embryos. The expressions of dorsal-ventral progenitor patterning genes including Nkx2.2, Nkx6.1, Pax6 and Pax7 seem unaffected in b-cat-r4LOF, although the neural progenitors are not well aligned along the ventricular layer. In contrast, conditional stabilization of β-catenin in r4 (b-cat-r4GoF) displays a reversal of Sox2/Tuj1 population imbalance. Interestingly, progenitor patterning genes such as Nkx2.2, Pax6 are not expressed in b-cat-r4GOF rhombomere, while Nkx6.1 and *Pax7* remain expressed. What is more, the suppression of Pax6 by stabilized β-catenin seems cell-autonomous. These data suggest that a proper level of β-catenin is required for progenitor pool maintenance and patterning gene expressions in developing hindbrain. In addition, we identified Suppressor of fused (Sufu), a negative regulator of Hedgehog/Gli signaling pathway, as a potential modulator of *β-catenin* in r4, since loss of *Sufu* (Sufu-r4CKO) will lead to a rise in the protein amount and nuclear localization of active β-catenin. Sufu-r4CKO shows an expansion of Sox2+ neural progenitor pool together with a loss of Tuj1+ neurons. In *Sufu; β-catenin* compound loss-of-function mutant, the imbalance between progenitor/neurons is alleviated compared with Sufu-r4CKO. What is more, when Sufu is removed in the b-cat-r4GOF mutant, the progenitor proportion is further enhanced against neurons. In summary, our data indicate that Sufu may be a possible regulator of β-catenin signaling in controlling neural stem cell/ progenitor pool maintenance during mammalian hindbrain development.

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W2047

OPTOGENETIC INVESTIGATION OF EFFECTS OF BETA-CATENIN SIGNALING DYNAMICS ON ADULT NEURAL STEM CELL DIFFERENTIATION

Rosenbloom, Alyssa[1], Bugaj, Lukasz[2], Kane, Ravi[3] and Schaffer, David[1], [1]University of California, Berkeley, Berkeley, CA, U.S., [2]University of California Berkeley, Berkeley, CA, U.S., [3]Georgia Institute of Technology, Atlanta, GA, U.S.

Adult neural stem cells (NSC) contribute new neurons in specific regions of the brain to modulate learning and memory, and they represent therapeutic targets due to their potential roles in disease pathologies and promise in cell replacement therapies. The adult NSC microenvironment is highly dynamic, with signaling molecules likely varying in concentration and duration at timescales ranging from electrophysiological activity, to circadian rhythm, to environmental cues. Wnt signaling activates β-catenin signaling, which regulates the differentiation of adult hippocampal neural stem cells in vivo and in vitro. To address the question of how dynamics in signaling impacts cell function, our lab developed a tunable optogenetic system to modulate β-catenin signaling via Cry2 oligomerization of the LRP6 intracellular domain (Nature Methods, 2013). Similar to Wnt3a activation of the canonical Wnt pathway, blue light illumination of Cry2-LRP6c expressing NSCs induces robust neuronal differentiation. This raises the question: do stem cells differentiate simply when the integral of a signal during a given temporal window exceeds a key threshold, or do dynamics in signal presentation matter? Continuous illumination at different light intensities in vitro resulted in a progressive, saturable increase in neuronal differentiation from 5% to 60%. However, variation in signaling intensity over time yielded different results. Specifically, we observed that initial pulses of light for variable duration, or oscilllating illumination at frequencies >12 hours, yielded considerably less neuronal differentiation than in cells that received the same overall signal dosage but with continuous illumination. Furthermore, this signal stimulation followed by signal loss led to increased apoptosis, indicating exposure to the differentiation signal also rendered cells dependent upon it for survival, potentially offering a mechanism for removal of incompletely or poorly differentiated stem cells from tissue. In sum, these results harness optogenetics to demon-

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strate that not only the overall dosage of a signal but temporal dynamics in its presentation can exert a strong impact on stem cell behavior, work that offers further insights into the biology and translational potential of adult neurogenesis.

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W2049

NEURONAL DIFFERENTIATION OF ENGINEERED INDUCED PLURIPOTENT STEM CELLS AS AN APPROACH TO STUDY THE PATHOPHYSIOLOGY OF PARKINSON'S DISEASE

Sivapatham, Renuka[1,2], Pei, Ying[3], Gerenscer, Akos A[1], Meyer, Morten[2], Rao, Mahendra S[4] and Zeng, Xianmin[1,5], [1]Buck Institute for Research on Aging, Novato, CA, U.S., [2]University of Southern Denmark, Novato, CA, Denmark, [3]Buck Institute for Research on Aging, Novato, CA, U.S., [4]NxCell Science, Novato, CA, U.S., [5]XCell Science, Novato, CA, U.S.

Parkinson's disease (PD) is one of a class of neurodegenerative diseases, which cause a progressive loss of specific subclasses of neurons. Both familial and sporadic version of the disease has been identified and several of the genes underlying familial PD have been identified. Many of these genes appear to affect mitochondrial biology and some may interact with each other. PINK-1 is a mitochondrial protein kinase that was identified as a gene regulated by the AKT/PTEN pathway. Mutations in the gene encoding PINK-1 are a cause of early-onset, autosomal-recessive PD. In this study, we used isogenic PINK1 induced pluripotent stem cells (iPSC) to show that mutations in PINK1 alter neuronal proliferation. The same phenotype was confirmed in an isogenic *PARK2* null line. Whole genome expression profiling at various stages of differentiation confirmed the mitochondrial phenotype and identified pathways altered by PINK1 dysfunction. The mitochondrial phenotype was also seen in non-midbrain neurons differentiated from the PARK2 null line, as was the functional phenotype of reduced proliferation in culture. Our results are consistent with current model of PINK1 function where damaged mitochondria are targeted for degradation via a PARK2/PINK1 mediated mechanism.

W2051

MIGRATORY CHARACTERISTICS OF SKIN-DERIVED NEURAL PRECURSORS: A NOVEL STEM CELL REPLACEMENT THERAPY FOR ALZHEIMER'S DISEASE.

Truong, Nguyen T.A.[1], Lowe, Aileen[2], Valenzuela, Michael[3] and Duncan, Thomas[2], [1]The University of Sydney, Camperdown, Australia, [2]The University of Sydney, Sydney, Australia, [3]School of Psychiatry, University of New South Wales, Sydney NSW, Australia

Skin-derived neural precursors (SKNs) are a novel source of autologous stem cells with the potential to overcome current issues impeding regenerative therapy for neurodegenerative diseases. While their capability for proliferation and neuronal differentiation in vitro has been well-established, and their therapeutic potential to restore neuronal connectivity demonstrated in aged rats, the migratory characteristics of SKNs have yet to be characterised and is therefore the focus of this study. SKNs were generated from adult canine and human skin biopsies using our unique 2-step culture method. In vitro chemotaxis assays were performed using the IBIDI microfluidic device to identify molecular cues of SKN migration. To study migration in vivo, fluorescently-labelled SKNs were transplanted into the aged rat hippocampus, followed by histological analysis at days 3, 7, 10 and 140 post-transplantation to track their displacement, maturation and integration over time. In vitro, canine SKNs exhibited random movements in the absence of chemoattractants, as indicated by $p > 0.05$ in the Rayleigh test for chemotactic potential and lack of moving direction. Conversely, directed migration was induced by concentration gradients of growth factors BDNF ($p < 0.0001$), IGF-1 ($p = 0.0015$) and VEGF ($p = 0.0056$). Calculating the Euclidean distance travelled by SKNs showed the greatest migratory potential occurred when exposed to BDNF ($p < 0.05$) and VEGF ($p < 0.001$). In vivo, transplanted canine SKNs migrated extensively over large distances by day 7 post-transplantation, with a predilection to the CA1 hippocampal subregion. In vitro results demonstrate a strong migratory potential of SKNs, and is supported by in vivo findings that show canine SKNs migrate extensively following transplantation, differentiating into mature neurons and populating all hippocampal subregions. More importantly, despite their non-neural origin, SKNs respond to chemotactic factors known to be important for migration of endogenous hippocampal neural precursors, suggesting a similar migratory mechanism to hippocampal neurogenesis. Ongoing studies will identify other molecular regulators of SKN migration and further characterize the migration of human SKNs in the aged rat hippocampus.

W2053

SUFU REGULATES THE SPECIFICATION AND PROLIFERATION OF NEURAL PROGENITORS VIA DISTINCT MECHANISMS IN THE DEVELOPING NEOCORTEX

Yabut, Odessa[1], Fernandez, Gloria[1], Huynh, Trung[1], Ng, Hui-Xuan[1], Yoon, Keejung[2] and Pleasure, Samuel[1], [1]University of California San Francisco, San Francisco, CA, U.S., [2]Sungkyunkwan University, Suwon, Gyeonggi-do, Korea

Neural progenitors in the embryonic neocortex must be tightly regulated to generate the proper number and subtypes of neurons and glial cells in the mature neocortex. Our studies reveal that the intracellular protein, Suppressor of Fused (Sufu), regulate the specification and proliferation of cortical progenitors via distinct molecular mechanisms. Conditional deletion of Sufu in cortical progenitors at early stages of corticogenesis (E10.5; Emx1$^{cre/+}$;Sufu$^{fl/fl}$) results in the ectopic activation of Sonic Hedgehog (Shh) signaling due to the complete degradation of both Gli3 activator (Gli3A) and repressor (Gli3R). These defects lead to the misspecification of Pax6+ and Tbr2+ progenitors and the progressive loss of Tbr2+ progenitors. Also, widespread abnormalities in progenitor proliferation are present in a region-specific manner (i.e. the dorsomedial regions exhibit an increase in progenitor proliferation while the dorsal regions exhibit a decrease). Indeed, RNA-Seq analyses of the E12.5 Emx1$^{cre/+}$;Sufu$^{fl/fl}$ identified altered expression of prominent cell cycle and proliferation genes. In contrast, conditional deletion of Sufu at mid-corticogenesis (E13.5; hGFAP$^{cre/+}$;Sufu$^{fl/fl}$) does not disrupt the specification of progenitors but only cause an increase in the proliferation of neuronal and glial precursors. However, unlike in the Emx1$^{cre/+}$;Sufu$^{fl/fl}$ mice, Gli3A and Gli3R proteins are present. This suggests that Sufu regulates specification and/or proliferation using distinct mechanisms during corticogenesis. These findings emphasize the need to further dissect the molecular properties of cortical progenitors spatially and temporally, and how these properties influence the function of critical regulators, such as Sufu, in the specification and proliferation of neuronal and glial lineages.

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W2055

AMYLOID BETA-ACTIVATED mTORC1 INCREASES CYCLIN D1/CDK4 AND CYCINE/CDK2 LEVELS LEADING TO TAU HYPER-PHOSPHORYLATION-INDUCED NEURONAL APOPTOSIS

Han, Ho Jae[1], Lee, Ki Hoon[1], Kim, Dah Ihm[1], Choi, Gee Eun[1], Ko, So Hee[1], Lee, Hyun Jik[1], Ryu, Jung Min[2] and Mohamed, Gabr Amr Ahmed[1], [1]Seoul National University, Seoul, Korea, South, [2]Chonnam National University, Gwang-ju, Korea, South

Alzheimer's Disease (AD) is neurodegenerative disorder induced by Aβ resulting in Tau hyperphosphorylation, which is associated with neuronal cell apoptosis. As there has been no defined therapeutic target for AD, we investigated critical signaling pathway regulating Aβ-induced apoptosis in SK-N-MC. In our results, Aβ increased cleaved caspase 3 expression and apoptosis in a dose-dependent manner. We also demonstrated that Aβ increased ROS generation, followed by HIF-1α expression. In addition, Aβ stimulated mTOR phosphorylation, which is inhibited by HIF-1α siRNA transfection. Our results show that Aβ-induced mTOR activation increased CDK2 and CDK4 mRNA expressions, but not CDK1. Moreover, we confirmed that inhibition of autophagy by Aβ-induced mTOR activation increased CDK2 and CDK4 accumulation, which are blocked by autophagy inducer. mTOR-induced CDKs upregulation contributed to Tau hyperphosphorylation. Furthermore, we confirmed that both mTOR and CDKs inhibition and mTOR-independent autophagy induction prevented Aβ-induced apoptosis. Especially, mTOR inhibition had more protective effect than either CDKs inhibition or autophagy induction. In conclusion, we demonstrated that Aβ-activated mTOR by HIF-1α regulated both transcription and autophagy of CDK2 and CDK4 which are essential for tau hyperphosphorylation leading neuronal cell apoptosis.

REPROGRAMMING

W2061

APPROACHES TO ACHIEVE CONSISTENT REPROGRAMMING FROM HUMAN BLOOD CELLS

MacArthur, Chad C., Sridharan, Mahalakshmi and Lakshmipathy, Uma, Thermo Fisher Scientific, Carlsbad, CA, U.S.

A major use of induced pluripotent cells (iPSCs) is the ability to create patient-derived cells that may be used in a number of different applications including disease modeling, drug screening, and personalized medicine. Fibroblasts collected by skin punch biopsy are a common

source of cells used to create iPSC, but this collection process is more inconvenient to the patient than a standard blood draw. Various sources of blood cells have been used for the generation of iPSC such as CD34+ cells from bone marrow or umbilical cord, total mononuclear cells from peripheral blood, and a single drop of blood from a finger prick. Here, we evaluate different blood cell types from different sources for relative consistency in generating iPSCs. CD34+ cells consistently provided a high level of iPSC generation both under feeder- dependent and feeder-free conditions. Mononuclear cells from peripheral blood cells comprised of several subcell types including CD34+, erythroblastoid, T- and B- cells, can be consistently reprogrammed using an optimal combination of culture media and cytokines using Sendai virus. However, 5-fold greater numbers of cells are required compared to CD34+ cells. In order to enable reprogramming with smaller volumes, a single drop of blood was successfully used to generate iPSC, albeit with varying efficiency based on the donor age. This further highlights the need for identifying sources of variability and optimizing workflows required for consistent iPSC generation from small sample sizes. The relative convenience of collection and the high number of reprogrammable cells make blood a viable source of cells to generate patient-derived iPSC.

W2063

IMPAIRED STAT3 FUNCTION AND ATTENUATED REPROGRAMMING IN HIPSCS FROM PATIENTS WITH AUTOSOMAL DOMINANT HYPER IgG SYNDROME

Chen, Guibin, Laboratory of Cardiovascular Regenerative Medicine, Center for Molecular Modeling, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, U.S.

The induced pluripotent stem cell (iPSC) technology offers a powerful tool to reprogram somatic cells to a pluripotent state, which provides an opportunity to achieve various types of stem cells for basic research and regenerative medicine. While the protocol to achieve pluripotency in human somatic cells is well established, the precise molecular mechanisms regulating reprogramming have not been fully elucidated, including the roles played by the JAK/STAT3 signaling pathways. It has been demonstrated that the Signal transducer and activator of transcription 3 (*Stat3)* plays a central role for pluripotency maintenance of mouse iPSCs, and for the complete reprogramming of mouse somatic cells. Although activation of *STAT3* was dispensable in maintaining human pluripotent stem cells, understanding of the STAT3-mediated reprogramming in human somatic cells still remains unachieved. Autosomal dominant Hyper IgE Syndrome (AD-HIES) is a rare, primary immunodeficiency that arises from mutations in *STAT3.* To investigate the role of *STAT3* in reprogramming in human somatic cells, we generated iPSCs from dermal fibroblasts and umbilical vein endothelial cells (HUVECs) isolated from AD-HIES patients. We found that the reprogramming efficiency of both HUVECs and fibroblasts is significantly reduced in AD-HIES comparison with that of health donor. Over-expression of *STAT3* in fibroblasts of HIES rescue the defect of reprogramming efficiency while knock-down expression of *STAT3* in fibroblast of health donor mimic the results generated from AD-HIES. The results indicated that the activation of *STAT3* in human somatic cells is indispensable in reprogramming. Furthermore, We investigated the mechanism in more details and found that *STAT3* is critical in reprogramming of somatic cells through regulating of *NANOG* and *NANOG* p8 expression. Notably, these HIES-iPSCs generated 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, and are able to successfully differentiate into all three germ layers in vitro. Therefore, our finding demonstrates the novel functions of *STAT3* enhancing its reprogramming efficiency in human somatic cells.

W2065

DEFINING AN ESSENTIAL TRANSCRIPTION FACTOR PROGRAM FOR MAMMALIAN INNER EAR SENSORY HAIR CELL FATE USING LINEAGE REPROGRAMMING

Gopalakrishnan, Suhasni[1], Menendez, Louise[2], Llamas, Juan[1], Tao, Litao[1], Makamura, Welly[1], Ichida, Justin[1] and Segil, Neil[1], [1]Broad CIRM Center, Keck School of Medicine, University of Southern California, Los Angeles, CA, U.S., [2]Broad CIRM University of Southern California, Los Angeles, CA, U.S.

Sensory hair cells located within the inner ear are vital for our sense of hearing and balance. Hair cells are specialized cells that detect movement in their environment through mechanotransduction. These cells are remarkably sensitive to both genetic and environmental stress, including aging, excessive noise, and exposure to a number of otherwise therapeutic drugs, such as aminoglycoside antibiotics and chemotherapy agents. Mammals, including humans, are incapable of sensory hair cell regeneration, leading to permanent hearing and balance impairments in millions of people worldwide. Extremely limited availability and accessibility of biological material is a critical roadblock to translational research for hearing loss. To overcome this problem, we used viral transduction of lineage specific transcription factors in human and mouse fibroblasts to generate induced hair cells (iHCs). The iHCs are positive for hair cell markers such as parvalbumin, MyosinVI, otoferlin, and display polarized phalloidin staining. Additionally, the iHCs appear to have functional mechanosensitive channels based on rapid uptake of FM4-64 dye and the known ototoxin gentamicin. Furthermore,

gene expression profiles of iHCs and primary hair cells are similar, and iHCs exhibit subtype specific markers of cochlear and vestibular hair cells. We are currently pursuing a systematic identification of the key molecular networks that specify and maintain hair cell fate and subtype specification using a combination of single cell analysis and chromatin profiling (ATACseq) by comparing iHCs to the primary hair cells. Strikingly, we find that the reprogramming transcription factors induce robust conversion of in vitro cultured inner ear supporting cells. The supporting cells are normally resistant to transdifferentiation into hair cell-like cells, suggesting that these factors could be used for *in vivo* reprogramming to stimulate hair cell regeneration. These studies establish our approach as a scalable in vitro mammalian platform to generate hair cells to investigate and elucidate molecular mechanisms of sensory hair cell development and physiology, in vitro disease modeling, and drug discovery.

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W2067

DEVELOPMENT OF AN ENABLING SYSTEM FOR HIGH EFFICIENCY REPROGRAMMING AND CRISPR-BASED GENOME EDITING

Kehler, James[1], Pachiappan, Manickam[1], Yokoe, Hiroko[1], Liu, Binjun[1], Gebeyehu, Gulilat[1], Auerbach, Jonathan[1], Jessee, Joel[1], Chen, Alice E.[2] and Yang, Miranda[2], [1]MTI-GlobalStem, Gaithersburg, MD, U.S., [2]BioTime, San Diego, CA, U.S.

Two major recent advances in cell biology have ushered in an age of functional human genetics. Reprogramming of human somatic cells to induced pluripotent stem cells (iPSC) is now performed routinely in many labs studying various aspects of biology to capture unique genotypes and phenotypes. Another emergent technology enabling labs to perform controlled experimentation through precise genome editing is CRISPR/Cas9. Pluripotent stem cells are an excellent starting point for genome editing. However, the process has several technical bottlenecks making it somewhat cumbersome. In order to streamline the workflow and increase throughput while simultaneously enhancing accuracy, we have developed an integrated transfection and culture system in which cells can be reprogrammed and the resultant iPSC can undergo genome editing. This platform consists of defined growth medium, substrate and non-enzymatic passaging solution that support single cell plating and clonogenic expansion, and unique reagents and methodologies for efficient nanovesicle-mediated transfer of macromolecules for reprogramming and editing with high efficiency and low toxicity. By optimizing unique chemistries and the timing of delivery, we have created a flexible platform for the efficient delivery of CRISPR/Cas9 as a DNA plasmid or Cas9 mRNA in combination with small guide RNAs, or double stranded oligomers expressing gRNA, supporting robust indel formation. Using this platform, we have established optimal conditions for high levels of cellular transfection at low confluency.

W2069

REACTIVE OXYGEN SPECIES - MEDIATED IMPAIRMENT OF THE MESENCHYMAL TO ENDOTHELIAL TRANSITION IN DIABETIC HYPERGLYCEMIA

Lai, Li, Houston Methodist Research Institute, Houston, TX, U.S.

Endothelial-to-mesenchyme transition plays a major role in fibrotic disorders including the cardiac fibrosis that is associated with diabetes. Recently, the reverse phenomenon i.e., Mesenchyme-to-endothelial transition (MEndoT) has been discovered as a physiological response to myocardial ischemia. These induced endothelial cells (iECs) contribute to the expansion of the microvasculature so as to improve perfusion in the ischemic tissue. It is known that angiogenesis is impaired in diabetes mellitus, and contributes to poor wound healing and diabetic foot ulcers. This study is to test whether high levels of glucose impair MEndoT and whether inhibition of reactive oxygen species (ROS) can ameliorate this transition. Previously we have developed a non-viral methodology to generate iECs which comprises a formulation containing a TLR3 agonist and EC growth factors. We assessed the effect of high glucose conditions on iEC generation. Inhibitors of most sources of ROS were imposed during the process. We observed that high glucose conditions markedly reduced the generation of iECs, an effect that could be reversed by inhibitors of mitochondrial or cytoplasmic ROS generation, as well as ROS scavengers. The quality of iECs generated under high glucose conditions was also impaired, as assessed by endothelial function assays, e.g. tube formation on matrigel. This functional impairment of iECs could also be reversed by antioxidants. We further assessed transdifferentiation in vivo by matrigel plug assay. In a subcutaneous pocket we embedded matrigel containing human fibroblasts together with the iEC formulation in vehicle- or streptozotocin-treated SCID mice. After 2 weeks we analyzed the percentage of cells staining for human CD31 within the matrigel plugs. We observed a reduction in human CD31+ cells in the hyperglycemic mice, an effect which was reversed by co-administration of N-Acetyl-Cysteine in the matrigel. In conclusion, our study identified for the first time the role of hyperglycemia upon MEndoT, a newly discovered response to tissue ischemia, and provides a mechanism for understanding the impairment in endothelial transdifferentiation in diabetes mellitus.

W2071

EFFECTS OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS ENGINEERED WITH OCT4/SOX2 ON ANTI-INFLAMMATORY

Li, Qiang, Han, Sei-Myoung, Song, Woo-Jin, Park, Sangchul, Ryu, Min-Ok, Lee, Ji-Ye, Jeon, Kee-Ok, Lee, Bo-Yeon, Kweon, Kyeong, Chae, Hyung-Kyu, Kim, Hyeon-Jin, Kim, Su-Yeon, Yang, Hye-Mi and Youn, Hwa-Young, College of Veterinary Medicine, Seoul National University, Seoul, Korea, South

During the inflammation process, macrophages as main innate immune cell play a central role in mediating many different immunopathological phenomena including the overproduction of pro-inflammatory cytokines. Recent works have been shown that *Oct4* and *Sox2* as main self-renewal factor enhance proliferation and pluripotency of adipose tissue-derived mesenchymal stem cells (ATMSCs). However, anti-inflammatory effects of ATMSCs overexpressing Oct4 and Sox2 (Oct4/Sox2-ATMSCs) have not been determined. The aim of the present study was to evaluate the anti-inflammatory effects of Oct4/Sox2-ATMSCs. In vitro, green-fluorescent protein (GFP) (control) and Oct4/Sox2-ATMSCs were cultured for 48 h and the supernatant (conditioned media) was collected to treat lipopolysaccharide (LPS)-induced Raw 264.7 cells. Subsequently, the levels of inflammatory cytokines expression were determined using quantitative real- time polymerase chain reaction analysis. In LPS induced systemic inflammatory mice models, GFP- and Oct4/Sox2-ATMSCs ($1x10^7$ cells/kg) were injected intraperitoneally and monitored by survival rate and sick score (diarrhea, eye condition, activity and condition of their fur). Oct4/Sox2-ATMSCs group further decreased expression of pro-inflammatory cytokines (IL-1β, IL-6. and TNF-α) from Raw 264.7 cells than GFP-ATMSCs group. Comparison to GFP- with Oct4/Sox2-ATMSCs injected mice, the total sick score was reduced to 1.53 fold, while the survival rate was increased by 11.1%. Although further studies of mechanisms are needed, these results suggest Oct4/Sox2-ATMSCs may be developed as a novel therapy strategy of inflammatory diseases.

W2073

REPROGRAMMING CELLS IN THE BRAIN: THE INFLUENCE OF CONVERSION FACTORS AND ENVIRONMENT ON NEURONAL IDENTITY

Pereira, Maria[1,2], Ottosson, Daniella Rylander[1,3], Torper, Olof[1,3] and Parmar, Malin[1,3], [1]Lund University, LUND, Sweden, [2]Lund University, Experimental Medical Science Department, Lund, Sweden, [3]Lund University, Lund, Sweden

Cellular reprogramming is a new and rapidly emerging field where somatic cells can be turned into pluripotent stem cells or other somatic cell types simply by expression of specific combinations of genes. By viral expression of neural fate determinants, it is possible to directly reprogram mouse and human fibroblasts into functional neurons, termed induced neurons (iNs). Direct neural conversion can also be performed in vivo, where resident glia is reprogrammed into neurons. We have developed a Cre-dependent AAV-based vector system that efficiently converts astrocytes and NG2-glia into functional neurons in vivo (Torper et al., 2015). When converting striatal glia using Ascl1, Lmx1a and Nurr1, the majority of the new neurons are GABAergic and Glutamatergic, despite that the same genes give rise to dopaminergic neurons when fibroblasts or astrocytes are converted in vitro (Caiazzo et al., 2011; Addis et al., 2011).

In this study, we explore how cell fate identity is governed during conversion. In the first set of experiments, the same conversion factors (Ascl1, Lmx1a and Nurr1) are used to convert glia in different regions of the brain. After 12 weeks, the subtype identity of the resulting neurons in the striatum, cortex and globus pallidus is analyzed based on morphology, neurotransmitter identity and functional properties. In the second set of experiments, different factor combinations are injected into the striatum and the subtype identity of the resulting cells is analyzed after 12 weeks.

Together, the results will give a comprehensive picture of how conversion factors and environment influence neuronal identity after conversion of glia into neurons *in vivo*.

W2075

DIRECT CONVERSION OF PATIENT SPECIFIC HUMAN NEURAL STEM CELLS FROM NIEMANN-PICK DISEASE TYPE C PATIENT-DERIVED FIBROBLAST

Shin, Ji-Hee[1], Sung, Eun-Ah[1], Yu, Kyung-Rok[1], Choi, Soon Won[1], Seo, Yoojin[1], Kang, Insung[1] and Kang, Kyung-Sun[2], [1]Seoul National University, Seoul, Korea, South, [2]Adult Stem Cell Research Center, Seoul National University, Seoul, Korea, South

Niemann-Pick disease type C (NPC) is a neurodegenerative and lysosomal lipid storage disorder, characterized by abnormal accumulation of unesterified cholesterol and glycolipids, which is caused by mutations in NPC1 or NPC2 genes. Here, we report the generation of human induced neural stem cells from NPC patient-derived fibroblasts (NPC-iNSCs) using only two reprogramming factors without going through the pluripotent state. NPC-iNSC lines were stably expandable and showed trilineage neural differentiation potential. However, NPC-iNSCs displayed cholesterol accumulation, defective self-renewal, and neuronal differentiation, suggesting that NPC-iNSC lines retain main features of NPC disease. We found that valproic acid (VPA), a histone deacetylase (HDAC) inhibi-

tor, significantly ameliorated the cholesterol accumulation and restored cholesterol homeostasis. Furthermore, VPA corrected the impaired self-renewal and neuronal differentiation of NPC-iNSC lines. Taken together, these findings suggest that NPC-iNSCs could provide a powerful platform for pathological study or drug screening in a patient specific manner and that this direct conversion technology may extend to other human neurodegenerative diseases.

W2077

A NOVEL SCREENING PLATFORM TO IDENTIFY DRUGS WHICH REPROGRAM PANCREATIC CANCER

Villarino, Nicholas, Sanford Burnham Prebys, La Jolla, CA, U.S.

A Novel Screening Platform to Identify Drugs which Reprogram Pancreatic Cancer

Pancreatic Ductal Adenocarcinoma (PDA) is the 4th leading cause of cancer in the US and has a predominantly lethal prognosis. Unlike most other cancers, patient lifespan post-PDA diagnosis has not changed in over 50 years, emphasizing the need for a breakthrough in therapeutic research. PDA originates in acinar cells, which secrete proteolytic digestive enzymes into the duodenum. In response to oncogenic Kras, these cells undergo acinar to ductal metaplasia and become highly proliferative. Our lab has shown that expression of many basic helix loop helix (bHLH) transcription factors declines as PDA progresses, suggesting a role for bHLH proteins in pancreas homeostasis. Indeed, we showed that overexpression of the bHLH protein E47 in PDA cell lines and patient derived xenograft lines results in a return to a quiescent, acinar-like state in part through the activity of the cell cycle inhibitor p21 (CDKN1A). In order to translate these findings into a clinical setting, our group developed a screening platform to identify inducers of bHLH activity and carried out a drug screen of 4375 FDA approved compounds. Among the hits we identified the statin class of drugs as significant inducers of bHLH activity. Characterization of these drugs is being performed on three unique human PDA cancer cell lines, one of which is a recent patient-derived line. We have determined that this class of compounds inhibits the cell cycle, greatly upregulates the tumor suppressor p21, and induces acinar differentiation markers. Given that statins are in clinical trials for PDA, the data establish the ability of this novel screening assay to identify pharmaceuticals of interest for pancreatic cancer.

W2079

DIRECTED REPROGRAMMING OF HUMAN CELLS TOWARD ASTROCYTES AS A DISEASE MODELING PLATFORM

Kohyama, Jun[1,2], **Zhou, Zhi**[1,3], Sanosaka, Tsukasa[1], Banno, Satoe[1], Chai, MuhChyi[1], Blanchi, Bruno[2], Ge, WeiHong[2], Okano, Hideyuki[1] and Sun, Yi Eve[2,4], [1]Keio University, Tokyo, Japan, [2]UCLA Medical School, Los Angeles, CA, U.S., [3]Japan Society for the Promotion of Science, Tokyo, Japan, [4]Tongji University, Shanghai, China

Accumulated evidence indicates that astrocytes, one of the three major cell types in the central nervous system, play important roles in the pathophysiology of neurological and psychiatric disorders in cell-autonomous and/or non-cell-autonomous manners. Therefore, studying disease mechanisms from a gliocentric perspective helps to fully elucidate disease etiologies. However, spontaneous differentiation of human pluripotent stem cells into astrocytes requires long culture period, hindering intensive studies. Here, we report the development of an efficient and prompt method to directly convert mouse and human fibroblasts into functional astrocyte-like cells (iA cells) by defined factors. We also show the applicability of our system to human neural stem cells yielding mature iA cells within two weeks in high purity. Using this system, we attempted to establish disease-in-a-dish models of Alexander disease and Rett syndrome, and successfully recapitulated some aspects of disease phenotypes. Taken together, this technology provides novel perspectives to better understand the complex pathophysiologies of various neurological disorders, facilitating future drug screening and the development of novel therapies.

IPS CELLS

W2083

GENERATION OF DISEASE-SPECIFIC IPSCS AND DEVELOPMENT OF TRANSGENIC CELL LINES FOR CYSTIC FIBROSIS DISEASE MODELLING AND DRUG SCREENING

Schubert, Madline[1], Merkert, Sylvia[1,2], Haase, Alexandra[1,2], Engels, Lena[1,2], Haller, Ralf[1,2], Lachmann, Nico[1,3], Moritz, Thomas[1,3], de la Roche, Jeanne[1,4], Tümmler, Burkhard[1,5], Galietta, Luis[6] and **Martin, Ulrich**[1,2], [1]Hannover Medical School, Hannover, Germany, [2]Leibniz Research Laboratories For Biotechnology And Artificial Organs, Hannover, Germany, [3]Institute of Experimental Hematology, Hannover, Germany, [4]Institute for Neurophysiology, Hannover, Germany, [5]Clinic for Paediatric Pneumology, Allergology and Neonatology, Hannover, Germany, [6]Istituto Giannina Gaslini, Genova, Italy

The genetic disorder cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene coding for a cAMP-activated chloride-channel. So far, immortalized cell lines overexpressing mutant CFTR-variants have been used to screen compound-libraries. In fact, CFTR-modulators have been identified, but show modest effects at best. Obviously, the complexity of the mutant CFTR-maturation and turnover kinetics including the influence of genetic modifiers require the use of advanced personalized cellular models that closely recapitulate the properties of the clinically most affected organs. To address these unmet needs we focus on the generation of induced pluripotent stem cells (iPSCs) from CF-patients homozygous for F508del mutation with mild and severe phenotype and with known intragenic recombination. CF-iPSCs were generated via reprogramming of CD34^{+} cells isolated from small volumes of non-mobilized peripheral blood. The resulting CF-iPSCs were analysed regarding their karyotype, pluripotency status and potential to differentiate. Moreover, different transgenic iPSC lines were generated overexpressing a halide sensitive yellow fluorescent protein (eYFP) monitoring CFTR-function, in combination with the overexpression of an artificial CFTR or an endogenous CFTR^{tomato} fluorescence reporter. Several CF-iPSC lines were established and characterized in detail. The generated eYFP reporter cell lines showed stable transgene expression also during in vitro differentiation. The differentiation of eYFP expressing CFTR^{tomato} reporter iPSCs towards cholangiocytes revealed eYFP^{pos}/tomato^{pos} cells, displaying CFTR channel specific response after Forskolin application. Preliminary measurements revealed that the endogenous CFTR expression level of differentiated cells should be sufficient to analyze CFTR function via the halide sensitive eYFP. Hence, the stable integration of the eYFP into CF patient-specific iPSCs in combination with integration of the CFTR^{tomato} reporter should enable disease modelling of F508del-based CF with regard to the individual genetic context and the implementation of high-throughput screening for the identification of novel correctors and potentiators of CFTR-trafficking mutations.

Funding Source: German Center for Lung Research (DZL, 82DZL00201), the German Research Foundation (Cluster of Excellence REBIRTH, EXC 62/3) and the Mukoviszidose Institut GmbH (S0

W2085

USING HUMAN INDUCED PLURIPOTENT STEM CELLS TO INVESTIGATE THE CONTRIBUTION OF RISK VARIANTS AND AGING TO THE ONSET AND PROGRESSION OF ALZHEIMER'S DISEASE

Brookhouser, Nicholas David, Raman, Sreedevi, Potts, Chris and Brafman, David, Arizona State University, Tempe, AZ, U.S.

Developing therapies for the treatment of Alzheimer's disease (AD) requires an understanding of the mechanisms that cause the disease. Animal models of AD have provided important insights but do not display important AD-related pathologies and have not been useful in modeling the complex genetics associated with "sporadic" AD. Although the majority of AD patients are sporadic, multiple genetic risk variants have been identified, the most powerful and prevalent of which is the E4 variant of Apolipoprotein E (APOE) gene. Compared to individuals with an APOE 3/3 genotype, heterozygosity for the E4 allele increases AD risk by 3 fold, and homozygosity for the E4 allele increases risk up to 12 fold. Amyloid-dependent and -independent mechanisms have been postulated to explain the APOE4 effect, but currently how APOE4 modulates AD disease risk, especially during aging, remains unclear. To that end, we are generating a diverse set of human induced pluripotent stem cell (hiPSC) lines from AD and non-demented control (NDC) patients with no (i.e. APOE 3/3) and two (i.e. APOE 4/4) copies of the E4 allele. We are using these hiPSCs to elucidate the potential genetic, molecular, and cellular mechanisms by which the APOE 4 allele contributes to AD onset and age-related disease progression. By using a novel 3D cortical neuronal culture model and genome-wide expression analysis (RNA-seq), we are identifying unique gene expression profiles that are independently defined by APOE genotype, disease status, and age. Future bioinformatic analysis will reveal candidate genetic, biochemical, and signaling pathways that will provide more definitive relationships between APOE genotype and AD onset and age-related progression. In the future, we will investigate how modulation of these candidate target genes and pathways regulates the manifestation of AD-related phe-

notypes. Such future investigations will have significant impact on the design of molecularly targeted therapeutics to treat AD.

Funding Source: Arizona Alzheimer's Consortium

W2087

GENOME EDITING IN HUMAN INDUCED PLURIPOTENT STEM CELLS

Chen-Tsai, Ruby, Diaz, Robert, Yan, Lin and Kong, Ling-Jie, Applied StemCell, Inc., Milpitas, CA, U.S.

Genome editing/correction in patient-specific induced pluripotent stem cells (iPSCs) offers one of the most promising approaches for personalized therapy in regenerative medicine. CRISPR/Cas9 has been widely used as an effective gene editing tool in the past two years for site-specific gene modification including insertion, deletion and nucleotide replacement. CRISPR works most efficiently in gene knockout through the non-homologous end joining (NHEJ) pathway. However for DNA knockin, the efficiency of CRISPR mediated homology-directed repair (HDR) remains low, limiting its application in therapeutics. Our TARGATT™ technology allows site-specific gene insertion at a higher efficiency in a genomic safe harbor locus. We will discuss the results on knockin, knockout and point mutation efficiency in iPSCs using CRISPR/Cas9. We will also report using a "master" iPSC line for efficient site-specific insertion of large DNA fragments. With TARGATT™ and CRISPR, we are empowered with a complete set of genome editing tools to manipulate human iPSCs.

W2089

DEGRADATION OF SACCHARIDE ABO EPITOPES IN FABRY AND SCHINDLER DISEASE iPSC MODELS

Dobrovolny, Robert[1], Rybova, Jitka[1], Asfaw, Befekadu[1], Poupetova, Helena[1], Kuchar, Ladislav[1] and Ledvinova, Jana[1,2], [1]Charles University, First Faculty of Medicine, Prague, Czech Republic, [2]First Faculty of Medicine, Charles University in Prague, Prague 2, Czech Republic

Fabry disease (FD) is an X-linked defect of lysosomal α-galactosidase A (agalA, GLA gene). The enzymatic defect leads to intralysosomal accumulation of glycolipid moieties with terminal α-linked galactose, predominantly globotryaosylceramide (Gb3) in tissues. Clinically the disorder is multisystemic with symptoms including angiokeratomas, cardiomyopathy and kidney disease. Another group of substrates that are affected by deficiency of agalA is blood group B epitope that contains α-galactose. Even though an increased amount of B active substances are detected in FD patients it has never been shown that blood group B would have any detrimental effect on severity of the disease. It has long been hypothesized that reason for these findings is partial overlap of substrate specificity between agalA and related α-N-acetylgalactosaminidase (NAGA). To verify that NAGA can degrade the substrates of aGalA, especially polar B- glycolipids with longer saccharide chains (e.g. B-6-2), we have prepared iPSC based model of FD (aGalA KO), NAGA KO (in fact model of Schindler disease) and double KO with both aGalA and NAGA disrupted. The KO cell lines were generated from healthy individual iPSC using the CRISPR/Cas9 protocol. The successful generation of desired iPSC lines was confirmed by detection of pluripotency marker, demonstration of deficient activities and sequencing of the GLA and NAGA loci. The lines were also differentiated to multiple cell types. The degradation study of aGalA and NAGA substrates was performed by pulse-chase experiments with three tritium labeled substrates. 1) globotetraosylceramide (Gb4), degradation precursor of Gb3. 2) A-6-2, blood group A glycolipid that serves as control of NAGA enzymatic block as it contains α-N-acelylgalactosamine and 3) B-6-2, typical blood group B glycolipid that was found to be partially degraded in FD cell cultures. The expected partial degradation of B-6-2 in aGalA KO lines and total block of degradation in double KO cultures was not observed. Based on three independent metabolic experiments, it seems that even in double KO lines the B-6-2 is partially degraded in contrast to Gb3. These results suggest that there may be another glycosidase that can degrade polar substrates of aGalA. The generated iPSC will be used for further characterization of these catabolic pathways.

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W2091

GENETIC DETERMINANTS OF GENE EXPRESSION IN A COLLECTION OF 215 HUMAN INDUCED PLURIPOTENT STEM CELLS

DeBoever, Christopher, Jakubosky, David, Arias, Angelo, D'Antonio-Chronowska, Agnieszka, Li, He and **Frazer, Kelly**, University of California San Diego, La Jolla, CA, U.S.

In this study, we examined gene expression regulation in a collection of 215 human induced pluripotent stem cells (iPSCs). We systematically reprogrammed fibroblasts from a diverse set of 215 individuals and performed transcriptome sequencing for the iPSCs (at passage 12) as well as germline whole genome sequencing. Using these data, we identified expression quantitative loci (eQTL) for 5,816 genes including markers of pluripotency such as POU5F1 (OCT4), LCK, IDO1, and CXCL5. We found 99 genes whose eQTL lead variant was a biallelic copy number variant (CNV) and 1,043 significant CNV-expression associations in total. We also identified 98 genes whose expression was associated with multi-allelic CNVs. Tran-

scription factor (TF) binding sites for NANOG, SP1, JUND, JUN, and other TFs were highly enriched for disruption by lead eQTL variants implicating these factors as important regulators of gene expression. Lead eQTL variants were also enriched for GWAS hits from several phenotypes such as LDL cholesterol, height, and mean corpuscular volume indicating that characterizing iPSC regulatory variants is relevant for a number of diverse phenotypes. We used various functional genomics datasets (H1-hESC DNase I HSs, TF ChIP-Seq peaks and ChIA-PET data) to fine map putative causal eQTL variants and found variants that disrupt TF binding sites are enriched for 3D interactions with gene promoters. We compared the power to detect eQTLs using iPSC to other tissue types and found that hiPSCs are relatively well-powered for genetic association analyses. In separate analyses, we used allele specific expression to investigate the rate of X chromosome reactivation after reprogramming and showed that it is heterogeneous in that it occurs at different rates across the X chromosome and is correlated with XIST *and* TSIX expression levels in the iPSCs. We also found evidence that imprinting can be retained in iPSCs suggesting that reprogramming does not completely erase imprinting. Our work demonstrates the utility of iPSCs for genetic association analyses, helps define the role of CNVs in the regulation of gene expression and identifies novel TFs as potential key regulators in stem cells. Additionally, it provides information on the heterogeneity of X reactivation in iPSCs and the resetting of imprinted loci after reprogramming.

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W2093

DEVELOPING ONLINE OPEN RESOURCES FOR THE HIPSCI iPSC CATALOGUE

Harrison, Peter, Streeter, Ian, Bradley, Holly Zheng, Datta, Avik, Fairley, Susan, Lowry, Ernesto, Consortium, HipSci and Clarke, Laura, EMBL-European Bioinformatics Institute, Cambridge, U.K.

The Human Induced Pluripotent Stem Cells Initiative (HipSci) is a collaboration of institutes and individuals across genomics, proteomics, cell biology and clinical genetics with the aim of creating an open iPSC resource. It includes a large number of phenotypically ‘normal’ donors to allow the study of how common genetic variations affect cellular phenotypes. It is generating over 500 iPSC lines from healthy volunteers and individuals with genetic disease through the projects strong links with the UKs National Health Service (NHS). Consortium researchers are employing these lines to conduct cellular genetic studies to discover how genomic variation impacts on cellular phenotypes and to identify new disease mechanisms. A key aspect of this project is its open access model of data sharing and community engagement, with the lines being made available through the European Collection of Authenticated Cell Cultures (ECACC). Our role at EMBL-EBI is to coordinate the transfer, storage and distribution of the data generated by consortium members for each of the cell lines, with a particular focus on development of online resources to provide the greatest impact from this large open project. The HipSci portal that we have developed www.hipsci.org provides a central catalogue of all of the cell lines generated by the project, providing direct links to all open access assay data and added value resources such as a visual display of data through Trackhubs that utilise the EBIs new Trackhub registry. Alongside powerful metadata searches we are currently developing the capability to search lines based on their underlying genetic variation data, enabling querying by specific mutations or particular alleles which will importantly include managed access lines for which the user has an access agreement in place.

Funding Source: The HipSci project is funded by the Wellcome Trust and Medical Research Council.

W2095

REPROGRAMMING GENES (OSKM) CONTROLS DIFFERENTIATION OF iPSCs INTO HEPATOCYTE-LIKE CELLS

Jeong, Jaemin[1], Lee, Seung Bum[2], Kang, Kyo Jin[3], Kim, Yohan[3] and Choi, Dongho[3], [1]Department of Surgery, Seoul, CA, Korea, South, [2]Lab. of Radiation Exposure & Therapeutics, National Radiation Emergency Medical Center,Korea Institute of Radiological & Medical Science, Seoul, Korea, [3]Hanyang University, Seoul, Korea

Induced pluripotent stem cells (iPSCs) that have become an important tool of human diseases therapy have generated by introducing four Yamanaka factors using retro viurs. However, these genes are integrated into the host genome so that cannot use for the clinical application. Foreign genes results in insertion mutation, residual expressions, and reactivation of transgenes during differentiation. In this report, we introduce transgene-free iPSC generation method and its differentiation into hepatocytes. We generate transgene-free iPSCs with Cre-loxp system that is removing of integrated transgenes from the genome. To remove transgene from iPSCs colony, Cre recombinase was treated for 2hrs and then cultured until colonies grow out. We confirmed that transgene is not expressed in genome by PCR. These colonies were expressed Tra-1-81, Oct4, Nanog, Sox2 and Myc as well as Tra-1-60. Though without transgene, pluripotent marker expression was not altered. And we find efficient differentiation methods iPSCs into homogeneous population of functional hepatocytes. For endoderm differentiation,

activing A was treated for 6 days, and HGF and FGF4 were treated for 5 days. hiPSC-derived hepatocytes is expressed not only hepatocyte specific markers, but also have liver functions, such as albumin, AFP, ASGPR, and glycogen storage and cytochrome P450 acitvity. In conclusion, we have generated a transgene-free iPSCs and functional hepatocytes in vitro, and these results represent a step forward for clinically applicable patient-specific hepatocytes for cell-based therapeutics.

W2097

MODELING AND FUNCTIONAL CORRECTION OF STRUCTURAL VARIATIONS IN NORMAL OR PATIENT-DERIVED IPS CELLS USING ENGINEERED NUCLEASES

Kim, Dong-Wook, Yonsei University College of Medicine, Seoul, Korea, South

Structural variations include chromosomal inversions, trinucleotide repeats and translocations etc. These are complicated and difficult to model or correct in the genome using programmable nucleases. Here, I introduce strategies we use to edit these structural variations. As an example, there is hemophilia A. Hemophilia A is an X-linked genetic disorder caused by mutations in the F8 gene, which encodes the blood coagulation factor VIII. Almost half of all severe hemophilia A cases result from two gross (140-kbp or 600-kbp) chromosomal inversions that involve introns 1 and 22 of the *F8* gene, respectively. A hemophilia A disease model (inversion) was first conducted in normal induced pluripotent stem cells (iPSCs) by causing double-strand breaks by TALEN inside the intron 1 homolog of the F8 gene, with an efficiency of 1.4%. The inverted model cells mimicked the disease phenotype (i.e., no expression of the *F8* gene), and interestingly, the inverted segment could be reverted to its normal state using the same TALEN mainly via HR. Furthermore, we recently generated iPSCs from hemophilia A patients with two types of inversion genotypes and used CRISPR-Cas9 nucleases to revert these chromosomal segments back to the WT situation. We isolated inversion-corrected iPSCs with frequencies of up to 6.7% without detectable off-target mutations based on whole-genome sequencing or targeted deep sequencing. Endothelial cells differentiated from corrected iPSCs expressed the *F8* gene and functionally rescued factor VIII deficiency in an otherwise lethal mouse model of hemophilia. Our results therefore provide a proof of principle for modeling and functional correction of large chromosomal rearrangements in normal or patient-derived iPSCs and suggest potential therapeutic applications.

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W2099

CRISPR-MODIFIED IPS CELLS TO VALIDATE NEW TARGETS FOR CYSTIC FIBROSIS

Li, Hongmei Lisa[1], Agrawal, Pankaj[1], Mahoney, John[2,3], Gupta, Manav[4], Schlaeger, Thorsten[1,2], Gerard, Craig J.[1,2], Kim, Carla F.[1,2] and Daley, George Q.[1,2], [1]Boston Children's Hospital, Boston, MA, U.S., [2]Harvard Medical School, Boston, MA, U.S., [3]Cystic Fibrosis Foundation Therapeutics, Lexington, MA, U.S., [4]Harvard University, Boston, MA, U.S.

Cystic Fibrosis (CF) is a genetic disorder that affects more than 30,000 people in the United States. CF particularly affects the lungs and digestive system, and disrupts the normal function of epithelial cells that make up the sweat glands in the skin and line passageways inside the lungs, liver, pancreas, and digestive and reproductive systems. CF is caused by autosomal recessive mutations in the CF transmembrane conductance regulator (CFTR) gene, which leads to abnormal transport of chloride and sodium across the respiratory epithelium, resulting in thick secretions. It is well known that CF presents in a broad range of severity, which may be related to the molecular nature of the CFTR mutations in individuals as well as to modifier loci. We have identified rare CF patients who have nearly normal lung function despite living many decades with their CFTR gene mutation. By whole exome sequencing of DNA from these "long term non-progressor (LTNP)" CF patients, we have discovered rare genetic variants in candidate modifier loci. We have generated iPS cells from typical and LTNP CF patients, and performed CRISPR-mediated repair of the CFTR mutation. We have differentiated these mutant and repaired CF-iPS cell lines into lung epithelia cells and confirmed the ability of iPS cells from typical and LTNP to make differentiated NKx2.1 positive cells. Efforts are underway to perform physiologic assays of CFTR channel function to demonstrate the effect of both the CFTR mutation and the candidate gene modifiers. Our in vitro studies should inform the physiologic mechanisms by which candidate disease-modifying mutations function in patients with extreme CF phenotypes, thereby pointing towards critical therapeutic targets to ameliorate disease.

Funding Source: Cystic Fibrosis Foundation Therapeutics Fellow

W2101

GMP-COMPATIBLE IPSC DERIVATION FROM HUMAN UMBILICAL CORD BLOOD AND TISSUE ACROSS MULTIPLE DONORS

Mack, Amanda[1], Faust, Elizabeth[1], Monroe, Kevin[1], Wachowiak, MS, Ryan[1], Wang, Wen Bo[1], Brown, Katherine[2], Skiles, Matthew[2] and Brown, Heather[2], [1]Cellular Dynamics International, Inc- a Fujifilm company, Madison, WI, U.S., [2]CBR Systems Inc, San Bruno, CA, U.S.

Induced pluripotent stem cells (iPSCs) can be generated from newborn tissues stored in one of the world's largest cord blood banks, Cord Blood Registry®, using an integration-free, clinically applicable reprograming method. We demonstrate the ability to generate iPSCs from umbilical cord blood units processed manually using a Ficoll gradient or on the fully automated AutoXpress® (AXP) platform (Cesca Therapeutics). AXP and Ficoll processed cord blood units were cryopreserved for up to 5 and 10 years respectively. Resulting iPSCs exhibit characteristic gene expression profiling, do not contain residual reprogramming factors, are normal in karyotype, and match the identity of the original starting material. This study also compares reprogramming capacity of cord blood and mesenchymal stem cells isolated from thawed umbilical cord tissue, previously cryopreserved as a composite material, from the same donor. Furthermore, we demonstrate that less than 1 million cells can be used as starting material for iPSC derivation making for a tractable system when tissue amounts and types are limited. This study serves as a proof of principle that stem cells from multiple newborn tissues cryopreserved at a cord blood bank can be used as starting material for iPSC reprogramming under conditions compatible for subsequent transition to a GMP facility for clinical applications.

W2103

A NOVEL METHOD USING CONDITIONALLY REPLICATING ADENOVIRUS FOR SPECIFICALLY KILLING TUMORIGENIC CELLS DERIVED FROM PLURIPOTENT STEM CELLS

Mitsui, Kaoru, Ide, Kanako and Kosai, Ken-ichiro, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

Human pluripotent stem cells (hPSCs), are promising candidate cell sources for regenerative medicine. However, the risk of formation of tumors, including teratomas and cancers originating from contaminating undifferentiated and transformed cells, hinder therapeutic applications of hPSC-based regenerative medicine. Most previous studies focused on reducing the risk of carcinogenesis, such as improvement in generation of hiPSCs or differentiation methods. However, these indirect tumor suppression approaches cannot completely abolish tumorigenic risk. Here, we present a novel "oncolytic virus" strategy that can directly target and kill tumorigenic cells derived from hPSCs. Conditionally replicating adenoviruses (CRAs), also called oncolytic adenoviruses, can selectively replicate in and kill cancer cells; consequently, CRAs represent attractive anticancer drugs. Previously, we developed a method for generating CRAs that can target cancers with multiple cancer-specific factors (m-CRAs); this approach further increased cancer specificity without reducing the anticancer effects, and demonstrated that survivin-responsive m-CRA (Surv.m-CRA) is one of the most promising anticancer agents, in two respects: superior cancer specificity (*i.e.,* safety) and therapeutic efficacy relative to clinically tested telomerase reverse transcriptase *(TERT)*-responsive m-CRAs (Tert.m-CRAs), and strong anticancer effects against currently incurable cancer stem cells. We demonstrate that m-CRAs may also be useful as novel anti-tumorigenic agents in hPSC-based therapy. We show that the survivin promoter was more active in undifferentiated hPSCs than the TERT promoter, whereas both promoters were minimally active in differentiated normal cells. Accordingly, Surv.m-CRA killed undifferentiated hPSCs more efficiently than Tert.m-CRAs; both m-CRAs exhibited efficient viral replication and cytotoxicity in undifferentiated hPSCs, but not in co-cultured differentiated normal cells. In addition, Pre-infection of hPSCs with m-CRA abolished in vivo teratoma formation following hPSC implantation into mice. Thus, we demonstrate that m-CRAs, in particular Surv.m-CRAs, are potentially useful as both potent anticancer drugs and as novel anti-tumorigenic agents in hPSC-based regenerative medicine.

W2105

STUDYING THE IMPACT OF RET MUTATIONS IN HIRSCHSPRUNG DISEASE PATHOGENESIS USING INDUCED PLURIPOTENT STEM CELLS

Lau, Sin-Ting, Lai, Frank, Tam, Paul Kwong-hang and **Ngan, Elly**, The University of Hong Kong, Hong Kong, Hong Kong

Hirschsprung (HSCR) disease is a complex congenital disorder and is attributed to failure of enteric neural crest cells (NCCs) to fully colonize the bowel, leading to bowel obstruction and megacolon. Genetic lesions that affect NCC development (proliferation/survival, migration and differentiation) may lead to HSCR disease. RET/GDNF signaling is crucial for ENCC development and RET is the most predominant susceptibility gene for HSCR. Genetic mutations in coding (CD) and non-coding (NCD) regions of *RET* are respectively associated with the severe (total colonic aganglionosis, TCA) and mild (short segment, S-HSCR) forms of HSCR. In addition, many of these genetic lesions directly lead to reduced or defective RET/GDNF signaling, suggesting that these mutations may impair NCC development. The goal of the studies is to in-

vestigate how these *RET* CD and NCD mutations affect NCC development. Recently, we have successfully established and characterized three induced pluripotent stem cell (HSCR-iPSC) lines from patients who presented with S-HSCR and TCA, harboring *RET* NCD and CD variant/mutations, respectively. Moreover, we have used CRISPR/CAS9 system to generate an isogenic mutant iPSC with homologous deletion of *RET* gene. In combination of dual inhibition of BMP and TGFβ and activation of WNT signaling, vagal NCCs (HOX3+, HOX4+ and HOX5+) were obtained from these human iPSC lines. Consistently, NCCs derived from all these diseased iPSC lines carrying *RET* mutations showed defects in migration and neuronal differentiation. Importantly, the expression of genes involved in migration and neuronal differentiation was dysregulated, accompanied by reduced RET expression. These results suggest that RET/GDNF signaling is crucial for both differentiation and migration of human NCCs and its disruption may lead to HSCR disease.

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W2107

REVERSION OF FMR1 METHYLATION AND SILENCING BY EDITING THE TRIPLET REPEATS IN FRAGILE-X iPSC-DERIVED NEURONS

Park, Chul-Yong[1], Halevy, Tomer[2], Lee, Dongjin R.[1], Sung, Jin Jea[1], Lee, Jae Souk[1], Yanuka, Ofra[2], Benvenisty, Nissim[2] and Kim, Dong-Wook[1,3], [1]Yonsei University College of Medicine, Seoul, Korea, South, [2]Hebrew University, Jerusalem, Israel, [3]Yonsei University College of Medicine, Seoul, Korea

Fragile X syndrome (FXS) is the most common form of inherited intellectual disability, resulting from a CGG repeat expansion in the fragile X mental retardation 1 (*FMR1*) gene. Here we report a strategy for CGG repeat correction using CRISPR/Cas9 for targeted deletion in both embryonic stem cells and induced pluripotent stem cells derived from FXS patients. Following gene correction in FXS induced pluripotent stem cells, *FMR1* expression was restored and sustained in neural precursor cells and mature neurons. Strikingly, after removal of the CGG repeats, the upstream CpG island of the *FMR1* promoter showed extensive demethylation, an open chromatin state, and transcription initiation. These results suggest a silencing maintenance mechanism for the *FMR1* promoter that is dependent on the existence of the CGG repeat expansion. Our strategy for deletion of trinucleotide repeats provides new insights into the molecular mechanisms of FXS and future therapies of trinucleotide repeat disorders.

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W2109

NEURONS AND GLIAL CELLS GENERATED FROM INDUCED PLURIPOTENT STEM CELLS OF PATIENT WITH MUCOPOLYSACCHARIDOSIS TYPE II AS A MODEL OF CNS INVOLVEMENT

Rybova, Jitka[1], Ledvinova, Jana[1], Sikora, Jakub[1], Knesplova, Irena[1], Kuchar, Ladislav[1] and Dobrovolný, Robert[2], [1]First Faculty of Medicine, Charles University in Prague, Prague 2, Czech Republic, [2]Charles University, Prague, Czech Republic

Mucopolysaccharidosis type II (MPSII, Hunter syndrome) is an X-linked recessive lysosomal storage disorder caused by mutations in the iduronate-2-sulfatase gene (IDS*,* Xq28). The emzymatic defect of IDS leads to an accumulation of two main glycosaminoglycans (GAG) - dermatan sulfate and heparan sulfate in tissues throughout the body leading to skeletal deformities, hearing loss, airway obstruction, hepatosplenomegaly, cardiomyopathy and progressive neurological impairment. Previous studies in MPSII mouse model focused on the mechanism of the CNS pathology of the disease. However, it has been repeatedly shown that mouse models does not always reflect human disorders accurately. Modeling human diseases with induced pluripotent stem cells (iPSC) has remarkable potential to generate new insights into understanding disease pathogenesis and to study possible therapeutic approaches. Here we report successful reprograming of patient's blood cells with MPSII into iPSC by non-integrating Sendai virus protocol. Generated iPSC expressed pluripotency markers and can be differentiated to all three germ layer. We differentiated generated iPSC into neurons and glial cells for pathophysiology studies due to poor understanding of GAG storage pattern in human neural cell types and inpact of enzyme replacement therapy (ERT) on these cells. Immunofluorescent analysis confirmed presence of β-Tubulin III and MAP2 positive neurons, GFAP positive astrocytes and CNPase positive oligodendrocites. Lysosomal aggregation was detected in all neural-iPSC of patient by co-localization of neural markers with lysosomal marker Lamp1 and Cathepsin D. Analysis by electron microscopy showed lysosomal abnormalities mainly in glial-iPSC of patients compared to control. Quantitative determination of GAG by photometric methods revealed moderate increase in GAG level in neural-iPSC with deficient IDS activity.

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W2111

XENO-FREE SUSPENSION CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELLS

Shoji, Shinichiro[1], Yanagihara, Kana[1], Furue, Miho Kusuda[2] and Tsukahara, Masayoshi[3], [1]Kyowa Hakko Bio Co., Ltd, Tokyo, Japan, [2]National Institutes of Biomedical Innovation, Health and Nutrition, Ibaraki Osaka, Japan, [3]Kyowa Hakko Kirin Co.,Ltd., Tokyo, Japan

Human induced pluripotent stem cells (hiPSCs) can be routinely maintained on extracellular matrix (ECM)-coated surface without feeder cells. Recently, several methods have been reported to culture hiPSCs in three-dimensional suspension culture. These studies, however, rely on media that have been optimized for culturing hiPSCs on two-dimensional ECM-coated surface and require frequent medium change to remove Rho-associated protein kinase (ROCK) inhibitor. Here, we developed novel xeno-free culture media optimized specifically for suspension culture of hiPSCs. Interestingly, these hiPSCs in suspension culture exhibited higher expression of several key pluripotency markers than hiPSCs maintained on an ECM-coated plate, suggesting distinct intracellular signaling of hiPSCs in floating cell aggregates. Addition and removal of ROCK inhibitor is not required, reducing effort of medium change.

W2113

THE DEVELOPMENT AND CHARACTERIZATION OF HUMAN IPSCS DERIVED MYOFIBROBLASTS

Suyama, Satoshi, Hiroto, Miwa, Takahashi, Koji and Takemoto, Hiroshi, SHIONOGI&CO.,LTD., Osaka, Japan

Neural Crest Cells (NCCs) are the source of the peripheral nervous system, craniofacial tissues and adult tissue-specific stem cells such as those found in the kidneys. These adult tissue-specific stem cells contribute to tissue homeostasis and regeneration. A misregulation of adult tissue-specific stem cells causes a variety of diseases. It was reported that myofibroblasts which lead to development of fibrosis are derived from tissue specific stem cells like mesenchymal stem cells. In this study, we made myofibroblasts form Neural Crest-Like Cells (NCLCs) which are derived from human iPSCs. Expression of Col1a1 in human iPSCs derived myofibroblasts was very high, and was increased by TGF-beta stimulation. These results indicate that human iPSCs derived myofibroblasts can be useful tools for studying fibrosis in vitro.

W2115

GLUTAMINE OXIDATION IS ESSENTIAL FOR CELL SURVIVAL OF HUMAN PLURIPOTENT STEM CELLS

Tohyama, Shugo, Fujita, Jun, Hishiki, Takako, Tanosaki, Sho, Someya, Shota, Hattori, Fumiyuki, Suematsu, Makoto and Fukuda, Keiichi, Keio University School of Medicine, Tokyo, Japan

Human pluripotent stem cells (PSCs) such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) uniquely depend on aerobic glycolysis to generate adenosine triphosphate (ATP). However, the importance of oxidative phosphorylation (OXPHOS) has not been elucidated. Minute research of amino acid profiles revealed that glutamine oxidation was essential for survival of human PSCs. Under glucose- and glutamine-depleted conditions, human PSC quickly died due to loss of ATP. The metabolome analyses using [U-^{13}C]-labeled glucose or [U-^{13}C]-labeled glutamine unraveled that human PSCs poorly oxidize pyruvate and that glutamine was a main energy source for OXPHOS, because human PSCs poorly expressed aconitase 2 (ACO2) and isocitrate dehydrogenase 2/3 (IDH2/3) which were important enzymes to utilize pyruvate-derived citrate in the tricarboxylic acid cycle. In contrast, human PSC-derived differentiated cardiomyocytes with mature mitochondria were able to take advantage of lactate-derived pyruvate to synthesize ATP via OXPHOS under glucose- and glutamine-depleted conditions with lactate because of higher expression of ACO2 and IDH2/3. Interestingly, the metabolome analysis using [U-^{13}C]-labeled lactate showed cardiomyocytes could produce glutamate and glutamine from lactate under these conditions. This distinguished metabolic feature of human PSCs allows us to prepare clinical-grade cell sources by eliminating residual undifferentiated stem cells and purifying differentiated cardiomyocytes, which prevents tumor formation in stem cell therapy using human PSCs.

W2117

AUTOMATIC AND NON-INVASIVE IPSC EVALUATION SYSTEM BASED ON IMAGE ANALYSIS OF THE MICRO CELL STRUCTURES

Wakui, Takashi[1], Matsumoto, Tsuyoshi[1], Matsubara, Kenta[1], Kawasaki, Tomoyuki[2], Yamaguchi, Hiroshi[1] and Akutsu, Hidenori[2], [1]FUJIFILM, Kanagawa, Japan, [2]Natl Ctr for Child Health & Development, Tokyo, Japan

Recently, the need for automatic induced pluripotent stem cells (iPSCs) production system is rapidly increased due to the expansion of the iPSC research for regenerative medicine and new drag discovery. Although an automatic cell evaluation method is crucial for producing scalable and uniform iPSCs in large quantity, the evaluation

method still remains as a manual way by visual inspection of operators. Visual inspection causes inter- and intra-observer variations and leads to a bottleneck in expansion and industrialization of iPSC production. To overcome these limitations, we have developed an automatic and non-invasive iPSC evaluation system based on the image analysis of the micro cell structures such as nucleoli and nuclei, which are observation targets by stem cell experts in conventional visual inspection. The image recognition techniques realize the highly sensitive and robust detection of microscopic features of iPSCs and the quality index of iPSCs is calculated by the detected features. In this study, we examined our developing iPSCs evaluation system using several iPSC lines. The multi quality indices of human iPSCs were accessed for cellular morphology and stem cell-surface marker (SSEA4, TRA1-81) expression. Our results demonstrated that the iPSCs evaluation system produced reliable correspondence with the visual scores for all human iPSC lines. Thus, our providing the micro cellular structural features would be valuable to detect cellular quality and to automatic scalable expansion system. Integrating this evaluation system with automatic culturing equipments, a fully-automated production of iPSC can be realized. This technology can be widely utilized in scalable expansion of human pluripotent stem cells for further application.

W2119

ABNORMAL MITOCHONDRIAL TRANSPORT AND MORPHOLOGY AS EARLY PATHOLOGICAL CHANGES IN HUMAN MODELS OF SPINAL MUSCULAR ATROPHY

Xu, Chong-Chong[1], Denton, Kyle[1], Wang, Zhi-Bo[1], Zhang, Xiaoqing[2] and Li, Xue-Jun[1,3], [1]University of Connecticut Health Center, Farmington, CT, U.S., [2]Tongji University, Shanghai, China, [3]Stem Cell Institue University of Connecticut, Farmington, CT, U.S.

Spinal muscular atrophy (SMA), the leading genetic cause of infant mortality, is caused by mutations in the survival of motor neuron 1 (*SMN1*) gene, which leads to specific degeneration of spinal motor neurons and subsequent muscle atrophy. How the deficiency of SMN, a ubiquitously expressed protein, leads to specific degeneration of spinal motor neurons remains unknown. Here, we examined the role of SMN in mitochondrial axonal transport and morphology in human motor neurons by generating SMA type 1 patient-specific induced pluripotent stem cells (iPSCs) and differentiating these cells into spinal motor neurons. The initial specification of spinal motor neurons was not affected, but these SMA spinal motor neurons specifically degenerated following long-term culture. Moreover, at an early stage in SMA spinal motor neurons, but not in forebrain neurons, mitochondrial number, area, and transport were significantly reduced in axons. Knocking down of SMN expression led to similar mitochondrial defects in spinal motor neurons derived from human embryonic stem cells, confirming that SMN deficiency results in impaired mitochondrial dynamics. Finally, the application of N-acetylcysteine (NAC) mitigated the impaired mitochondrial transport and morphology, and then rescued motor neuron degeneration in SMA long-term cultures. Furthermore, NAC ameliorated the reduction in mitochondrial membrane potential in SMA spinal motor neurons, suggesting that NAC might rescue apoptosis and motor neuron degeneration by improving mitochondrial health. Together, our data demonstrate that SMN deficiency results in abnormal mitochondrial transport and morphology and subsequent reduced mitochondrial health, which are implicated in the specific degeneration of spinal motor neurons in SMA. In the future, a deeper understanding of how and why mitochondrial dynamics and function are impaired will provide valuable insights into identifying potential therapeutics for SMA.

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W2121

THERAPEUTIC POTENTIALS OF EQUINE IPS CELLS AND MSCS FOR MUSCULOSKELETAL DISEASES IN MICE AND HORSES

Lee, Eun-Mi[1], Kim, Ah-Young[2], Lee, Eun-Joo[1] and **Jeong, Kyu-Shik**[1], [1]Kyungpook National University, Daegu, Korea, South, [2]Kyungpook Nattional University, Daegu, Korea, South

Musculoskeletal diseases are common in horses. Stem cells have gained attention due to their potential as therapeutic agents for musculoskeletal injuries and disorders. In this study, we successfully generated equine iPSCs and applied equine stem cells, including MSCs and iPSCs, for the musculoskeletal therapy. Equine ASCs were reprogrammed into iPSCs using polycistronic lentiviral vector and tested them for pluripotent characteristics. Subsequently, established E-iPSCs were transplanted into muscle-injured rag/mdx mice. Histopathology showed that E-iPSCs transplanted mice exhibited enhanced muscle regeneration compared to controls. In addition, E-iPSC-derived myofibers were observed in injured muscles, although undifferentated cells also existed. And finally, to investigate therapeutic potential of equine stem cells in horses, equine MSCs and E-iPSCs transplanted into skeletal system-injured horses. Symptom and condition were generally improved in all horses. In conclusion, in this study, we showed that E-iPSCs successfully generated respectively from equine ASCs, and showed transplanted equine MSCs and E-iPSCs into injured musculoskeletal

system have capacity to induced musculoskeletal regeneration, although challenges remain for safety assurance.

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IPS CELLS: DIRECTED DIFFERENTIATION

W2127

HIGHLY EFFICIENT DIFFERENTIATION OF PORCINE INDUCED PLURIPOTENT STEM CELLS TO NEURAL PROGENITOR CELLS REQUIRES SYNERGISTIC CONTRIBUTION OF DUAL SMAD INHIBITION AND HIGH LOCALIZED CELL DENSITY

Kim, Eunhye and Hyun, Sang-Hwan, College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea, South

Neural progenitor cells (NPCs) derived from induced pluripotent stem cells (iPSCs) are promising candidates for autologous neural cell therapy. However, prior to transplantation, preclinical study of safety and efficacy are needed in large animal models such as pigs which have similar neural anatomy and physiology. In this study, we focused on the influence of initial cell density and relevant signaling pathways during neural commitment of porcine iPSCs into NPCs. The porcine iPSCs seeded at different densities (low: 40,000- ; mid: 60,000- ; high: 80,000 cells per cm^2) to neural induction medium containing the dual SMAD inhibitors (dSMADi), SB431541 (TGF-b inhibitor) and LDN 193189 (BMP4 inhibitor) to promote neural induction. After neural induction using dSMADi, the high density group showed significantly higher number of colonies than the low and mid density group (0.8 ± 0.4 and 2.4 ± 0.5 vs. 10.2 ± 0.9, P< 0.01). By day 14; 81.9 % of high density group treated dSMADi derived colonies containing robust neural rosette (NR) structures while low density group failed to form neural rosettes. The mRNA expression of pluripotency marker (*NANOG*) which was clearly detected in porcine iPSCs was found to significantly decrease in the iPSC-derived neural rosettes of control group and disappeared in those of dSMADi group. The NR structures derived from high density group treated dSMADi showed representative NR markers such as *PLAG1* and neural stem cell markers such as *NESTIN* and *VIMENTIN*. The mRNA expression levels of markers (astrocyte marker - *GFAP*; oligodendrocyte marker - *MBP*; and neuronal marker - *TUJ1*) were detected in further differentiated cells from NR. These results highlight our ability to define an efficient method for production of porcine iPSC-derived neural precursors that mimic human neural system.

Funding Source: This work was supported by a grant from the Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ011077, PJ011288), Rural Development Administration, Republic of Korea.

W2129

INCREASED MITOCHONDRIAL FISSION CONTRIBUTES TO HIGH GLUCOSE-INDUCED ATTENUATION OF ANESTHETIC CARDIOPROTECTION IN STEM CELL-DERIVED CARDIOMYOCYTES

Bai, Xiaowen, Canfield, Scott, Zaja, Ivan, Yan, Yasheng and Bosnjak, Zeljko, Medical College of Wisconsin, Milwaukee, WI, U.S.

Hyperglycemia has been shown to attenuate cardioprotective effects of isoflurane. Previous studies have indicated that reactive oxygen species (ROS) and increased mitochondrial fission may play a role in cardiomyocyte death during ischemia-reperfusion injury. The aim of this study was to investigate, for the first time, the role of glucose-induced increase in ROS production and subsequent increase of mitochondrial fission on the attenuated anesthetic cardioprotection in a clinically relevant model of human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). Cardiomyocyte differentiation from iPSC was characterized by observing cell contractility and analyzing sarcomeric a-actinin and connexin 43 expression using immufluorescence staining. iPSC-CMs were exposed to varying glucose conditions (5, 11, 25 mM) for 24 hours. Mitochondrial permeability transition pore (mPTP) opening, cell viability, and ROS generation were used to assess the effects of various treatment conditions. Mitochondrial fission was monitored using confocal microscopy. Expression of activated dynamin-related protein 1 (Drp1), a key protein responsible for mitochondrial fission was assessed by western blot. The results showed that elevated glucose conditions (11 and 25 mM) significantly increased ROS generation, while only the 25 mM high glucose condition induced mitochondrial fission and increased the expression of activated Drp1 in spontaneously contracting iPSC-CMs. Isoflurane delayed mPTP opening and protected iPSC-CMs from oxidative stress in 5 and 11 mM glucose conditions. Scavenging ROS with Trolox or inhibiting mitochondrial fission with mdivi-1 restored the anesthetic cardioprotective effects in iPSC-CMs in 25 mM glucose conditions. Collectively, human iPSC-derived cardiomyocytes is a useful, relevant model for studying isoflurane cardioprotetion, and can be manipulated to recapitulate complex clinical perturbations. We demonstrate that the cardioprotective effects of isoflurane in elevated glucose conditions can be restored by scavenging ROS or inhibiting mitochondrial fission. These findings may con-

tribute to further understanding and guidance for restoring pharmacological cadioprotection in hyperglycemic patients.

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W2131

THE SCALABLE DERIVATION OF OFF-THE-SHELF AND GENETICALLY ENHANCED HEMATOPOIETIC CELLULAR THERAPEUTICS FROM NAIVE HUMAN INDUCED PLURIPOTENT STEM CELLS

Clarke, Raedun Laurie[1], Groff, Brian[1], Bjordahl, Ryan[2], Abujarour, Ramzey J.[1], Robinson, Megan[1], Lynn, Chris[1], Raynel, Sarah[1], Moreno, Stacey[1], Lan, Weijie[1], Lee, Tom[1], Burrascano, Michelle[1], Bonello, Greg[1], Rogers, Paul[1], Abbot, Stewart[1], Shoemaker, Dan[1] and Valamehr, Bahram[1], [1]Fate Therapeutics, Inc., San Diego, CA, U.S., [2]Fate Therapeutics, San Diego, CA, U.S.

Encouraging clinical outcomes in T and natural killer (NK) cell adoptive therapy have garnered hope and excitement. However, limitations associated with autologous lymphocyte engineering and manufacturing will most likely reduce the successful treatment of large number of patients. Harnessing the power of human induced pluripotent stem cell (hiPSC) technology to generate an "off-the-shelf" source of hematopoietic cellular therapeutics with enhanced potency through genome-engineering of persistence, targeting and safety mechanisms represents a powerful approach to make cell-based immunotherapies available to a wide range of patients. To advance the promise of hiPSC technology it is essential to efficiently and reproducibly generate the diverse repertoire of immune effector populations, through a robust and scalable process. Additionally, because the in vitro derivation of effector lymphocytes is complicated by the existence of distinct waves of blood cell formation during embryonic development, our ability to accurately recapitulate the stages of early embryonic hematopoiesis towards the definitive program is vital to the successful derivation of effector lymphoid cells. Here we describe a method for the derivation of hematopoietic cells from naïve hiPSCs in a highly scalable manner, void of an embryoid body intermediate, under serum- and feeder-free conditions. This platform represents a well-defined, small molecule-driven, staged protocol that can readily be translated to meet current good manufacturing practice requirements. The derived hematopoietic population is definitive in nature as determine by Notch dependency and exhibits multi-lineage potential, as demonstrated through the formation of functional T and NK lymphoid cells. Hematopoietic cells generated can be successfully cryopreserved and banked, serving as a highly-stable feedstock for subsequent derivation of various cell types for therapeutic use. Furthermore, we demonstrate that when properly designed, hiPSC-level engineered modalities such as controllable safety systems, continue to remain functional during the hematopoietic differentiation process. Our study indicates that ground state hiPSCs are an ideal source for "off-the-shelf" hematopoietic cell-based immunotherapies.

W2133

ASTROCYTE PATHOLOGY IN A CRISPR/CAS9 ISOGENIC IPS CELL MODEL OF FRONTOTEMPORAL DEMENTIA CAUSED BY MUTANT TAU PROTEIN

Hargus, Gunnar[1,2], Hallmann, Anna-Lena[2], Arauzo-Bravo, Marcos J.[3,4], Ehrlich, Marc[2], Schöler, Hans R.[5], Kuhlmann, Tanja[2] and Zaehres, Holm[5], [1]Columbia University, New York, NY, U.S., [2]University of Muenster, Muenster, Germany, [3]Basque Foundation for Science, Bilbao, Spain, [4]Biodonostia Health Research Institute, San Sebastián, Spain, [5]Max Planck Institute for Molecular Biomedicine, Muenster, Germany

Mutations in the *MAPT* gene encoding the microtubule-associated protein TAU can cause frontotemporal dementia (FTD), a group of neurodegenerative diseases leading to early-onset dementia. These *MAPT* mutations result in excessive accumulation of phosphorylated TAU protein within neurons but also glial cells in various brain areas including the frontal and temporal cortex and the substantia nigra leading to progressive degeneration at these sites. We have recently shown that induced pluripotent stem cell-derived neurons from FTD patients with *MAPT* mutations demonstrate pronounced TAU pathology, disturbed neurite outgrowth and increased oxidative stress. Here, we investigated the role of astrocytes on neural degeneration by applying an isogenic stem cell model of FTD. We applied CRISPR/CAS9 technology to repair the pathogenic N279K MAPT mutation in iPS cell-derived neural stem cells and differentiated mutation-carrying and gene-corrected progenitor cells into mature astrocytes. Both groups showed an equal astrocyte differentiation potential and similar functional characteristics as evidence by comparable responses in L-glutamate uptake assays and propagation of calcium waves. However, FTD astrocytes expressed increased levels of *4R TAU* isoforms, acquired phenotypes of reactive astrocytes and exhibited changes in whole genome expression profiles. Interestingly, co-culture experiments of FTD astrocytes and healthy neurons did not reveal significant effects on neurite outgrowth but FTD astrocytes induced robust changes in gene expression profiles in previously healthy neurons. Our isogenic stem cell model of FTD thus pro-

vides a suitable platform to further elucidate the role of glial cells in the pathogenesis of FTD.

W2135

DOWNREGULATION OF A G PROTEIN-COUPLED RECEPTOR INHIBITS CARDIOMYOCYTE DIFFERENTIATION WHILE POTENTIATES ENDOTHELIAL CELL DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

Jha, Rajneesh[1], Singh, Monalisa[2], Wu, Qingling[2,3], Preininger, Marcela K[2,3] and Xu, Chunhui[2,3], [1]Emory University School of Medicine, Atlanta, GA, U.S., [2]Division of Pediatric Cardiology, Department of Pediatrics, Emory University School of Medicine and Children's Healthcare of Atlanta, Atlanta, GA, U.S., [3]Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, GA, U.S.

Differentiation of human pluripotent stem cells (hPSCs) to cardiomyocytes and endothelial cells is tightly regulated by signaling molecules and transcription factors. Hence, understanding molecules involved in their lineage commitment and differentiation is important for exploring their potential use for cell therapy and drug testing. Here, we have identified a G Protein-coupled receptor (GPCR) that is transiently upregulated during early stages of growth factor-mediated cardiomyocyte differentiation from hPSCs. Knockdown of the GPCR using short hairpin RNA (shRNA) did not affect undifferentiated cell morphology, growth and expression of pluripotency markers, but reduced the expression of mesodermal and endodermal lineage marker expression upon the growth factor induction. Moreover, knockdown of the GPCR also significantly reduced the expression of key cardiac transcription factors and other cardiomyocyte-associated genes, leading to poor differentiation. Interestingly, knockdown of the GPCR directed mesoderm towards endothelial cells as shown by upregulated expression of endothelial cell markers. Differentiation of endothelial cells was further confirmed by in vitro functional assays including their ability to form capillary-like structures and uptake acetylated low density lipoproteins. These data suggest that the GPCR is an important molecule for both cardiac and endothelial cell lineage commitment.

W2137

MODELING THE BLOOD-BRAIN BARRIER OF ADRENOLEUKODYSTROPHY (X-ALD) PATIENTS USING DIRECTED DIFFERENTIATION OF HUMAN iPSCs INTO BRAIN MICROVASCUALAR ENDOTHELIAL CELLS (BMECS)

Lee, Catherine[1], Azarin, Samira[2] and Tolar, Jakub[1,3], [1]University of Minnesota, Minneapolis, MN, U.S., [2]University of Minnesota, Minnepolis, MN, U.S., [3]Stem Cell Institute, University of Minnesota, Minneapolis, MN, U.S.

X-linked adrenoleukodystrophy (X-ALD) is an inherited metabolic storage disorder caused by mutations in the ABCD1 gene. ABCD1 is a peroxisomal transporter protein responsible for transporting very long-chain fatty acids (VLCFAs) from the cytosol into the peroxisome for subsequent degradation. Accumulation of VLCFAs causes demyelination in the long tracts of the spinal cord and the progressive axonopathy seen in adrenomyeloneuropathy (AMN). A more severe phenotype that only affects males is cerebral ALD (CALD). CALD is characterized by rapid inflammatory demyelination in the brain and death within a few years. The molecular mechanisms responsible for the onset and progression of CALD remain poorly understood. Due to the absence of biomarkers to predict this conversion, MRI of the brain remains the only tool to detect the progression at an early state. We are using a previously published method for directed differentiation of human pluripotent stem cells into blood brain barrier (BBB) endothelium with iPSCs from patients with CALD. This protocol includes a singularized iPSC seeding approach that greatly enhances the reproducibility of this model. Key questions include (1) are there differences in BBB integrity and (2) BMEC gene expression between patients with CALD and wild type (WT) controls. qRT-PCR and immunocytochemistry of the BMEC markers claudin-5, GLUT-1, PECAM-1, occluding, and p-glycoprotein are used to assess BMEC cell identity. To assess barrier integrity across these cells we are using trans endothelial electrical resistance (TEER) and a sodium fluorescein permeability assay. Preliminary results indicate decreased TEER and increased sodium fluorescein permeability of BMECs derived from CALD-iPSC compared to WT-iPSC. To investigate differences in gene expression, we will be performing RNA-seq of these BMECs to identify potential biomarkers contributing to the onset of CALD. Additionally, the BBB represents an additional challenge for therapies that target the brain in patients with X-ALD. This protocol provides us with a renewable source of BBB endothelium from X-ALD patients that can be used as a disease model to test chemicals or enzymes that can cross the BBB of X-ALD patients to halt the progression of this disease.

W2139

DERIVATION AND FACS-MEDIATED ISOLATION OF RETINA PROGENITORS FROM HUMAN PLURIPOTENT STEM CELLS

Muniz, Alberto and Barberi, Tiziano, Texas Biomedical Research Institute, San Antonio, TX, U.S.

Human pluripotent stem cells (hPSCs) constitute a promising resource for use in cell-based therapies and a valuable in vitro model for studying early human development and disease. There is a lot of interest in using these cells to derive retinal elements such as Retinal Pigmented Epithelium (RPE) and neural Retinal Progenitor Cells (RPCs) that may provide a source of specialized retinal cells. While isolation of RPE has been achieved and hPSC-derived RPE cells are currently used in clinical trials, major limitations in the advancement towards translational and pre-clinical testing in retinal disease include the specific selection and expansion of RPCs from heterogeneous cell populations. To fill this gap, we have derived RPCs under specific hPSC culture conditions that allow these progenitors to be detectable within 3 weeks of differentiation. Significantly, using a three-color FACS sorting strategy we were able to isolate them as Pax6+/Chx10+ RPCs from the rest of the differentiating cells. Purified RPCs have been maintained and passaged for up to14 weeks in post-sorting cultures. These cells then showed differentiation toward multiple retinal cell types including Muller, Bipolar, Amacrine, Horizontal, Ganglion and photoreceptors. Our study demonstrates that RPCs can be isolated from a heterogeneous cell population of differentiated hPSCs and remain viable. Additionally, these RPCs retain the ability to generate all retina subtype cells. Carefully selected and expanded RPCs will provide platforms to develop drug discovery and therapeutic strategies for retinal degenerative disease.

W2141

ESTABLISHMENT OF A MODY DISEASE MODEL IN INDUCED PLURIPOTENT STEM CELLS USING CRISPR

Rasmussen, Mikkel[1], Honoré, Christian[2], Holst, Bjoern[3], Schmid, Benjamin[3], Hansson, Mattias[2] and Clausen, Christian[3], [1]Bioneer A/S, Hørsholm, Denmark, [2]Novo Nordisk, DK-2760 Måløv, Denmark, [3]Bioneer A/S, Hoersholm 2970, Denmark

Maturity onset diabetes of the young (MODY) is a rare inherited form of diabetes leading to impairment of beta cell function before the age of 25. The most common form of MODY is caused by the duplication of a cytosine at position 872 in the HNF1a gene (MODY3; P291fsinsC), which is believed to lead to nonsense mediated decay (NMD) and haploinsufficiency. In this study, the HNF1a P291fsinsC mutation was introduced into induced pluripotent stem cells (iPSCs) derived from a healthy individual by use of CRISPR-Cas9 technology. Restriction fragment length polymorphism and sequencing analysis was used to identify a heterozygous and a homozygous HNF1a P291fsinsC line. The gene-edited iPSC lines displayed a normal karyotype, and the top three top most likely off-target sequences were unaffected. To investigate the disease etiology in more detail, the gene edited and the wild-type iPSC lines were differentiated toward pancreatic endoderm (PE). Flow cytometry analysis showed that 75-85% co-expressed the PE markers PDX1 and NKX6.1 at this stage, which was confirmed by immunocytochemistry. qPCR analysis showed that HNF1a transcripts were significantly downregulated in the heterozygous (42%) and the homozygous (38%) HNF1a P291fsinsC lines compared to the wild-type line, whereas transcript levels of HNF4a, PDX1 and NKX6.1 remained unchanged. Attempts to rescue HNF1a transcript levels by treatment with various NMD inhibitors are currently ongoing. The presented cellular model could be useful for elucidating disease mechanisms related to early-onset diabetes.

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W2143

STUDYING THE FUNCTION OF HUMAN HIPPOCAMPAL NEURONS IN A DISH

Sarkar, Anindita, Mei, Arianna, Paquola, Apua, Bardy, Cedric, Kim, Hyung Joon, Japelli, Roberto and Gage, Fred H., Salk Institute for Biological Studies, La Jolla, CA, U.S.

The hippocampus is the site of learning and memory in the brain and a growing body of evidence suggests some dysfunctions in the hippocampal circuitry of patients affected by psychiatric and neurological disorders. More specifically, synaptic transmission deficits in hippocampal neurons have been implicated in schizophrenia, major depression disorder, and bipolar disorder. The availability of human ES and pluripotent stem cells (hPSCs) offers an opportunity to generate lineage-specific neuronal subtypes to investigate mechanisms of human diseases in brain regions such as the hippocampus. In this study, we investigated the development and functioning of a human hippocampal neuronal circuitry derived from human ES cells. We utilized a two-step human stem cell based, directed differentiation protocol to generate hippocampal neurons in a monolayer culture. In the first step, we have used HuES6 cells to generate neuronal progenitor cells (NPC) with forebrain-specific identities. In the second step, we further differentiated these NPCs to generate a neuronal culture enriched in mature hippocampal neurons. We confirm that these neurons in culture express appropriate molecular markers, and proven to

be active as they fire mature spontaneous and evoked action potentials. We used microfluidic devices, as an in vitro platform that allowed us to study synaptic connectivity between neurons. To identify the nature of different neuronal hippocampal types, we have developed promoter specific lentiviral reporters. We demonstrated the establishment of a functional neuronal network as read by time-lapse multielectrode extracellular electrophysiology recordings during differentiation. We show that this model recapitulates many of the features of hippocampal neuronal circuitry. The ability to mimic the developmental process of disease-relevant cell types in an in vitro setting is important for providing insights into the mechanisms of neurodevelopmental disorders. Furthermore, our finding is relevant to the factors regulating neuronal network as it offers mechanistic insight into the development of lineage-specific network and represents a promising tool for future disease modeling studies.

Funding Source: Bob and Mary Jane Engman Laboratory for Schizophrenia Research, NARSAD, Takeda Pharmaceuticals International, Inc. and a training Grant (TG2-01158) from the California Institute of Regenerative Medicine

W2145

ENGINEERING PATIENT SPECIFIC NEURAL TISSUE USING CELL PERMEABLE TRANSCRIPTION FACTORS

Willerth, Stephanie[1], Robinson, Meghan[1] and Vaidyanathan, Ranjani[2], [1]University of Victoria, Victoria, BC, Canada, [2]iProgen Biotech, Vancouver, BC, Canada

Pluripotent stem cells can become any cell type found in the body. However, one of the major challenges when working with pluripotent stem cells is producing a highly homogenous population of differentiated cells which can then be used for various applications such as cell therapies or drug screening. Previous work has shown that overexpression of the transcription factor Asc1 using viral vectors can reprogram fibroblasts directly in neurons. Asc1 plays a key role in neural development and here we characterize how recombinant Asc1 functionalized with intracellular protein delivery technology (Asc1-IPTD) can be used to influence human induced pluripotent stem cells (hiPSCs) to differentiate into neurons. We evaluated a range of protein concentrations to determine the most effective concentration for generating neurons from hiPSCs cultured in DMEM/F12 media. Next, we looked at the frequency of Asc1-IPTD supplementation on differentiation and found that one time supplementation is sufficient enough to trigger the neural differentiation process. We also found that the Asc1-IPTD was efficiently taken up by the hiPSCs and by 7 days, enabled rapid differentiation into TUJ1-positive and NeuN-positive population with neuronal morphology. The neurite length and branch points were higher than in neural progenitor population obtained from using a neural induction protocol. The results are reproducible, with no additional trophic factors and neural enrichment media required. This work validates Asc1-IPTD as a powerful tool for engineering neural tissue from pluripotent stem cells. We are currently evaluating if this novel protein construct can directly reprogram human fibroblasts into neurons.

Funding Source: Natural Sciences and Engineering Research Council

W2147

GENERATION OF RETINAL PHOTORECEPTORS FROM CGMP-MANUFACTURED HUMAN IPSC LINE

Zhu, Jie, Cifuentes, Helen, Reynolds, Joseph and Lamba, Deepak, The Buck Institute for Research on Aging, Novato, CA, U.S.

Retinal degeneration often results in the loss of photoreceptors, which leads to permanent vision loss. Generating transplantable photoreceptors using human induced pluripotent stem cells (iPSCs) to replace lost or dysfunctional photoreceptors holds a promise to treat a variety of retinal degenerative diseases. Developing effective methods to produce retinal cells including photoreceptors using available cGMP-manufactured human iPSC lines is a critical step for advancing cell replacement therapy to clinical application. This study aims to make transplantable photoreceptors using a cGMP-manufactured iPSC line. The generated retinal cells were tested for their differentiation capability and integration in a host mouse retina. An iPSC line, derived from CD34+ cord blood cells in cGMP-compliant conditions at Lonza Bioscience, was obtained from Dr. Zeng's lab. The iPS cells were directed to gain retinal cell fate via a modified version of our previous directed differentiation protocol (Lamba et al 2006). The cells were induced to optic field stage for 1 week using small molecules to inhibit Wnt, BMP and TGFβ signaling pathways along with IGF-1 in culture medium. The cells were then expanded to generate neuro-retina and RPE. Neuro-retinal progenitor cells were manually isolated from RPE and expanded further to a relatively pure population of retinal neurons including differentiated photoreceptors by 3 months of culture based on expression of PAX6, OTX2, TUJ-1, CRX and AIPL1. The GFP labeled retinal cells were injected into the subretinal space of 4-6 week old IL2rγ$^{-/-}$ recipient mice to analyze their ability to integrate into the ONL layer of host retina. The eyes were collected at 2 months post transplantation for analysis by ICC. We observed robust integration of photoreceptors with typical mature photoreceptor morphology and presence of Otx2, Recoverin, and Rhodopsin markers in the integrated cells. This study provides strong evidence that transplantable photoreceptors can be generated from a

cGMP-manufactured human iPS cell line which could then be fast-tracked to the clinic.

T2149

PREDICTING HEPATIC, MESENCHYMAL AND CARDIAC STEM CELL FATE THROUGH GLOBAL PROTEIN EXPRESSION PROFILE IN HUMAN PLURIPOTENT STEM CELLS

Laco, Filip, Woo, Tsung Liang and Oh, Steve, Bioprocessing Technology Institute, Singapore, Singapore

Differentiation of human pluripotent stem cells (hPSCs) can be induced via a small molecule that inhibits GSK (CHIR99021). Dose dependent addition of growth factor BMP-4 showed differentiation into all germ layers including certain endoderm and mesoderm linages such as cardiac, muscle-mesenchymal and hepatic. While reproducibility was demonstrated with embryonic stem cell lines, it has been observed that quantitative differences between induced pluripotent stem cell lines and embryonic stem cells lead to unpredictable and often low differentiation results, as shown with cardiac differentiation. hPSCs genomic variations of germ-layer associated genes were shown to predict their potential to induce neural specification. Therefore, we believe and demonstrate that predispositions of endo-mesoderm consecutive development to cardiac, mesenchymal or hepatic linage is assessable at the proteome level in stem cells for a single step GSK inhibition differentiation protocol. We differentiated over 10 hPSCs from different origins and analysed >100 protein levels and cell populations before and during the differentiation period. Our Data shows that the cell lines can be easily directed with high to low CHIR99021 concentration from cardiac to mesenchymal and hepatic linage. The cell lines have individual dose dependencies; a few cell lines are restricted in their lineage diversion. Further, our preliminary data shows that variation in several protein markers among them pluripotency marker (Nanog) correlate with the cardiac differentiation results. Our data demonstrates that single GSK inhibition can robustly induce endo- and mesoderm linages differentiation such as cardiac, mesenchymal and hepatic in many platforms. Further, we indicated potential markers for cardiogenic predisposition that can be assessed at the proteome level. Moreover, we hypothesize that the measured differences in protein levels of hPSCs could be an indicator for the small molecule doses driven linage differentiations.

IPS CELLS: EPIGENETICS

W2151

COMPARATIVE FUNCTIONAL PROFILING OF HUMAN STEM CELLS

Gunes, Ceren[1], Paszkowski Rogacz, Maciej[1], Khattak, Shahryar[2], Dahl, Andreas[2] and Buchholz, Frank[1], [1]TU Dresden, Medical Faculty, Dresden, Germany, [2]CRTD / DFG-CTR for Regenerative Therapies Dresden - Cluster of Excellence, Dresden, Germany

Regenerative medicine aims to regenerate or replace tissues or organs, which cannot function properly due to damage, aging, etc. Characteristics of stem cells, self-renewal and differentiation, render stem cell research one of the main foci of regenerative medicine. Beyond most of the stem cell research done on model organisms, no previous study applied a systematic comparison on human stem cells (hSC), due to great genetic diversity between individuals. Hence we perform a comparative study by genome-wide RNAi screens on human hematopoietic stem cell (HSC), iPSC, and neural stem cell (NSC). To be able to have cells with the same genetic background, HSC derived from a healthy donor, are reprogrammed to iPSC, which are differentiated to NSC. After obtaining these three hSC, RNAi screens on each cell type are performed employing a pooled epigenetic shRNA library. Cells, infected with pooled shRNA library, are selected for their cell-specific markers and transduction control marker. Sorted cells are analyzed by next generation sequencing. Genes being up- or down-regulated over time are selected in two ways: change in gene expression within the cell type (stem cell vs. differentiated), or among three cell types, showing which genes are being specifically or commonly expressed. So far the RNAi screen on HSC and iPSC were performed, and our preliminary data suggests several strong candidates, being differentially expressed in CD34+ vs. the differentiated population (iPSC screen data being analyzed). Further analysis and validation experiments will be performed for a deeper investigation.

Funding Source: Sonderforschungsbereich (SFB) 655

W2153

BMP4 IS A MODIFIER OF FBXL10-PRC1 COMPLEX IN SOMATIC CELL REPROGRAMMING

Zhou, Wei Zhi[1], Liu, Jing[1], Yang, Xuejie[1], Wang, Xiaoshan[1], Chen, Jiekai[2] and Pei, Duanqing[1], [1]Guangzhou Institutes of Biomedicine and Health, Guangzhou, China, [2]Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, China, Guangzhou, China

The multidomain chromosomal proteins Fbxl10 and Fbxl11 are in the same protein family. Both of them have many protein domains including Jmjc, CxxC, PHD zinc finger, Fbox, LRR and share the similar function. However, we found that their function in reprogramming model is factor-dependent. In OKS(Oct4,Sox2,Klf4) system, Fbxl10 and Fbxl11 have similar promotion, while in Oct4 and Oct4 plus Id1(OD) system, the effect of Fbxl11 declines sharply. Futher studies demonstrate that Fbxl10 improves reprogramming in Oct4 and OD system through Fbxl10-PRC1 complex while Fbxl11 is not the member of PRC1 complex. Meanwhile, we found that BMP4 signal inhibits Fbxl10 function specifically in Oct4 and OD system, but Fbxl11 is not inhibited. The inhibition by BMP4 signal is mediated through interaction between Smads and Fbxl10. The protein interaction weakens the binding of Fbxl10 PRC1 complex to genome globally and leads to the upregulation of the PRC1 target genes which inhibits reprogramming. Therefore, these studies not only provide information for the elaborate difference between Fbxl10 and Fbxl11 which have the same protein domains, but also provide new viewpoint for the understanding of the relationship between signaling pathway and epigenetic modulation in cell fate decision.

CHROMATIN IN STEM CELLS

W2155

ROLE OF TET2 IN REGULATING AGE-RELATED DECLINE IN NEUROGENESIS AND COGNITION IN THE MOUSE BRAIN

Gontier, Geraldine[1], Iyer, Manasi[2], Wheately, Elizabeth[2] and Villeda, Saul A.[2], [1]UCSF, San Francisco, CA, U.S., [2]University of California, San Francisco, San Francisco, CA, U.S.

The discovery of neural stem cells (NSC) in the adult brain has incited possibilities for restoring cognitive dysfunction in the elderly by enhancing neurogenesis. However, advancements remain necessary in our understanding of how the normal aging process affects stem cell function in the brain. Interestingly, recent studies have identified DNA methylation as a potential driver of brain aging. A global decrease in methylation and DNA methyltransferases expression within the hippocampus during aging is associated with impaired NSC maintenance and proliferation. Furthermore, in the central nervous system DNA can be actively de-methylated by the Ten-eleven translocation family of proteins (Tet1-3). Interestingly, we detected a decrease in Tet2 expression in the adult hippocampus during aging. While Tet2 has been implicated in hematopoietic stem cell renewal and inflammation, its role in the context of adult neurogenesis or brain aging has yet to be investigated. We hypothesized that age-related decrease in Tet2 expression in the hippocampus negatively regulates adult neurogenesis and associated cognitive function. Using a lentiviral-mediated RNA interference approach, we abrogated Tet2 expression in the young adult hippocampus resulting in a decrease in NSC number and lower levels of neurogenesis. Moreover, decreased Tet2 expression elicited cognitive impairments in contextual fear conditioning and spatial learning and memory. Lastly, we made use of a temporally controlled NSC-specific conditional Tet2 knock out mouse model and observed similar hippocampal-dependent cognitive impairments after Tet2 gene excision. Collectively, these data suggest that age-related changes in Tet2 expression contribute to impairments in adult neurogenesis and associated learning and memory processes.

GERMLINE CELLS

W2159

CD49f-IGF-IR SIGNAL MEDIATES THE EXPRESSIONS OF PLURIPOTENT TRANSCRIPTION FACTOR Oct4 IN MOUSE GERMLINE STEM CELLS

Peng, Syue Wei[1], Lin, Chien-Chia[1], Kuo, Hung Chih[2] and **Huang, Yen Hua**[1], [1]Taipei Medical University, Taipei, Taiwan, [2]Academia Sinica, Taipei, Taiwan

Stem cell niche is known to regulate stem cells self-renew and differentiation. Our previous studies have demonstrated that the niche hypoxia maintains the Oct-4 level through HIF-2α-IGF-1R signal loop in mouse germline stem cells. However, how the niche extracellular matrix cooperates with hypoxia-derived signals still remains largely unknown. In this paper, we found the $CD49f^{+}AP^{+}$GSCs showed significant activity of alkaline phosphatase and expressed stemness-related genes/proteins particularly when cultivated on laminin-coated plates. Treatment of laminin or IGF-I both dose-dependently increased the expression levels of CD49f, IGF-IR, HIF-2α, and Oct4 in AP^{+}GSCs. Knockdown of endogenous IGF-1R using shRNA effectively suppressed the expressions of CD49f; and knockdown the CD49f significantly suppressed the expression of IGF-IR vice versa. Importantly, knockdown of HIF-2α not only suppressed the Oct4 expression levels,

but also dramatically suppressed the levels of CD49f as well as the IGF-IR. Furthermore, double knockdown of IGF-IR and CD49f synergistically suppressed the expressions of HIF-2α and Oct4 evidenced by western blotting and immunocytochemical staining combined with confocal image. Together with these results demonstrated that CD49f-IGF-IR-HIF-2α signaling loop maintains the Oct4 expression in early AP$^+$GSCs. Findings in this study would provide insights into niche extracellular matrix and endocrinology underlying the early pluripotent germ line development.

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W2161

MODELING HUMAN GERM CELL DEVELOPMENT BY PLURIPOTENT STEM CELLS

Wang, Yuan, East China Normal University, Shanghai, China

Infertility affects more than 15% of couples with half due to male factors. So far, the etiology of male infertility has yet to be understood, partially due to the difficulties to obtain a large quantity of diseased human gametes. Although a number of studies demonstrated successful derivation of germ cells from human pluripotent stem cells, in vitro recapitulation of germ cell development remains to be one of the most fundamental challenges in biology. In this study, we developed a serum-free and xeno-free culture condition, and successfully induced spermatogonial stem cells (SSC)-like cells and haploid cells from human embryonic stem cells. The identity of these germ cell-like populations were further confirmed by genome-wide expression analyses compared to their in vivo counterparts. More importantly, pluripotent stem cells (PSCs) from non-obstructive azoospermic (NOA) patients or PSCs treated with inhibitor for androgen receptor displayed various defects during in vitro differentiation toward germ cell lineages. Taken together, our study provides a powerful platform to mimic germ cell development in vitro and as well a useful tool to dissect molecular mechanism toward understanding of male infertility.

TOTIPOTENT/EARLY EMBRYO CELLS

W2163

INTRODUCING PRECISE GENETIC MODIFICATION INTO HUMAN 3PN EMBRYOS BY CRISPR/CAS-MEDIATED GENOME EDITING

Fan, Yong, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

As a powerful technology for genome engineering, the CRISPR/Cas system has been successfully applied to modify the genomes of various species. Here we demonstrate that CRISPR/Cas-mediated genome engineering can generate precise genetic modification in early human tripronuclear (3PN) embryos. 3PN zygotes with three pronuclei are commonly found during the in vitro fertilization process, and regarded as invariably pathological and not suitable for transplantation. By co-injecting Cas9 mRNA, gRNAs, and DNA donor, we successfully introduced the naturally occurring *CCR5Δ32* allele into early human 3PN embryos. In the embryos containing the engineered *CCR5Δ32* allele, however, the other alleles at the same locus could not be fully controlled, as they either remained wild type, or contained indel mutations. This work has implications for the development of therapeutic treatment of genetic disorders, and it demonstrates the significant technical issues remaining to be addressed. We advocate preventing any application of genome editing in the human germline until after a rigorous and thorough evaluation and discussion undertaken by the global research and ethics communities.

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W2165

IDENTIFICATION OF KEY FACTORS CONQUERING DEVELOPMENTAL ARREST OF CLONED EMBYRO BY COMBINING EMBRYO BIOPSY AND SINGLE-CELL SEQUENCING

Liu, Wenqiang[1], Liu, Xiaoyu[2], Wang, Chenfei[1], Gao, Yawei[3] and Gao, Shaorong[1], [1]Tongji University, Shanghai, China, [2]Graduate School of Peking Union Medical College, Beijing, China, [3]Tongji University, Shanghai, China

Differentiated somatic cells can be reprogrammed into totipotent embryos through somatic cell nuclear transfer (SCNT). However, most cloned embryos arrest at early stages and the underlying molecular mechanism remains largely unexplored. Here, we first developed a SCNT embryo biopsy system at 2- or 4-cell stage, which allows us to trace the developmental fate of the biopsied embryos precisely. Then through single-cell transcriptome sequencing of SCNT embryos with different developmental

fates, we identified Kdm4b which can reset the histone H3 lysine 9 trimethylation, a barrier responsible for 2-cell arrest of cloned embryos. Moreover, we discovered another histone demethylase Kdm5b, accounts for the arrest of cloned embryos at 4-cell stage through single-cell analysis. Co-injection of Kdm4b and Kdm5b can restore transcriptional profiles of SCNT embryos and greatly improve the blastocyst development (over 95%) as well as the production of cloned mice. Our study therefore provides an effective approach to identify key factors responsible for the developmental arrest of somatic cell cloned embryos.

EMBRYONIC STEM CELL DIFFERENTIATION

W2169

COMPARISON STUDY ON CHARACTERISTICS OF PLURIPOTENT STEM CELL-DERIVED RPE ORIGINATED FROM AN AMD PATIENT AND A HEALTHY DONOR

Jung, Sookyung[1], Go, Eun Sol[1], **Lee, Jeoung Eun**[1], Chung, Young Gie[2], Song, Won Kyung[3] and Lee, Dong Ryul[1], [1]CHA University, Gyeonggi-do, Korea, [2]CHA Health Systems, Los Angeles, CA, U.S., [3]CHA Bundang Medical Center, Gyeonggi-do, Korea

Age-related macular degeneration (AMD) is the major cause of blindness among old people, and is characterized by progressive degeneration and loss of retinal pigment epithelium (RPE) and photoreceptor cells in the macular region leading to loss of central, high-acuity vision. RPE transplantation might preserve or restore vision in AMD patients or in patients with other diseases in which vision loss is associated with dysfunctional RPE. Because of their proliferation and differentiation abilities, hES derived RPE cells represent a potentially unlimited resource for cell replacement therapy for AMD. Besides, transplantation of autologous RPE using patient-specific pluripotent stem cells (PSC) might be the best choice for patients because there is no risk of immune rejection. In this study, we compared the characteristics of somatic cell nuclear transfer (SCNT)-hES and hiPSC derived RPE originated from the same donor, and analyzed the RPE function between AMD patient (NT5) and healthy donor (NT4). To testify the differentiation ability of SCNT-hES and hiPS to RPE, we differentiated 2 SCNT-hESC lines (CHA-hES NT4 and NT5), 4 iPSC lines (iPS-NT4-S1 and E15, iPS-NT5-S1 and S9) and MA09 (ACT) as a hES control to RPE. The RPE cells show pigmented polygonal shape which is the typical RPE morphology. We confirmed the expression of RPE-related markers such as PAX6, BEST1, MITF, ZO1 and RPE65 in all RPE cells. In addition, these cells showed the normal state of epithelial polarization and phagocytosis activity as functional RPE cells. Although we still need to demonstrate the effect of RPE on photoreceptor cell rescue *in vivo*, our results indicate that the production of functional RPE cells can be generated from patient-specific PSC both AMD patient and healthy donor in accordance with the independence of reprogramming methods.

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W2171

EFFECTS OF HEAVY METALS ON GROWTH AND NEURONAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS

El Majdoubi, Mohammed, McDonald, Circe and Zagzoog, Monia, Dominican University of California, San Rafael, CA, U.S.

Neurotoxic effects of heavy metals on the developing brain are a major public health concern. Because of the limitations of animal-based models and traditional cell culture models of neuronal development, the mechanisms of developmental neurotoxicity are poorly understood. In recent years, embryonic stem cell (ESC)-derived neuronal models have been developed and offer distinct advantages over traditional in vivo and in vitro model systems for investigating the effects of neurotoxins. In vitro neuronal differentiation recapitulates several critical processes involved in the development of the nervous system such as migration, differentiation, and synaptogenesis. Here, we cultured feeder-independent mouse embryonic stem cells and induced their differentiation into neurons using retinoic acid. Using this model of developing neurons, we assessed developmental neurotoxicity of four heavy metal compounds found in the environment: mercury, cadmium, lead, and manganese. Changes in cell viability, morphology, and replication rates were monitored at each step of the developmental process. The efficiency of neuronal differentiation was determined by calculating the proportions of cells that are immuno-positive for MAP-2, a cytoskeleton protein unique to neurons. Undifferentiated ESCs were generally more sensitive to higher physiological doses of all four compounds, which inhibited cell proliferation and induced apoptosis. Lower physiological doses of these compounds did not impact ESCs proliferation but did interfere with their neuronal differentiation. These results are consistent with findings in animal-based models and show that the neuronal differentiation of ESCs is a useful model system for investigating developmental neurotoxicity of environmental chemicals at the cellular level. We are currently using this model to characterize developmental neurotoxicity of endocrine disruptors such as Polybrominated diphenyl ethers (PBDE).

W2173

GENE REGULATORY NETWORKS IN MESENDODERM DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS

Carpenedo, Richard[1], Tanner, R. Matthew[1,2], Perkins, Theodore[1,2] and Stanford, William[1,2], [1]Ottawa Hospital Research Institute, Ottawa, ON, Canada, [2]University of Ottawa, Ottawa, ON, Canada

Pluripotent stem cells (PSCs) hold tremendous potential as a cell source for applications in regenerative medicine, in vitro drug screening, and studies of human development and disease. A significant challenge preventing PSCs from being adopted in such applications is efficient generation of mature, functional cell populations. A comprehensive, systems-level understanding of the molecular mechanisms underlying differentiation, including regulation of transcription and post-transcriptional regulatory mechanisms, will be highly beneficial toward production of functional tissue from PSCs. The objective of this project is to draft and test a multi-level gene regulatory network of human PSC mesendoderm (ME) differentiation comprising transcription, post-transcriptional modification, and translation state. To induce ME differentiation, H9 hESCs were seeded as single cells in chemically defined E8 media, which was then replaced with basal media supplemented with BMP4 and Activin A (termed BA). Morphological changes were observed in BA conditions compared to E8 and basal media (E6) controls as early as 24 hrs post-treatment. Expression of pluripotency genes was reduced in BA compared to E8 after 48 hrs, while expression of ME genes, including *T*, *MIXL1*, *GSC* and *EOMES,* was observed exclusively in BA. RNA-seq was performed at 6-hr intervals throughout the 48-hr differentiation time course to assess transcriptomic changes. Hierarchical clustering and principal component analysis of transcriptomic data indicated that BA-treated cells had distinct expression profiles from E8 or E6, and that consecutive BA time points clustered sequentially. Similarly, sequencing of small RNA and ribosome-protected RNA libraries was done to assess changes in miRNA expression and global translation-state during ME differentiation. Integration of these data sets is expected to yield insights into regulatory mechanisms involved in ME commitment. Furthermore, our global translation-state analysis allows us to draft regulatory networks with a focus on identifying post-transcriptional control elements (such as miRNA) that mediate discrepancies between transcription and translation. Thus, using an unbiased systems-biology approach, we will identify previously uncharacterized regulators of ME development.

W2175

INVESTIGATING THE MECHANISM OF A MULTI-STATE MODEL OF WNT SIGNALING

Cutts, Josh and Brafman, David, Arizona State University, Tempe, AZ, U.S.

The WNT signaling pathway plays a critical role in many developmental processes as well as the maintenance of tissue homeostasis in adults. In addition, dysfunction in WNT signaling results in numerous human diseases. Canonical WNT signaling is classically described by the ‘two-state’ model. This model posits that in the ‘off’ state in the absence of a WNT ligand, cytoplasmic β-catenin is continuously degraded by the action of the APC/Axin/GSK-3β destruction complex. In the ‘on’ state in the presence of WNT ligands, this protein destruction complex is disrupted, allowing β-catenin to translocate into the nucleus where it interacts with the DNA-bound TCF/LEF proteins to regulate target gene expression. However, this ‘two-state’ model does not adequately explain the mechanisms by which WNT signaling can elicit distinct patterns of target gene expression and cell responses at specific signaling thresholds. For example, in the development and patterning of many tissues, the WNT pathway attains different levels of activity through gradients of WNT signaling activity. In turn, the positional information supplied by these WNT signaling gradients produces the appropriate spatial pattern of cellular differentiation. Elucidating the mechanisms of how a graded WNT signal leads to precise changes in transcriptional responses has been difficult because the lack of an in vitro model where WNT signaling molecules cause distinct cellular phenotypes at different concentrations. To that end, we have developed an in vitro human pluripotent stem cell (hPSC)-based model that recapitulates the same in vivo developmental effects of the WNT signaling gradient on the anterior-posterior (A/P) patterning of the neural tube during early development. Using this model along with genome-wide expression analysis (RNA-seq) and DNA binding analysis (ChIP-seq), we are uncovering the mechanisms by which specific levels of WNT activity are translated into precise transcriptional responses and cell identities. Overall, the new insights gained from this research will lead to the better understanding of how various WNT pathway activity levels lead to cancer or other pathological conditions.

Funding Source: Arizona Alzheimer's Association

W2177

INDUCTION OF THE HEMATOENDOTHELIAL LINEAGE USING HUMAN PLURIPOTENT STEM CELLS

Faal, Tannaz, Scarfone, Vanessa and Inlay, Matthew A., University of California, Irvine, Irvine, CA, U.S.

Bone marrow transplants (BMT), also known as hematopoietic stem cell transplants (HSCT), are performed to treat a large variety of blood disorders. Hematopoietic stem cells (HSCs) exhibit tremendous regenerative capacity, as a single HSC has the potential to reconstitute the entire blood system. Despite this, BMT/HSCT is performed rarely due to possible side effects of the procedure such as graft rejection. One goal of regenerative medicine is the generation HSCs from patient-specific pluripotent stem cell lines (hPSC) to treat blood diseases. However, despite decades of effort, the generation of functional HSCs from hPSC lines has yet to be conclusively achieved. While recent progress has been made in generating definitive hematopoietic progenitor cells – defined by the potential to give rise to all blood cell types – the majority of protocols to generate blood cells from hPSC lines result in primitive blood cells that are biased towards making red blood cells or other myeloid cells. Our goal is to better understand the earliest steps of hematopoietic commitment to direct cells to definitive fates. Hematopoietic cells are thought to arise from a hematoendothelial precursor called hemogenic endothelium. To generate hemogenic endothelium, hPSC clusters are plated onto an overconfluent layer of a bone marrow stromal line called OP9 and analyzed for CD34 and CD43 marker expression, which signals endothelial and hematopoietic differentiation, respectively. In the presence of serum-containing media, hPSCs rapidly differentiate into mesodermal lineages, and produce CD34+ endothelial cells in a few days. However, we found that hematopoietic induction correlated with upregulation of primitive markers such as CD235a and CD41. To redirect hematoendothelial cells to a more definitive fate, we explored switching the culture media from a pro-differentiation serum-containing media to serum-free media designed to maintain hPSC pluripotency. We found that a combination of serum-containing and serum-free media promotes expansion of putative hemogenic endothelium and definitive hematopoietic cells and prevents primitive induction. We hypothesize that this protocol could direct hemogenic endothelium towards definitive hematopoietic progenitors capable of engraftment and reconstitution of the blood system.

Funding Source: National Institute of Neurological Disorders and Strokes (NINDS/NIH) Training Grant Award#NS082174

W2179

A RAPID AND EFFICIENT METHOD TO OBTAIN INDUCED ENDOTHELIAL PROGENITOR LIKE CELLS FROM HUMAN EMBRYONIC STEM CELLS

Zhao, Yan[1,2], Zhao, Hao[1], Li, Zili[2], Ouyang, Qi[1,2], Sun, Yi[1,2], Zhou, Di[1,2], Xie, Pingyuan[2], Zeng, Sicong[1,2], Dong, Lingfeng[1], Wen, Hua[1], Lu, Guangxiu[1,2], Lin, Ge[1,2] and **Hu, Liang**[1,2], [1]School of Basic Medical Science, Central South University, Changsha, China, [2]National Engineering and Research Center of Human Stem Cells, Changsha, China

Endothelial progenitor cells (EPCs) can promote the formation of cardiovascular and angiogenesis, and can be used in a variety of areas like cardiac regeneration, gene therapy, development of tissue-engineered vascular grafts, and prevascularized tissue transplants. EPCs can be isolated from various sources, such as the peripheral blood, bone marrow, adipose tissue, and umbilical cord blood. However, isolating EPCs from these sources were difficult to expand in culture and may affect both quantity and quality of EPCs for clinical use. Thus, pluripotent stem cells such as human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) have been highlighted as alternative sources of EPCs. Previous efforts to differentiate hESCs into EPCs were expensive and time-consuming. To check which transcriptional factor is important in hematopoiesis and endothelial cell development, we established conditional overexpression system in hESCs for 18 transcription factors that were reported responsible for hematopoiesis. Among them, only overexpression of FLI1 and c-MYC could differentiate hESCs to hematopoietic lineage. Moreover, overexpression of FLI1 as well as activation of PKC simultaneously could rapidly and efficiently differentiate hESCs to endothelial progenitor like cells (iEPCs), while neither FLI1 overexpression nor PKC activation alone could differentiate hESCs to iEPCs. By only 3-day induction, hESCs were differentiated to spindle-like cells that were consistent in appearance with EPCs. Flow cytometry analysis reavealed that the rate of CD31/CD144 double positive cells was more than 90%, which was much higher than previously reported in other literatures. Expression of endothelial relate genes CD144, CD31, CD34, FLK-1, VWF2 dramatically increased during induction while pluripotency genes OCT4, SOX, NANOG, KLF4 decreased gradually. Like EPCs, iEPCs could also uptake low-density lipoproteins, strongly expressed UEA-1 and vWF. Interestingly, iEPCs showed better capillary-like structures forming ability in matrigel both in vitro and in vivo than EPC and HUVEC. Detailed mechanism of this directional differentiation may help us better under-

stand angiogenesis and develop new method to generate EPCs from pluripotent cells for regenerative medicine.

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W2181

AUTOMATED PLATFORM FOR POPULATION BASED DISEASE MODELING OF STEM CELL DERIVED CELL TYPES

Johannesson, Bjarki[1], Lallos, Gregory[1], Zimmer, Matthew[1], Browne, Daniel[1], Napolitano, Chris[1], Hahn, Aana[1], Sun, Bruce[1], Shang, Linshan[1], Brenner, Katie[1], Nelson, Philip[2], Ando, Mike[2], Dimon, Michelle[2], Coram, Marc[2], Solomon, Susan L.[1], Berndl, Marc[2] and Noggle, Scott[1], [1]New York Stem Cell Foundation, New York, NY, U.S., [2]Google Inc, Mountain View, CA, U.S.

Directed differentiation of pluripotent stem cells has become one of the cornerstones of cell-based disease modeling. Together with somatic cell reprogramming, which allows for the generation of patient specific stem cells, this is a powerful tool for studying genotype-phenotype relationships in vitro. In severe monogentic diseases, this relationship is often pronounced and can be demonstrated experimentally using cell lines from a low number of affected individuals. However, genetically complex diseases such as type 1 and type 2 diabetes have to be modeled using larger cohorts of patient-derived cells. Protocols for directed differentiation are frequently optimized using a single cell line and show inconsistent results when applied to cohorts of genetically diverse stem cell lines. This lack of robustness incorporates experimental variability that can mask subtle phenotypes and represents a major limitation for large-scale studies where multiple cell lines have to be differentiated and analyzed in parallel. To address this problem, we are developing automated techniques that enable the standardized creation and analysis of stem cell derived tissues at scale. Our approach combines active machine learning techniques with our new automated culture systems, laying the groundwork for true population based, in vitro disease modeling.

W2183

INVESTIGATING THE ROLE(S) OF XIST RNA BINDING PROTEINS IN X CHROMOSOME INACTIVATION (XCI)

Korsakova, Elena[1], Pandya-Jones, Amy[2] and Plath, Kathrin[2], [1]CSUN, Northridge, CA, U.S., [2]University of California Los Angeles, Los Angeles, CA, U.S.

X Chromosome Inactivation is an essential developmental step in the maturation of placental female embryos. Upon exit from the pluripotent state of the inner cell mass cells of the embryo proper, XCI results in transcriptional silencing of one of the two X chromosomes that is heritable through all subsequent cell divisions. XCI is thought to compensate for the imbalance in X-linked gene dosage between female (XX) and male (XY) cells. The critical regulator of XCI is the long non-coding RNA *Xist*, which coats the future inactive X chromosome to initiate transcriptional silencing and facultative heterochromatin formation. RNA footprinting experiments on *Xist* revealed a set of sites that were protected by bound proteins. Here we investigate the role of these protein binding sites in XCI by deleting them using the CRISPR-Cas9 system. Their role(s) in mediating *Xist* RNA coating of the Xi will be assessed by FISH. If Xist cloud formation is observed in the mutant cells, the ability of Xist to induce transcriptional silencing and promote epigenetic change will be evaluated by combination FISH/ immunofluorescence experiments. This investigation will help elucidate which regions of the *Xist* RNA require trans-acting factors to execute coating and epigenetic regulation, with the larger goal being the identification of these factors and their mechanisms of action.

W2185

MODELLING THE IMPACT OF MATERNAL DRUG USE ON HEPATOCYTE DIFFERENTIATION

lucendo-Villarin, Baltasar[1], Panagiotis, Filis[2], Iredale, John P[1], O'Shaughnessy, Peter J[3], Fowler, Paul A[4] and Hay, David[5], [1]University of Edinburgh, Edinburgh, U.K. of Great Britain and Northern Ireland, [2]University of Aberdeen, Aberdeen, U.K. of Great Britain and Northern Ireland, [3]University of Glasgow, glasgow, U.K. of Great Britain and Northern Ireland, [4]University of Aberdeen, Aberdeen, U.K., [5]University of Edinburgh, edinburgh, U.K.

Maternal alcohol and drug consumption exposes the foetus to harmful xenobiotics. This can impair human liver development, resulting in long-term consequences for the affected offspring after birth. While hepatocyte models exist to study the effect of xenobiotics on human development, their drawbacks outweigh the advantages. Therefore, other sources of human foetal hepatocytes have been explored. Pluripotent stem cells (PSCs) represent an exciting cell resource which can be scaled to demand and then directed to differentiate down the lineage of interest. The focus of this study was to understand how xenobiotic exposure induces changes in hepatic gene expression during hepatocyte differentiation. For this purpose, we generated PSC-derived hepatoblast populations using a defined and efficient procedure and then exposed them to many compounds found in cigarette smoke, such as cotinine, polycyclic aromatic hydrocarbons, cadmium, arsenic, and cobalamin. Following this, cells were direct-

ly differentiated to hepatocyte-like cells with hepatocyte gene expression, redox potential, metabolic function, and serum protein production analysed. We believe that these studies represent important progress towards understanding the effect of maternal drug consumption on the human foetus.

Funding Source: MR/L010011/1

W2187

DECIPHERING THE GENETIC CONTRIBUTION TO SCHIZOPHRENIA

Mitchell, Jana[1], Kamitaki, Nolan[2], Nahme, Ralda[1,3], McCarroll, Steven[2,3] and Eggan, Kevin Carl[1,3], [1]Harvard University, Cambridge, MA, U.S., [2]Harvard Medical School, Boston, MA, U.S., [3]The Broad Institute of Harvard and MIT, Cambridge, MA, U.S.

Genome-wide analyses have linked variation in ~100 genomic regions to Schizophrenia (SCZ). This provides for the first time a list of candidate genes that potentially influence disease. To generate hypotheses about how these genes contribute to disease, we must first establish when and where in the brain they are expressed. We then need to determine how genetic variation associated with disease alters the function of these genes in specific neural cell types. While the vast majority of variation lies within non-coding regions of the genome, we need to determine the effects of variation on regulation of gene expression, in both direction and magnitude. As many of these genes are potential targets of novel therapeutics, we need to unambiguously establish their expression profiles in normal and disease cells. We have used directed differentiation of Human embryonic stem cells to generate neural cell types implicated in the pathogenesis of SCZ, including cortical excitatory neurons and inhibitory interneurons. By sampling cells over the course of maturity, we have systematically measured the trajectory of expression of genes linked to SCZ. We show that distinct clusters of candidate genes are expressed in specific neural cell types. A critical aspect of our analyses is to determine the effects that SCZ-linked variation has on candidate gene expression. We have approached this question by measuring changes in allele-specific expression at loci heterozygous for ‘risk’ polymorphisms. We have developed an exhaustive probe set to measure the allelic skew of >700 genes located within the vicinity of SCZ regulatory polymorphisms, and measured the extent to which variation at each haplotype acts to increase or decrease their expression. In aggregate, this information provides novel insight into the function of these genes in neurodevelopment, suggests how changes in gene expression might influence the pathogenesis of SCZ, and ultimately reveals targets for downstream drug development.

W2189

HOXA-PATTERNED HEMOGENIC ENDOTHELIUM DIFFERENTIATED FROM HUMAN PLURIPOTENT STEM CELLS RESEMBLES AGM AND GENERATES FETAL HAEMATOPOIETIC CELLS

Ng, Elizabeth S[1], Azzola, Lisa[1], Vlahos, Katerina[1], Yu, Qing Cissy[2], Bruveris, Freya F[1], Calvanese, Vincenzo[3], Phipson, Belinda[1], Maksimovic, Jovana[1], Januar, Vania[1], Elliott, David[4], Haylock, David N[5], Nilsson, Susan K[5], Saffery, Richard[1], Oshlack, Alicia[1], Mikkola, Hanna K A[6], Stanley, Edouard G[1] and Elefanty, Andrew G[1], [1]Murdoch Childrens Research Institute, The Royal Children's Hospital, Parkville, Australia, [2]Shanghai Institute of Biological Sciences, Shanghai, China, [3]University of California Los Angeles, Los Angeles, CA, U.S., [4]Murdoch Childrens Research Institute, Parkville, Australia, [5]CSIRO, Clayton, Australia, [6]University of California Los Angeles, Los Angeles, CA, U.S.

Hematopoietic stem cell (HSC) transplantation reconstitutes the blood cell compartment following myeloablative therapy or for patients with marrow aplasia. Because many patients do not have an optimal matched donor, the provision of HSCs from alternate sources, such as differentiated human pluripotent stem cells (hPSCs), is required. Despite considerable efforts, it has not been possible to efficiently generate repopulating HSCs from PSCs. Based on key roles for *SOX17* in hemogenic endothelium and in the earliest HSCs, and for *RUNX1C* in marking hematopoietic progenitors, we reasoned that reporter lines that marked cells expressing these genes would be valuable for identifying definitive hematopoietic lineages. In initial studies, we found that *RUNX1C* marked a subset of CD34$^+$ cells highly enriched for haematopoietic progenitors that homed to the bone marrow, but did not engraft immunocompromised mouse recipients. Exploring molecular differences between hPSC-derived and cord blood CD34$^+$ cells revealed that the RUNX1C$^+$CD34$^+$ cells failed to express *HOXA* genes. We found that modulating ACTIVIN and WNT signalling, timed to overlap with the peak expression of primitive streak genes, enhanced chromatin accessibility across the *HOXA* cluster and up-regulated *HOXA* expression, effectively providing a 'switch' from primitive to definitive hematopoiesis. This led to the formation of striking SOX17$^+$ vascular structures, which generated RUNX1C$^+$ haematopoietic cells, mimicking aspects of human aorta-gonad-mesonephros (AGM). The *HOXA*-expressing cultures sustained haematopoiesis longer than control cultures, evidenced by the prolonged generation of colony forming cells, which included erythroid precursors that had switched from embryonic to fetal globin expression. Our findings argue that *HOXA* codes established early in differentiation predict cellular poten-

tial and provide correct cell patterning for the specification of definitive hematopoietic lineages from hPSCs. Our identification of the relationship between specific signaling events and *HOXA* gene induction represents a significant step towards the generation of transplantable human hematopoietic stem cells from pluripotent stem cells.

W2191

FUNCTIONAL AND TOPOLOGICAL CHARACTERIZATION OF POISED ENHANCERS DURING EMBRYONIC STEM CELL DIFFERENTIATION

Rada-Iglesias, Alvaro[1], de la Cruz Molina, Sara[1], Tebartz, Christina[1], Respuela, Patricia[1], Kolovos, Petros[2], Nikolic, Milos[1,3], Grosveld, Frank[2] and Frommolt, Peter[3], [1]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Cologne, Germany, [2]Erasmus Medical Center, Rotterdam, Netherlands, [3]Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Germany, Cologne, Germany

Poised enhancers in embryonic stem cells (ESC) are *cis*-regulatory elements uniquely characterized by the simultaneous presence of transcription factors, co-activators (e.g.p300), H3K4me1 and, importantly, H3K27me3. Although correlative evidences suggest that poised enhancers facilitate the activation of somatic gene expression programs upon ESC differentiation, their functional relevance during this process has not been formally demonstrated. Initial epigenomic and functional annotation of poised enhancers in mouse ESC (mESC) suggested that, rather than being promiscuously used during the acquisition of somatic cell fates, poised enhancers were preferentially involved in the establishment of anterior neural identity. Using CRISPR/Cas9 to delete poised enhancers in situ, we formally showed that these cis-regulatory elements are necessary for the induction of major forebrain regulators. Since single poised enhancers were deleted at each of the analyzed loci, our data suggest that poised enhancers play an important and non-redundant role among the hierarchy of regulatory elements controlling the expression of anterior neural genes. In agreement with this major and early regulatory function, 4C-seq experiments revealed that poised enhancers established strong and specific physical interactions with their target genes already in the undifferentiated mESC state. Chielfy, additional 4C-seq experiments in EED$^{-/-}$ mESC showed that such pre-looped topological conformations are polycomb dependent. Together with epigenomic and transcriptional characterization of EED$^{-/-}$ mESC, we propose that, in addition to their well-established repressive function, polycomb proteins confer poised enhancers with the appropriate regulatory topology and, thus, facilitate the timely induction of forebrain master regulators. Overall, our work conclusively demonstrates the functional relevance of poised enhancers and illuminates a novel mechanism by which polycomb proteins might facilitate the proper establishment of developmental gene expression programs

W2193

SUPERIOR RED BLOOD CELL GENERATION FROM HUMAN PLURIPOTENT STEM CELLS VIA A NOVEL MICROCARRIER BASED EMBRYOID BODY PLATFORM

Sivalingam, Jaichandran[1], Lam, Alan[1], Chen, Allen[1], Yang, Bin Xia[2], Reuveny, Shaul[1], Loh, Yuin Han[3] and Oh, Steve[1], [1]Bioprocessing Technology Institute, Singapore, Singapore, [2]IMCB, Singapore, Singapore, [3]Institute of Molecular and Cell Biology, Singapore, Singapore

In vitro generation of red blood cells (RBCs) from human embryonic stem cells (hESC) and human induced pluripotent stem cells (hiPSC) appears to be a promising alternate approach to circumvent shortages in donor-derived blood supplies for clinical applications. Conventional methods for hematopoietic differentiation of hESC and iPSC rely on embryoid body (EB) formation and/or co-culture with xenogeneic cell-lines. However, these current methods are not amenable for scale-up and face obstacles for clinical translation due to the use of xenogeneic materials. Here we report the development of a serum-free and chemically defined platform using recombinant human Laminin-521 coated microcarriers for pluripotent stem cell expansion and hematopoietic differentiation of hESC and hiPSC. Improved survival and better quality EBs generated with the microcarrier-based method resulted in at least a 6 fold- improvement in hemangioblast expansion, potentially culminating in a 80-fold improvement in the yield of RBC generation as compared to a conventional EB-based method. Using the improved microcarrier-based differentiation protocol, we demonstrate successful differentiation of O-negative (Rhesus factor D negative) RBCs from donor hiPSC generated from finger-prick blood. Universal donor RBCs (blood type O-RhD negative) could prove to be extremely useful for future cell therapy applications such as emergency blood transfusions and for treating poly-immunized patients with recurrent transfusion needs. Thus, our microcarrier-based differentiation platform could prove to be an appealing strategy for future scale-up of hESC and hiPSC culture and for hematopoietic differentiation under defined conditions for large-scale generation of universal donor RBCs.

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W2195

ANTAGONISTIC INTERACTIONS BETWEEN PLURIPOTENCY & LINEAGE-SPECIFIC TRANSCRIPTION FACTORS IN PARAXIAL MESODERMAL SPECIFICATION OF HUMAN PLURIPOTENT STEM CELLS

Ting, Chiao-Hsuan, Natl Health Res Institutes, Zhunan Taiwan, Taipei City, Taiwan, Jiang, Shih-Sheng, National Institute of Cancer Research, National Health Research Institutes (NHRI), Zhunan, Taiwan, Hsu, Pei-Ju, Regenerative Medicine Research Group, Institute of Cellular and System Medicine (ICSM), National Health Research Institute (NHRI), Zhunan, Taiwan, Taipei City, Taiwan, Lee, Yu-Wei, Regenerative Medicine Research Group, Institute of Cellular & System Medicine, National Health Research Institutes (NHRI), Zhunan, Taiwan and Yen, B. Linju, Regenerative Medicine Research Group, Institute of Cellular&System Medicine, National Health Research Institutes (NHRI), Zhunan, Taiwan

Human pluripotent stem cells (hPSCs) have the capacity of unlimited self-renewal and capacity to differentiate into cell types of all three germ layers. These stem cells therefore represent excellent in vitro models for studying the molecular mechanisms involved in early human development and cellular lineage commitment. One of the most clinically relevant lineages is the paraxial mesoderm. Arising from the mesoderm, which is the second germ layer to be specified after the ectoderm, the paraxial mesoderm gives rise to cell types related to the skeleton including skeletal muscle, cartilage, and bone—all tissues which undergo 'wear-and-tear' which result in much aged-related dysfunction. Surprisingly, molecular understanding of how PSC undergo lineage commitment into paraxial mesoderm is still not well understood. Using the hPSCs of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), we sought to elucidate molecular mechanisms involved during this process. We found that knockdown of Sox2—one of the pluripotency transcription factors (TFs) and also the key TF in ectodermal specification—in hESCs and iPSCs leads to differentiation into mesendoderm. To further narrow the differentiation specification into paraxial mesoderm, we used transcriptome analyses and found a candidate lineage TF which is a downstream target of Sox2 downregulation. Previous reports have shown that the BMP and Wnt/b-catenin pathways are important in hPSC mesendoderm specification, and we found that fine-tuning of the Wnt/b-catenin pathway can result into specific commitment towards paraxial mesoderm. Our current work is to elucidate the direct molecular steps linking Sox2 downregulation to downstream paraxial mesodermal TF and Wnt/b-catenin pathway activation in hPSCs. We hope our work can lead to further understanding of early human development as well as develop rapid and efficient methods of obtaining paraxial mesodermal derivatives for therapeutic application.

W2197

SYNCTYTIOTROPHOBLAST GENERATION FROM HUMAN EMBRYONIC STEM CELLS

Yabe, Shinichiro[1], Alexenko, , Andrei P[1], Amita, Mitsuyoshi[1], Yang, Ying[1], Schust, Danny J[1], Ezashi, Toshihiko[2] and Roberts, R. Michael[1], [1]University of Missouri-columbia, Columbia, MO, U.S., [2]University of Missouri, Columbia, MO, U.S.

Syncytiotrophoblast (STB) is a conceptus-derived multi-nucleated syncytium of human placenta and forms the interface with the maternal blood for nutrient and gas exchange. Alterations in STB development in early pregnancy have been implicated in placental diseases, including preeclampsia. Unfortunately, we little know about the manner in which STB forms and functionally differentiates, especially in the peri-implantation period when invasive STB leads the establishment of the final villous hemochorial placenta and when the conceptus is particularly vulnerable to environmental influences and viral infection. Human embryonic stem cells (ESC) can be directed into the trophoblast lineage by exposing them to BMP4 and two inhibitors, A83-01 and PD173074 that block ACTIVIN A and FGF2 signaling, respectively (BAP treatment). During BAP treatment, multiple areas of syncytium became visible within colonies ~day 5, coincident with the production of placental hormones and their release into the culture medium. By day 8 syncytial areas range from ~40 to ~100 mm or greater in diameter. Here, we describe isolation of cytotrophoblast (CTB) and larger STB fractions by differential cell size-based filtration of dispersed colonies by filtering through nylon strainers with mesh sizes of 40 and 70 μm. The two most homogeneous fractions, the mainly mononucleated < 40 mm fraction and the syncytial > 70 μm fraction had DNA contents consistent with an average nuclear number of 1 and 37, respectively. RNAseq analysis performed on these cells demonstrated that BAP-differentiated hESC were comprised only of trophoblast, with no evidence of marker gene expression suggesting the presence of other lineages. The large syncytial cells (> 70 μm) showed significantly (P<0.001) higher gene expression of placental hormones, including *CGB/LH* family members, CGA*,* PGF*,* fusogenic retroviral envelope proteins (ERVW1, ERVFRD1) and several transporters, including SLC38A3*, -13A4* and *-40A, when compared to* the mononucleated cytotrophoblast fraction (< 40 μm). Several transcription factors, including CEBPB, PPARD, GATA2*,* HEY1, DLX3, GCM1, TFAP2C MSX2 and OVOL1 were also significantly up-regulated in the large STB. STB derived from ESC may provide an in vitro model of initial STB formation in early placenta stage that cannot be studied in vivo.

W2199

HUMAN PLURIPOTENT STEM CELL DERIVED ENTERIC NEURAL CREST LINEAGES FOR CELL THERAPY AND DRUG DISCOVERY IN HIRSCHSPRUNG DISEASE

Fattahi, Faranak[1,2], Steinbeck, Julius[1], Kriks, Sonja[1], Tchieu, Jason[1], Zimmer, Bastian[1], Kishinevsky, Sarah[1], Zeltner, Nadja[1], Mica, Yvonne[3], El-Nachef, Wael[4], Zhao, Huiyong[1], de Stanchina, Elisa[1], Gershon, Michael[5], Grikscheit, Tracy[4], Chen, Shuibing[2] and Studer, Lorenz[1], [1]Memorial Sloan-Kettering Cancer CenterDevelopmental Biology, New York, NY, U.S., [2]Weill Cornell medicine, Weill Graduate School of Medical Sciences, New York, NY, U.S., [3]Life Technologies, Inc., Carlsbad, CA, U.S., [4]Children's Hospital of Los Angeles, Los Angeles, CA, U.S., [5]Columbia University, New York, NY, U.S.

The human enteric nervous system (ENS) is derived from the neural crest and represents a complex network of ~500 million neurons with dozens of distinct neurotransmitter and hormone subtypes essential for gastro-intestinal (GI) function. The ENS has been called the "second brain" given its autonomy and complex cytoarchitecture. ENS defects are the cause of a broad range of human disorders including Hirschsprung's disease (HSCR), a congenital defect affecting 1 in 5,000 live births. HSCR is a caused by the developmental failure of ENS progenitors to migrate into the gastrointestinal tract, particularly the distal colon. Development of the human ENS is poorly understood given the lack of accessible tissue. Here we demonstrate the efficient derivation and isolation of ENS progenitors from hPSCs, and their further differentiation into functional enteric neurons. A particularly remarkable feature of hPSC-derived ENS lineages is their ability to migrate extensively within the developing chick embryo or adult mouse colon. Transplantation into the wall of the cecum in adult mice results in long-distance migration and rescue of Ednrb mutant mice indicating therapeutic potential for HSCR. We further established an EDNRB-/- hPSC-model of HSCR and performed a drug screen using this model. We identified Pepstatin as a compound that promotes migration in EDNRB-/- enteric precursors in vitro and, upon pre-transplantation treatment of cells in vivo. Our study establishes the first, to our knowledge, human PS-cell-based platform for the study of human ENS development and presents cell- and drug-based strategies for the treatment of HSCR.

EMBRYONIC STEM CELL PLURIPOTENCY

W2203

ROSCOVITINE, A SMALL PURINE-LIKE CDK INHIBITOR, TRIGGERS APOPTOSIS IN HUMAN EMBRYONIC STEM CELLS

Furmento, Verónica[1], García, Carolina[1], Videla Richardson, Guillermo Agustin[1], **Romorini, Leonardo**[2], Miriuka, Santiago[1], Sevlever, Gustavo[1] and Scassa, Maria Elida[1], [1]LIAN-FLENI, Buenos Aires, Argentina, [2]FLENI, Buenos Aires, Argentina, Ciudad de Buenos Aires, Argentina

Human embryonic stem cells (hESCs) are derived from the inner cell mass of the blastocyst during a stage of development defined by rapid cell division rates. These cells possess the unique characteristic of indefinite self-renewal while retaining an undifferentiated state. Protein kinase complexes formed by the association of cyclins and their catalytic subunits called cyclin dependent kinases (CDKs) represent the key molecules that orderly regulate progression through the cell cycle. In hESCs, elevated cyclin activity combined with lack of endogenous CDK inhibitors results in increased activity of CDK1 and CDK2 and consequently in diminished G1 and G2 cell cycle phases. Acute inhibition of CDK1 or CDK2 in proliferating somatic cells generally results in reversible cell cycle arrest without significant cell death. However, in other cellular contexts, CDK inhibitors could induce cell differentiation or apoptosis. Therefore, CDKs have cell-type specific functions, and compensatory roles exist among different CDK isoforms, which in turn could play pivotal roles in the regulation of proliferation and apoptosis. Herein, we used Roscovitine (ROSC), a small purine-like CDK inhibitor, to examine the role of CDK1 and CDK2 inhibition in WA09 hESC line. Initially, by XTT/PMS vital dye assays we found that ROSC exposure reduces WA09 cells viability in a dose-dependent manner. Interestingly, by Western blot analysis we determined that the loss in cell viability was accompanied by apoptotic features such as caspase-9 and caspase-3 activation and PARP-1 cleavage. Flow cytometric analysis of DNA content revealed that ROSC led to an increase in the percentage of cells in G2/M phase after a 16 h treatment, which coincided with nuclear accumulation and site-specific phosphorylation of p53 at Serine 46. Frequently, molecular inhibition of CDKs protects normal non-cycling cells from programmed cell death, however in the present study we found that, similar to what occurs in many types of cancer cells, in rapidly proliferating hESCs ROSC triggers apoptosis. Our findings further support the concept that CDKs activities might not be universally required for apoptosis in the same way that CDKs are universally required for cell cycle progression in all eukaryotic cells studied.

W2205

mRNA DEADENYLATION SAFEGUARDS THE PLURIPOTENT STATE

Hu, Guang, Zheng, Xiaofeng, Yang, Pengyi, Lackford, Brad, Bennett, Brian, Wang, Li, Fargo, David and Jothi, Raja, National Institute of Environmental Health Ssciences/National Institutes of Health, RTP, NC, U.S.

Poly(A)-tail length and mRNA deadenylation play important roles in gene regulation. However, how they regulate embryonic development and pluripotent cell fate is not fully understood. Here we present evidence that mRNA deadenylation governs the pluripotent state. We show that Cnot3, a component of the Ccr4-Not deadenylase complex, is required for mouse epiblast maintenance. It is highly expressed in blastocysts and its deletion leads to peri-implantation lethality. The epiblast cells in Cnot3 deletion embryos are quickly lost during diapause and fail to outgrow in culture. Mechanistically, Cnot3 C-terminus is required for its interaction with the complex and its function. Further, Cnot3 deletion results in increases in the poly(A)-tail lengths, half-lives, and steady-state levels of differentiation gene mRNAs. The half-lives of Cnot3 target mRNAs are shorter in ESCs and become longer during normal differentiation. Together, we propose that Cnot3 maintains the pluripotent state by promoting differentiation gene mRNA deadenylation and degradation, and we identify poly(A)-tail length regulation as a novel post-transcriptional mechanism that controls pluripotency.

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W2207

COMBINATORIAL CHEMICAL GENETICS IDENTIFIES ERK5 AS A KEY REGULATOR OF EMBRYONIC STEM CELL FATE

Findlay, Greg, MRC-PPU, Dundee, U.K.

Embryonic Stem Cells (ESCs) can self-renew or differentiate into potentially all specialised cell types, a phenomenon known as pluripotency. However, the mechanisms by which pluripotency and differentiation are controlled are poorly understood. To address this, we develop a high-throughput small molecule screen to identify novel kinase regulators of pluripotency. We find that XMD compounds, a series of selective Erk5 kinase/BET bromodomain inhibitors, promote primed pluripotency. We engineer XMD target selectivity to generate Erk5 specific tool compounds, which in combination with CRISPR/Cas9 mediated genome editing uncovers a novel function for Erk5 in pluripotency regulation. Mechanistically, Erk5 maintains naïve pluripotency by promoting expression of Krueppel-like transcription factors, in a manner which requires kinase activity, upstream activation by Mek5 and a C-terminal transcriptional activation domain. Finally, we show that Erk5 functions to specifically restrict cardiac mesoderm differentiation, suggesting that Erk5 plays a critical role in controlling pluripotency and lineage specialisation during ESC development.

W2209

METHODS AND ANALYSIS OF PLURIPOTENCY MARKERS AND CELL VIABILITY IN hESC CULTURED AS 3D AGGREGATES

Harkness, Linda[1], Chen, Xiaolo[1], Gray, Peter[1] and Davies, Anthony[2], [1]University of Queensland, St Lucia, Australia, [2]Queensland University of Technology, Brisbane, Australia

We have previously demonstrated that hESC static 3D suspension cultures, which utilise a thermo-responsive polymer and thus an enzyme-free method for passaging, can readily proliferate while maintaining key pluripotent markers. 3D culture techniques provide advantages for cells undergoing maintenance of pluripotency and differentiation as compared to the routinely used 2D culture systems as they more closely mimic the native environment of tissue. Robust methods for detailed analysis of 3D cultures are uncommon and needed to be established as part of the experimental set-up. Using an IN Cell 2000 Analyzer we established methods for 3D analysis of hESC aggregates and investigated how the aggregates are formed using fluorescent staining. From high content images, we analysed nuclear morphology and the expression of key pluripotency (OCT3/4, SOX2, Nanog) markers on fixed hESC aggregates. Analysis demonstrated that while over 90% of cells remained positive for the markers, there were differences in the intensity of expression. However, cells demonstrating decreased intensity appeared random within the aggregate. This data was additionally validated using flow cytometry where a broad range of signal intensities were observed. In addition, we investigated cell viability using live/dead cell staining (Hoechst/DRAQ7) within aggregates. Data demonstrated that over 80% of cells retain their viability within the aggregates – data comparable to our cell counts performed on aggregates following single cell suspension. In conclusion, we have developed new methods for 3D analysis of hESC aggregates which do not disrupt cell-cell interactions. Analysis using HCA and flow cytometry demonstrates that hESC remain viable and pluripotent throughout the aggregate.

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W2211

SINGLE CELL DISSECTION OF TRANSCRIPTIONAL REGULATORS IN HUMAN PLURIPOTENT STEM CELL STATE TRANSITIONS

Kimmey, Samuel, Borges, Luciene and Bendall, Sean C, Stanford University School of Medicine, Stanford, CA, U.S.

To date, some of the most insightful information on specific biological regulators were acquired through loss/gain of function experiments in the most basic of model organisms. Investigation of human Pluripotent Stem Cells (hPSC) established the precise abundance of key transcription factors, rather than their mere presence or absence, imparts distinct cell fates. This indicates a spectrum of sub-populations exist within hPSC cultures, defined by their difference in amount and combination of transcriptional regulators, yet such population-granularity is unobservable in bulk-assays commonly used. Additionally, inability to maintain genetic perturbations in hPSCs prevents investigating regulators in transient states or lineage restricted progenitors. To address these confounding issues, we generated stable hPSC lines with inducible short hairpin RNA targeting transcriptional regulators (demonstrated here with *OCT4*), and a panel of 40+ cellular parameters to measure using single-cell mass cytometry (CyTOF). This enables the simultaneous measurement of key features in hPSC, including regulatory factors (SOX2, LIN28A, etc.), behaviors (cell cycle, apoptosis, etc.) and phenotypic markers (SSEA3, TRA-1-60, etc.). In doing so, we reveal that cell state potential can be reorganized, where knockdown of genes such as *OCT4* can create new 'metastable' states within PSC culture. Furthermore, analysis using conditional–Density Rescaled Visualization can be employed to investigate dependency of various molecular regulators, cell behaviors, and lineage potential, on the amount of a given regulator. Our system now permits the interrogation of regulators during self-renewal and differentiation on a cell-by-cell basis. Not only does this allow direct observation of sub-populations, but molecular heterogeneity in the generated hPSC lines combined with single cell measurements permits every cell to be a unique observation – providing a multitude of experimental doses in each experiment. Future experiments will generate hPSC lines with constructs to target putative regulators of somatic cells, permitting the same powerful loss/gain of function studies otherwise difficult to study in the human in vitro, thus creating a toolkit to assess cell-state regulators in tissue-specific contexts similarly.

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W2213

ELUCIDATING THE GENETIC DETERMINANTS FOR EXIT OUT OF PLURIPOTENCY WITH A CRISPR-CAS9 GENOME-WIDE KNOCKOUT SCREEN

MacDougall, Matthew Steven and Merrill, Brad, University of Illinois at Chicago, Chicago, IL, U.S.

Pluripotent stem cells in the early mammalian embryo progress through developmental states in preparation for lineage specification during gastrulation. Two distinct states of pluripotency can be replicated in vitro by using mouse embryonic stem cells (ES) for a naive state and epiblast like cells (EpiLC) for a primed state. Naive ES cells can rapidly transition to the EpiLC primed state with simple changes to cell culture media. This transition is effectively irreversible, because most EpiLC die if they are returned to naive cell culture conditions. Previously, several genes have been shown to be necessary for the transition from naive to primed states, and inactivation of these genes prevents cell death when cells are switched between EpiLC and ES culture conditions. To screen for novel genes required for exit out of naive pluripotency, I performed a CRISPR-Cas9 genome-wide pooled knockout screen and targeted all protein coding genes in the mouse genome with ~90k unique sgRNAs. The screen yielded 30 high confidence candidates (FDR<5%), including 11 genes previously reported to be required for naive to primed transition, including: Tcf7L1, Zfp281, Tsc1, Tsc2, and Flcn. Many of the 19 novel pluripotency genes are known to be important for two protein complexes that carry out distinct, but related, cellular processes. The clustering of "hits" from the unbiased CRISPR screen to these discrete cellular processes indicates a previously unknown importance of these aspects of cell biology for controlling the state of pluripotency in stem cells. The mechanism underlying the role of these novel cellular complexes for establishing the primed state of pluripotency is currently being elucidated, and will be presented.

W2215

HUMAN PLURIPOTENT STEM CELLS DISPLAY MECHANISTICALLY INDEPENDENT REQUIREMENTS FOR ARGININE IN SURVIVAL AND MAINTENANCE OF PLURIPOTENCY

Ozawa, Hiroki and Enver, Tariq, University College London, London, U.K.

Recent studies show that human pluripotent stem cells (hPSCs) have significant differences in metabolism compared to differentiated cells which directly contribute to the control of physiology and cell fate, as well as widespread epigenetic remodeling. Amino acids are important in metabolism and methionine has been identified as a crucial regulator in hPSCs. Here, we show that arginine

is an essential amino acid for the maintenance of hPSCs in vitro. Complete arginine deprivation triggered oxidative stress and G0/G1 cell cycle arrest followed by p38/p53-dependent apoptosis. In low arginine concentrations however, hPSCs remained viable but potentially differentiated as indicated by decrease of NANOG expression, uncoupling pluripotency from apoptosis. We next dissected the molecular mechanisms underlying hPSCs arginine requirement. Arginine deprivation resulted in a rapid decrease of intracellular levels of its metabolites including ornithine and polyamines. We found that either ornithine or polyamines rescued the reduction of NANOG expression caused by arginine shortage. Knock-down or inhibition of either arginine or polyamine metabolic enzymes also led to a loss of NANOG expression. Polyamines have been shown to inhibit histone deacetylase (HDAC) activity. Therefore we examined the effect of arginine deprivation on histone acetylation state. Arginine deprivation induced HDAC activity and reduced the overall histone acetylation level which could be rescued by either ornithine or polyamine addition. The HDAC inhibitor, valproic acid reversed not only the loss of histone acetylation but also the decrease of NANOG expression. These findings demonstrate that hPSCs are dependent on arginine for survival and that polyamines derived from arginine maintain pluripotency in hPSCs through histone acetylation.

W2217

TOWARD THE EFFICIENT PRODUCTION OF GENETICALLY MODIFIED MARMOSET MODELS FOR REGENERATIVE MEDICINE

Sasaki, Erika[1,2], Okano, Hideyuki[3,4], Takahashi, Taukasa[1,2] and Okahara, Junko[2,4], [1]Keio University, Shinjuku-ku, Tokyo, Japan, [2]Central Institute for Experimental Animals, Kawasaki, Japan, [3]Department of Physiology, Keio University School of Medicine, Tokyo, Japan, [4]RIKEN Brain Science Institute, Wako, Japan

Non-human primates offer excellent, precise preclinical study systems for assessing the safety and efficacy of new therapies and drugs. In particular, non-human primates are expected to be models for regenerative medicine using pluripotent stem cells. The common marmoset (Callithrix jacchus) is a useful experimental animal in biomedical research because of its similarity to humans, high reproductive efficiency, and easy handling. Given its prolificacy, the marmoset is suitable for producing genetically modified animals. Recent progress in transgenic technologies in non-human primates has enabled the generation of many human disease models. Furthermore, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have been established in the marmoset and used in preclinical studies for regenerative medicine. We have generated ten independent transgenic marmoset lines using lentiviral vectors. To avoid obtaining non-transgenic animals by transgene integration failure, a fluorescent protein marker gene was introduced with the transgene. Fluorescent protein expression was confirmed at the eight-cell stage before embryo transfer to a surrogate mother. While producing these ten transgenic marmoset lines, it appeared that the promoters and fluorescent proteins affected embryonic development. Thus, in this study, the influence of each promoter and fluorescent protein on marmoset embryonic development was analyzed. Six promoters, elongation factor 1a, a modified chicken beta-actin promoter with a CMV-IE enhancer, cytomegalovirus, SynapsinI, early transposon promoter and Oct3/4 and Sox2 enhancers (EOS), ubiquitin, and five fluorescent protein markers, green fluorescent protein, Venus, Kusabira Orange, monomeric red fluorescent protein, and monomeric Cherry, were analyzed for their influence on embryonic development. The chi-square test results indicated that the birth rate of transgenic marmoset pups were affected by the promoter ($P<0.01$) and the fluorescent marker protein ($P<0.05$). Our results demonstrate that selections of promotor and fluorescent protein marker are critical steps in efficient transgenic marmoset production.

W2219

BETA-CATENIN ASSOCIATED REPRESSIVE PROTEIN COMPLEX BLOCKS DEVELOPMENTAL PROGRAMS TO MAINTAIN GROUND STATE EMBRYONIC STEM CELLS

Tao, MD, Fang[1,2], Gao, Xin[1], Zhao, Meng[1], Zhao, Chongbei[1], Li, Zhenrui[1,2], Qian, Pengxu[1], He, Xi (CiCi)[1] and Li, Linheng[1,2], [1]Stowers Institute for Medical Research, Kansas City, MO, U.S., [2]University of Kansas, Kansas City, KS, U.S.

Mouse embryonic stem cells(ESCs) grown in defined medium with inhibitors of MEK and GSK3 (2i) are in a naïve ground state. Although β-catenin is known important for maintaining ground state of ESC, the mechanism is not fully uncovered. Here we showed that β-catenin contributed to ESC ground state maintenance through global inhibition of developmental genes. We first found that absence of β-catenin impaired ESC self-renewal, however, core self-renewal circuitry such as Oct4, Sox2 and Nanog(OSN) as well as other pluripotency genes were still preserved. Transcriptome analysis showed that β-catenin deficient cells had increased development and lineage specific genes which led to ESC differentiation. To investigate how β-catenin restrains ESC from differentiation, we have further identified a β-catenin associated protein complex in ESCs including E2F6 and HP1γ. Consistently, E2F6 or Hp1γ defective cells also have compromised self-renewal and upregulated development genes, which suggested that β-catenin formed a repressive protein complex with E2F6 and Hp1γ to restrict differentiation for maintaining their ground state. ChIP-sequencing

revealed that β-catenin associated complex occupied both promoter and enhancer regions of developmental genes. More interestingly, β-catenin deficient cells have increased deposit of H3K4me3, an active histone modification mark on the developmental genes in ESCs. Overall, our study suggested that β-catenin formed a repressive protein complex with E2F6 and HP1γ to block ESC differentiation to maintain their ground state self-renewal.

W2221

WNT/β-CATENIN SIGNALING PROMOTES SELF-RENEWAL AND INHIBITS THE PRIMED-STATE TRANSITION IN NAÏVE HUMAN EMBRYONIC STEM CELLS

Xu, Zhuojin[1], Robitaille, Aaron[1], Berndt, Jason[1], Davidson, Kathryn C.[2], Fischer, Karin[3], Mathieu, Julie[1], Ruohola-Baker, Hannele[1], Porter, Jennie[1] and Moon, Randall[1], [1]University of Washington, Seattle, WA, U.S., [2]Australian Regenerative Medicine Institute, Clayton, Australia, [3]The University of Washington, Seattle, WA, U.S.

In both mice and humans, pluripotent stem cells (PSCs) exist in at least two distinct states of pluripotency, known as the naïve or primed states. Our understanding of the intrinsic and extrinsic factors that enable PSCs to self-renew in naïve and primed states, and ultimately, to exit self-renewal and undergo differentiation is incomplete. In naïve mouse embryonic stem cells (mESCs), secreted Wnt proteins support the naïve state. In naïve human embryonic stem cells (hESCs), Wnt/β-catenin signaling is active, and the expression of β-catenin target genes decreases during the transition from a naïve to primed state. However, the functions of Wnt/β-catenin signaling in naïve human pluripotency are largely uncharacterized. Here, we provide evidence that naïve hESCs have active endogenous Wnt/β-catenin signaling that is likely sustained by autocrine Wnt ligands. In addition, we show that an inhibitor of Wnt secretion reduces self-renewal in naïve hESCs, and that self-renewal is partially rescued by addition of recombinant Wnt3a. Whereas this suggests that the Wnt/β-catenin pathway is required for the efficient self-renewal of naïve hESCs, further experiments suggest it is dispensable for maintaining the expression of pluripotency markers. Finally, we demonstrate that inhibition of Wnt/β-catenin signaling in naïve hESCs promotes a more primed-like protein expression profile. Together, our results suggest that Wnt/β-catenin signaling plays an important role in the self-renewal of naïve hESCs and in the transition of naïve cells to a primed state.

EMBRYONIC STEM CELL CLINICAL APPLICATION

W2225

PREPARATION OF SEED STOCKS OF PLURIPOTENT STEM CELLS FOR HUMAN APPLICATION: "A STITCH IN TIME SAVES NINE"!

Stacey, Glyn[1], Hunt, Charles[1], Man, Jennifer Sui-Sum[1], Iriajen, Angela[1], Abranches, Elsa[1], O'Shea, Orla[2], Barbato, Michela[1], Chapman, Charlotte[1], Nowell, Craig[1] and Collins, Mary[1], [1]UK Stem Cell Bank, National Institute for Biological Standards and Control, Hertfordshire, U.K., [2]UK Stem Cell Bank, Potters Bar, U.K.

The raw and starting materials such as culture media components and production cell line/s require careful consideration in order to enable a reliable and safe cell therapy product. Regulators will often say "it is not possible to test safety into a product" and early and robust risk assessment and traceability of these materials is crucial to minimise risk to patients and investors. The UK Stem Cell Bank is a key component of the UK regenerative medicine infrastructure charged with procuring, processing (banking and testing) and distributing seed stocks of human pluripotent stem cell (hPSC) lines for research and human application and is equipped with laboratories designed to meet EU GMP. In its latest phase of development the UKSCB is focused on 41 hPSC lines established in the UK under appropriate conditions and traceability (i.e. Human Tissues Authority (HTA) licensed) and under principles established by the International Stem Cell Banking Initiative to enable them to be used for cell therapies. Key elements of the UKSCB process for these lines are ethics review, "due diligence", processing, storage and distribution; all of which are designed to meet the requirements of the UKSCB HTA license which has been maintained successfully for more than ten years. All cell lines are subject to ethical scrutiny by a national ethics committee, following which the UKSCB performs a due diligence protocol which establishes for each individual cell line, whether it could meet European requirements (i.e. EUTCD) and provide a potential starting material for clinical trials. The first of these cell lines has now been banked, representing the start of a pipeline of hPSC lines that will be made available for research or commercial development internationally, under a non-exclusive license. UKSCB has established a catalogue of the 41 lines and is now establishing a 'user group' to gather input on prioritisation of lines for banking and release. The UKSCB process and its pre-distribution catalogue of UK Steering Committee approved lines will be described and UKSCB invites other potential developers of cell therapies to engage with the UKSCB on the re-

quirements for such seed stocks and prioritisation of lines for early banking and release.

Funding Source: Medical Research Council UK

W2227

CPG METHYLATION UPSTREAM OF THE CTG REPEAT IN MYOTONIC DYSTROPHY TYPE 1 AFFECTED HUMAN EMBRYONIC STEM CELL LINES IS CORRELATED WITH MATERNAL INHERITANCE OF THE DISEASE AND A LARGER EXPANSION SIZE.

Barbé, Lise[1], Lopez-Castel, Arturo[2], Spits, Claudia[3], Seneca, Sara[4], Pearson, Christopher[5] and Sermon, Karen[3], [1]Vrije Universiteit Brussel (VUB), Brussels, Belgium, [2]Scientific and Business Director at Genera Biotech, Valencia, Spain, [3]Vrije Universiteit Brussel, Brussels, Belgium, [4]Centre for Medical Genetics, UZ Brussels, Brussels, Belgium, [5]Genetics and Genome Biology, The Hospital for Sick Children, Toronto, ON, Canada

The CTG repeat in the DMPK gene, causing myotonic dystrophy (DM1) when expanded, is located in a CpG island and is flanked by two CCCTC-binding factor (CTCF) binding sites. Epigenetic changes around expanded trinucleotide repeats (TNR) have been observed and a correlation with TNR instability has been suggested. We have investigated CTCF site methylation in three DM1 affected human embryonic stem cell (hESC) lines and their affected donors. We compared DNA bisulphite treatment combined with cloning of PCR products and Sanger sequencing, to a novel combination of DNA bisulphite treatment, PCR and massive parallel sequencing (MPS). Cloning of PCR products is labour intensive and is restricted in the number of epi-alleles that can be analysed, typically about 20. Massive parallel bisulphite sequencing sequences several thousands of DNA molecules of which we randomly selected 100 alleles for further analysis. Both techniques showed methylation upstream of the repeat for VUB03_DM1 and VUB24_DM1, but not for VUB19_DM1 and methylation downstream of the repeat for all three hESC lines. The CTG repeat size was found to be smaller in VUB19_DM1 (470 repeats) and than in VUB24_DM1 (1800) and VUB03_DM1 (2100 repeats). Furthermore, the embryo used for the generation of VUB19_DM1 inherited DM1 paternally, while for VUB03_DM1 and VUB24_DM1 the embryo inherited DM1 maternally. The DNA of the donors of the embryos from which the hESC lines were derived did not show methylation. In hESC, the difference in methylation status upstream of the CTG repeat could therefore either be attributed to a difference in repeat length or the inheritance pattern of DM1. Since all DM1 hESC lines showed methylation downstream of the CTG repeat, no such correlations could be made here. Only one study recently investigated the methylation pattern in DM1 hESC lines in a large region upstream of the CTG repeat which however did not overlap the region we have analysed (Yanovsky-Dagan et al., 2015). They found methylation in all hESC lines with >300 CTG repeats which is contrary to our data showing no methylation for VUB24_DM1 with 470 repeats, but in accordance with the other two VUB hESC lines with a larger expansion. Analysis of more hESC lines especially with paternal inheritance and large repeat sizes would reinforce our conclusions.

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CANCER CELLS

W2229

MU.S.SHI-1 REGULATES AKT-DERIVED IL-6 PARACRINAL MALIGNANCY AND CHEMORESISTANCE IN GLIOBLASTOMA

Chen, Hsiao-yun and Chiou, Shih-Hwa, National Yang Ming University, Taipei, Taiwan

Glioblastoma multiform (GBM) is one of the most deadly human malignant brain tumors with high risks of recurrence and poor treatment outcomes after clinical follow up. The RNA-binding protein Musashi-1 (MSI1) is a marker of neural stem/progenitor cells. Recent study showed that high expression level of MSI1 positively correlates with advanced grade of glioma, and that MSI1 increases the tumor growth of GBM. Herein, we explore the roles of MSI1 as well as the underlying mechanisms in the regulation of chemo-drug resistance and tumor formation of GBM cells. Our results demonstrated that overexpression of MSI1 effectively protected GBM cells from chemo-drug induced apoptosis through down-regulating pro-apoptotic molecules; whereas inhibition of AKT reduced the MSI1-induced anti-apoptosis and cell survival. We further showed that MSI1 robustly increased the expression and secretion of the pro-inflammatory cytokine IL-6, which can be reversed by blocking AKT activity. On the other hand, the secreted IL-6 enhanced AKT activity in a paracrine manner, forming a positive feedback regulatory loop with the MSI1-AKT pathway. Conclusively, our results demonstrated a novel drug resistance mechanism in GBM cells that MSI1 inhibits chemo-drug-induced apoptosis by promoting an AKT/IL6 regulatory circuit and suppressing several pro-apoptotic molecules. MSI1 regulates both cellular signaling and tumor-microenvironmental cytokine secretion to create an intra- and intercellular niche for GBM to survive chemo-drug attack.

W2231

BLOCKING OF PROGRAMMED DEATH LIAND 1 ENHANCES NATURAL KILLER CELL-MEDIATED ANTICANCER IMMUNITY TO MELANOMA CELL LINES

Heo, Woong and Bae, Jaeho, Pusan National University School of Medicine, Yangsan, Korea, South

Ionizing radiation has been used to treat cancer for a century. It effectively inhibits the growth of cancer cells by inducing cell death and anticancer immunity. Although ionizing radiation is somewhat immunogenic, it also increases the expression of immune suppressive proteins such as programmed death ligand 1 (PD-L1). PD-L1 is a ligand against programmed death-1 (PD-1) which contributes an inhibitory pathway of T cells, B cells, and dendritic cells in adaptive immunity. But their role during innate immunity remains poorly understood. With this point of view PD-1, it was supposed that PD-L1 might inhibit Natural Killer (NK) cells which are critical innate immune lymphocytes destroying virally infected or transformed cells through targeted cytotoxicity and further assisting other lymphocytes in the immune response by releasing inflammatory cytokines. Surface PD-L1 levels are increased by ionizing radiation in a dose-independent manner and increased PD-L1 suppresses the activity of the NK cell. When PD-1/PD-L1 inhibitor 2 which inhibit the interaction between PD-1 and PD-L1, was treated following irradiation, it could increase the cytotoxic effects of NK cells to cancer cells. It was suggested that treatment with PD-1/PD-L1 inhibitor might potentiate anti-tumor immunity induced ionizing radiation. In addition, since it was known that cancer stem cells (CSCs) are resistant to standard treatment including radiotherapy, NK cells are alternative modality to kill cancer stem cells. We evaluated the susceptibility to NK cells of CSCs compared to non-CSCs. Therefore, NK cell-based immunotherapy may have more benefit to eliminate cancer stem cells.

Funding Source: Department of Research Center, Dongnam Institute of Radiological and Medical Sciences, Gijang Busan, Republic of Korea 46033

W2233

IDENTIFICATION OF CALMIDAZOLIUM CHLORIDE THAT SELECTIVELY TARGETS CANCER STEM-LIKE CELLS

Lee, Jina, Yonsei University, Seoul, Korea

Cancer stem cells (CSCs), that are capable of self-renewal and differentiation, are considered as tumor-initiating cells in many cancers, including leukemias and multiple myelomas. CSCs are thought to be responsible for metastasis, relapse and drug resistance. Undifferentiated or poorly differentiated tumors are harder to control and it may need more strong treatment. Thus, finding a novel drug which is able to selectively induce either differentiation or apoptosis in CSCs is important. Calmidazolium chloride (CMZ) is known as a Calmodulin (CaM) antagonist and calcium channel protein inhibitor. CMZ can induce an increase in the intracellular calcium concentration which have an effect on cellular processes including apoptosis in various cell lines. However, little is known about the effects of CMZ on CSCs. Here, we examined the effects of CMZ on the embryonal carcinoma cell line F9 and embryonic stem cell line E14 (normal counterpart). In this study, embryonal carcinoma cell (ECC) can be used as an alternative model of CSCs. Our cell viability assay showed that growth inhibition of F9 cells by CMZ is profound compared to that of E14 cells. Furthermore, CMZ treatment induced Caspase-3 dependent apoptosis of F9 cells and accumulation of sub-G1 phase population. Real-time PCR showed that mRNA expression level of c-Myc was notably down-regulated in CMZ-treated F9 cells. c-Myc performs a central role in regulating proliferation, differentiation and apoptosis, and its deregulation is involved in majority of cancers. Taken together, these results imply that CMZ can promote growth inhibition of EC cells by inducing apoptosis and down-regulation of c-Myc expression, thereby implicating CMZ might have potential as a selective drug for CSCs.

W2235

PROTON ACTIVATES OGR1, GPR4 AND G2A HOMOLOGS OF ZEBRAFISH

Mochimaru, Yuta[1], Azuma, Morio[2], Negishi, Jun[3] and Tomura, Hideaki[3], [1]Meiji university, kawasaki , Japan, [2]University of Toyama, Toyama, Japan, [3]Meiji university, kawasaki, Japan

Local extracellular acidification is often occurred in the region of the tumor and inflammation. The acidification influences not only to the tumor and immune cell activities such as the progression and survival, but also to the therapeutic potential of transplanted progenitor cell survival and activities. Little is known, however, about the molecular mechanisms sensing the extracellular acidification. Mammalian ovarian cancer G protein-coupled receptor 1 (OGR1), GPR4 and G2A are belong to the family of G protein-coupled receptors. They are widely expressed in various cells, especially in immune cells. The receptors sense extracellular protons and activate intracellular signaling pathways through trimeric G-proteins although these receptors have originally reported that they were activated by the lipids such as sphingosylphosphorylcholine (SPC), lysophosphatidylcholine (LPC) and 9-HODE. So the receptors can be a candidate of the sensor of the extracellular acidification as described above. Zebrafish is a useful vertebrate model system to elucidate the molecular mechanism of the receptor functions in vivo. They grow outside from mother and have the transparent body. These characteristics enabled them to analyze

tumor progression and inflammation processes by using in vivo imaging. We found that OGR1, GPR4 and G2A homologs in the zebrafish genome database. This suggests these receptors may play a common physiological role in vertebrates; however, their characterizations have not been reported yet. In this study, we characterized a function of the receptor homologs, especially for their ligand specificity and signaling pathways by expressing them in HEK293 cells. We found these receptors also sense protons like the mammalian receptors and activates multiple signaling pathways.

W2237

METABOLIC BIOMARKERS OF PROSTATE CANCER STEM CELLS RADIORESISTANCE

Tyutyunnykova, Anna[1], Peitzsch, Claudia[1], Telegeev, Gennady[2] and Dubrovska, Anna[1,3], [1]OncoRay - National Center for Radiation Research in Oncology, Dresden, Germany, [2]The Institute of Molecular Biology and Genetics of NASU, Kyiv, Ukraine, [3]German Cancer Consortium (DKTK), Dresden, Germany

Radiotherapy is one of the main curative modalities for prostate cancer treatment. However, tumor radioresistance still remain a clinical challenge. Recent data suggest that tumor radioresistant cells and tumor initiating cells (TIC) share unique metabolic features. The deciphering of these metabolic pathways might be beneficial for the development of predictive biomarkers and novel treatment strategies. To analyze metabolic traits associated with emergence of radioresistance, we developed highly tumorigenic radioresistant (RR) isogenic sublines of the established prostate cancer cell lines DU145, 22RW1, LNCaP, PC3 by irradiation with multiple fractions of X-ray irradiation. Comparative analysis of the intracellular metabolites was performed using flow cytometry and mass spectrometry analysis. The data of the metabolic profiling were related to the results of comparative gene expression profiling. Whole genome gene expression analysis was performed for prostate TICs, parental and RR cells. Expression of the selected enzymatic proteins, which are differentially regulated in the parental and RR cells was targeted by using small interfering RNAs (siRNAs) or by pharmacological inhibition. The radioresistant cancer cells share many properties with TICs including a high tumorigenicity, enhanced expression of CSC markers (CD133, CXCR4, ABCG2, OCT4, NANOG) and high aldehyde dehydrogenases (ALDH) activity. Radioresistant prostate cancer cell sublines DU145-RR and LNCaP-RR have significantly lower baseline level of intracellular ROS as compared to parental cells that together with activated DNA repair can contribute to their higher radioresistance. Metabolic and gene expression profiling revealed that radioresistant prostate cancer cells possess a high expression of the anti-oxidative enzymes, inhibition the tricarboxylic acids cycle as well as alteration in the amino acid metabolism, pentose phosphate pathway and NADPH production which is essential for antioxidant defense and cell survival after radiotherapy. Our results indicate that prostate cancer cell undergo reprogramming of the essential metabolic pathways during the course of fractionated irradiation that affect their tumorigenic and radioresistant properties.

W2239

TARGETING SELF-RENEWAL AS A THERAPEUTIC APPROACH FOR ORAL SQUAMOUS CELL CARCINOMA

Simental, Alfred, Lee, Steve, De Andrade Filho, Pedro, Mirshahidi, Saied, Duerksen-Hughes, Penelope and **Yuan, Xiangpeng**, Loma Linda University, School of Medicine, Loma Linda, CA, U.S.

Evidence suggests that head and neck squamous cell carcinoma is organized as a cellular hierarchy sustained by a subpopulation of cancer initiating cells (CICs), or cancer stem cells. These CICs are believed to play major roles in local regional recurrences, distant metastases and patient survival, indicating that therapeutic approaches targeting this cell population would be highly beneficial to patient outcome. However, there is no evidence regarding whether targeting CICs is a clinical relevant approach in the treatment of oral cavity cancer. Here, we isolated tumorspheres from clinical specimens of oral cavity squamous cell carcinoma. The tumorspheres were able to self renew and recapitulate the original tumor phenotypes in xenograft models, suggesting that the tumorspheres function as CICs of oral squamous cell carcinoma. Further studies revealed that a canonical self-renewal regulator Bmi-1 expression could be detected in the tumorsphere cells with both transcriptional and translational level analyses. To explore whether the BMI-1 has any function in the tumorshpheres, we down-regulated BMI-1 activity with a small molecule inhibitor PTC-209. We observed that the down-regulation caused a sustained decrease in the tumorsphere growth, which was reflected by a combination of a reduced fraction of cells going through s-phase, a small population of actively cycling cells and an increase in cells undergoing apoptosis. In addition, the tumorsphere's self-renewal capability was attenuated after the PTC-209 mediated BMI-1 inhibition as evidenced by in vitro sphere-initiation assay and in vivo transplantation (with limiting-dilution) analysis. Furthermore, inhibiting BMI-1 by in vitro treatment of the tumorsphere cells with PTC-209 diminished the cell's tumor initiation capacity following xenotransplantation. To test whether targeting BMI-1 has a therapeutic benefit on oral cancer, BMI-1 inhibitor was given to tumor-bearing animals as a treatment. Such treatment halted the growth of preformed oral squamous cell carcinoma in subcutaneous xenograft models and reduced lung and liver metastases in tail vein metastatic models. Taken together, our data provide evidence that targeting oral CIC self-renewal by inhibiting

BMI-1 represents a novel therapeutic approach in treating oral cavity cancer.

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TECHNOLOGIES FOR STEM CELL RESEARCH

W3001

DUAL FLUORESCENCE-LABELLED MESENCHYMAL STEM CELLS FOR STUDIES ON ITS POTENTIAL EFFECTS IN A RAT SMALL INTESTINE ISCHEMIC-REPERFUSIONAL INJURY AND MASSIVE RESECTION MODEL

Chen, Yi-Hsin[1], Chen, Pin-Hua[1], Yang, Tsung-Lin[2], Kan, Daphne Wei-Chun[2], Chien, Chung-Liang[1], Ho, Hong-Nerng[3], Lai, Hong-Shiee[2] and **Chen, You-Tzung**[1], [1]National Taiwan University College of Medicine, Taipei, Taiwan, [2]National Taiwan University Hospital, Taipei, Taiwan, [3]Graduate Institute of Medical Genomics and Proteomics, College of Medicine and the Hospital, National Taiwan University, Taiwan, Taipei, Taiwan

Injuries such as trauma, inflammation, surgery, and arterial thrombosis can induce ischemic change of the intestine. Ischemic and reperfusional injury (I/R) will increase intracellular free radicals and alter the permeability and enzyme reaction of the bowel mucosa cells causing bowel dysfunction. Bone marrow derived mesenchymal stem cells (MSCs) are a part of the adult stem cells that have been successfully isolated and cultured for stem cell research for many years. MSCs not only have the ability to transdifferentiate into endoderm derivatives, they also have immunomodulation potential and the ability to attract host MSCs to the damaged site. Because of this, MSCs can be extensively applied in many categories of research including cell therapy, tissue engineering and regenerative medicine. The main purpose of this project is to use male Wistar rats as subjects to find out whether MSCs can protect ischemic and reperfusion injury of the intestine and reduce tissue damage in the massive small bowel resection model.

W3003

THE PB-TET-GOI INDUCIBLE SYSTEM FOR DIRECTED DIFFERENTIATION, REGULATED GROWTH FACTOR SECRETION, AND IDENTIFICATION OF QUIESCENT TUMOR CELL POPULATIONS

Akhtar, Aslam[1], Gowing, Genevieve[1], Svendsen, Clive[2], Danielpour, Moise[1] and Breunig, Joshua[3], [1]Cedars Sinai Medical Center - Regenerative Medicine Institute, Los Angeles, CA, U.S., [2]Cedars-Sinai Regenerative Medicine Institute, Los Angeles, CA, U.S., [3]Cedars-Sinai Medical Center, Los Angeles, CA, U.S.

Precise control of transgene expression is fundamentally important for the investigation of biological systems and for gene therapy in the clinic. To address this, we have created a genetic system for the stable, inducible and reversible genetic control of cell lineages derived from proliferating stem and progenitor cells. Specifically, we have constructed an optimized piggyBac-transposable system that integrates into the genome of proliferating cells through pBase-mediated transposition, circumventing the problems associated with plasmid dilution. The system incorporates the latest generations of tetracycline transactivators, reverse tetracycline transactivators, and variants in order to provide regulated "on" and "off" transgene expression using doxycycline (dox). This novel system termed *pB-Tet-GOI (piggyBac-Tetracycline inducible system for the expression of a gene of interest)* allows for precise temporal control of transgene expression in neural stem and progenitor cells both in vitro and in vivo, with robust inducibility and minimal leakiness. Furthermore, incorporation of fluorescent protein and luciferase allows for live imaging or bioluminescent detection, respectively, of cells expressing a gene of interest in vivo. We have used this system to direct differentiation of human neural progenitor cells to subtype specific neurons in vitro, reprogram mouse olfactory bulb interneurons to projection neuron-like subtypes in vivo, regulate growth factor secretion in vitro and in vivo, and identify quiescent tumor cell populations after oncogene misexpression in mouse neural stem cells in vivo. As various transgenes can be readily introduced into the system, this approach provides a robust and versatile strategy for the temporal regulation of transgene expression in vitro and in vivo.

Funding Source: Cedars-Sinai Regenerative Medicine Institute

W3005

A MACHINE LEARNING TECHNIQUE USES CELL LINEAGE MAPS TO IDENTIFY KEY REGULATORS OF STEM CELL DIFFERENTIATION

Carlin, Daniel, Pratt, Dexter and Ideker, Trey, University of California-San Diego, San Diego, CA, U.S.

Understanding the master regulators of pluripotency and differentiation is the key to inducing pluripotency, facilitating transdifferentiation and controlling lineage commitment. There is a need for analysis that takes into account unique cellular context, prior knowledge of gene regulatory networks, and established lineage relationships. To robustly identify regulatory events that occur at each step of a cellular differentiation hierarchy, we introduce a novel machine learning approach to transcriptional analysis. First, we summarize each sample by extracting evidence for regulation by transcription factors or epigenetic modification of topological domains. We then use known cell lineage maps to guide a machine learning regression task that learns the implied relationships between developing cell types and their regulation features. By assuming that developmentally related cell types have similar expression and regulatory profiles, we can share information about related cell types across the development graph. The change in importance of particular regulation features between cell types implicates specific regulators that are the most responsible for each step of a differentiation hierarchy. We demonstrate the efficacy of both the regulatory features and the development graph-guided regression in effectively capturing the unique features of cellular identity and identifying the key genomic regulators in a publicly available hematopoietic development expression dataset.

Funding Source: CIRM

W3007

COMPLEMENTARY SCREENING OF IPS CELLS: AUTOMATED PATCH CLAMP, EXTRACELLULAR FIELD POTENTIAL AND IMPEDANCE RECORDINGS

Dragicevic, Elena[1], Becker, Nadine[2], Thomas, Ulrich[2], Doerr, Leo[2], Bot, Corina T.[3], Rapedius, Markus[2], Obergrussberger, Ali[2], Haarmann, Claudia[2], Rinke, Ilka[2], Beckler, Matthias[2], Stoelzle-Feix, Sonja[2], Haedo, Rodolfo[3], George, Michael[2], Brueggemann, Andrea[2] and Fertig, Niels[2], [1]Nanion Technologies, München, Germany, [2]Nanion Technologies, Munich, Germany, [3]Nanion Technologies Inc., Livingston, NJ, U.S.

Novel reliable and predictive *in-vitro* cardiac safety or neurotoxicity screenings demand further development of automated, high-throughput compatible drug evaluation systems. Recently, induced pluripotent stem cells (iPSCs) emerged as the model of choice for cardiovascular risk assessment or neurotoxicity screening in-vitro assays. Here, we report newly developed specialized cell handling protocols for the use of iPSC cardiomyocytes and neurons on planar patch clamp systems. In addition, we implemented a new hybrid screening method that combines impedance (cell contractility) with MEA-like extracellular field potential (EFP) into routine screening of iPSC cardiomyocytes. Combined, above described electrophysiological methods, provide a non-invasive, label-free, high temporal resolution approaches for screening iPSC derived cardiomyocytes and neurons. Our chip-based approaches, allow parallel patch clamp recordings without compromising neither data quality nor sophistication regarding technical features. We developed miniaturized, modular system, with full integration in automated robotic platforms, which enables highly efficient, parallel ion channel screening with the chip-based approach in the industry standard microtiter plate format. We also developed minimized cell usage protocols, crucial for usage of cell lines with limited availability, or otherwise rare and expensive cells, such as (iPS) cell-derived cardiomyocytes or neurons. Here, we present patch clamp recordings of voltage gated ion channels (e.g. Nav1.5, hERG or Cav1.x) as well as ligand gated channels (GABA and glutamate receptors) from iPSC cardiomyocytes and neurons. Additionally, we present pharmacological investigations of reference compounds (e.g. E4031 and Verapamil) on cardiac contractility and EFPs of iPS cardiomyocytes. Taken together, these platforms together provide unmatched information on a compound's safety profile. Reduced cell usage, increased throughput and integration into robotic environments improve cost efficiency, precision and are speeding up the whole HTS process of drug development and safety screening.

W3009

ENGINEERING BIOACTIVE VASCULAR GRAFTS WITH A NOVEL LIGAND AGAINST αvβ3 INTEGRIN TO RECRUIT ENDOTHELIAL PROGENITOR CELLS FOR IMPROVED ENDOTHELIALIZATION AND PATENCY

Hao, Dake[1,2], Fan, Yahan[1], Wu, Yang[1], Xiao, Wenwu[3], Li, Yuanpei[3], Liu, Ruiwu[3], Pivetti, Christopher[1], Farmer, Diana[1], Lam, Kit[3] and Wang, Aijun[1], [1]Surgical Bioengineering Laboratory, Department of Surgery, University of California Davis, Sacramento, CA, U.S., [2]School of Pharmaceutical Sciences, Shandong University, Jinan, China, [3]Department of Biochemistry & Molecular Medicine, University of California Davis, Sacramento, CA, U.S.

Previously using K562 myeloid leukemia cells transfected with human αvβ3 integrin as living probes, we screened

high-throughput one-bead-one-compound (OBOC) combinatorial libraries and identified LXW7, a ligand that has high-specific binding affinity against αvβ3 integrin. In this project, using primary arterial endothelial cells (ECs) and cord blood derived endothelial progenitor cells (EPCs), we confirm that LXW7 has high-specific binding affinity for αvβ3 integrin on the surface of active ECs and EPCs. Culture surface coated with LXW7 strongly supported the attachment and proliferation of ECs and EPCs, and enhanced the phosphorylation of VEGF receptor 2 (VEGF-R2) and the activation of mitogen-activated protein kinase (MAPK) ERK1/2 in ECs. LXW7 supported limited attachment of platelets and did not support attachment of THP-1 monocytes. To test the in vivo function of LXW7, we functionalized small-diameter nanofibrous vascular grafts with LXW7 via CLICK chemistry and investigated its functions on endothelialization and graft patency in a rat carotid artery bypass model. Immunohistological analyses showed that at 1 week after implantation, the LXW7 modified vascular grafts possess significantly more $CD34^+$ EPCs in the middle segment of the luminal surface than the control grafts. At 6 weeks after implantation, mature endothelialization was present throughout the whole length of the LXW7 modified grafts while only a limited number of $CD31^+$ ECs were seen in the middle of the control grafts. Patency testing results confirmed that at 6 week after implantation, 5 out of 6 LXW7-modified vascular grafts remained patent versus in the control group only 1 out of 6 grafts was patent. Collectively, our results have demonstrated that LXW7 specifically supports EPC/EC attachment and functions, and can improve in situ endothelialization of vascular grafts. LXW7 and their derivatives hold great promise for endothelialization related tissue regeneration applications.

Funding Source: The project was supported by the departmental fund from the Department of Surgery, UC Davis and the National Natural Science Foundation of China

W3011

cGMP-APPLICABLE SINGLE USE BAG-BASED BIOREACTOR SYSTEM FOR LARGE SCALE PRODUCTION OF HUMAN PLURIPOTENT STEM CELLS

Huang, Patricia Hsiao-I, Lin, Chih-Min, Chen, Vincent Chang-Yi, Liu, Jian-Chang, Hua, Giau, Ye, Jing-Jing, Hsu, David and Couture, Larry A., Beckman Research Institute of City of Hope, Duarte, CA, U.S.

Human embryonic stem cell (hESC) and induced pluripotent stem cell (iPSC) hold great potential as sources for differentiating into specific cell types in regenerative medicine. Access to unlimited numbers of the sources is crucial for cellular based therapeutic applications, human disease modeling, and drug discovery. Here, we report a microcarrier-free scalable suspension culture system using serum-free defined media and cGMP-equivalent conditions in wave bag bioreactor system for large scale hESC and iPSC production. By optimizing critical process parameters including agitation rate, cell seeding density, splitting interval and growth factor, we demonstrate the consistency and reproducibility of the production process by serial passage several hESC/iPSC lines in 1 to 3 liter scale wave bag system with overall expansion, yield, viability, and maintenance of pluripotency. hESC/iPSC maintained in suspension as aggregates can be expanded up to $2x10^6$ cells/ ml with normal karyotype. In addition, the aggregates are able to further differentiate into downstream lineages such as cardiomyocytes. Moreover, a perfusion mode is also established with the wave bag bioreactor for large scale production of hESC/iPSC lines to significantly reduce the manual operations. Taken together, our suspension wave bag system provides a potent approach for scale-up /scale-out expansion of hESC/iPSC with potential of hands free for medium change during the process that facilitates the needs of providing hESC/iPSC for clinical and research applications.

Funding Source: CIRM (California Institute for Regenerative Medicine)

W3013

HUMAN INDUCED PLURIPOTENT STEM CELLS TO PREDICT AND MODEL NEUROTOXICITY IN HIGH THROUGHPUT AND HIGH CONTENT ASSAYS

Keminer, Oliver, Fraunhofer IME Screening Port, Hamburg, Hamburg, Germany, Braendl, Bjoern, UKSH Kiel, Kiel, Germany, Mueller, Franz-Josef, Zentrum für Integrative Psychiatrie, Kiel, Germany, Fischer, Rainer, Fraunhofer IME, Aachen, Germany and Pless, Ole, Fraunhofer IME ScreeningPort, Hamburg, Germany

A major challenge in characterizing the potential neurotoxic risk of chemical agents or drugs is the paucity of available and relevant hazard data sets. Currently, there are thousands of chemicals being evaluated for drug discovery purposes, however, only a relatively small number of these have been adequately characterized for adverse effects such as being causative agents for neurological disorders. Many studies have indicated that in vitroneurite outgrowth assays can be used to identify harmful chemical effects on developing nervous system and/or peripheral neurons and this approach has been widely adopted by the pharmaceutical industry for drug discovery and safety pharmacology. Due to the advances in stem cell technologies, we have developed an improved assay system that makes use of highly characterised hiPSC derived peripheral neurons (Peri.4U, Axiogenesis) according to a recently published assay protocol (Wheeler et al., 2015). We were able to develop and to validate a semi-automated 384-well High Throughput Assay, which combines a non-lytic Cell Viability Assay (RealTime-Glo™, Promega)

with a flexible High Content Screening (HCS) assay (Perkin Elmer, Opera) that detects neuron specific betaIII-Tubulin, activated Caspase-3 and nuclei staining. The assays offers Z′ >0.5 (Zhang et al., 1999) for "total neurite outgrowth intensity", "mean Caspase-3 intensity" and "nuclei number". To validate the assay we tested a commercial available toxicity collection (approximately 150 compounds) at 10 μM. Toxic Hits were profiled in 11-point dose-response studies. Using CSIRO Neurite Analysis (Perkin Elmer, Columbus) we were able to characterize neurite outgrowth in detail, including determining maximum neurite length, number of extremities, roots, segments and nodes. Due to the hypothesis that differences in damage among drugs reflect differences in their mechanisms of action we analysed neurite outgrowth in detail, including determining maximum neurite length, number of extremities, roots, segments and nodes (CSIRO Analysis, Columbus, PerkinElmer,). These data will be used to perform statistical association tests to identify and to describe phenotypic changes which are correlated with the mechanism of action of known and unknown compounds.

W3015

IMPROVED HUMAN PLURIPOTENT STEM CELL EXPANSION IN PERFUSED BIOREACTORS AT CHEMICALLY DEFINED CONDITIONS – PROCESS-DEPENDENT CHANGES OF THE ENERGY METABOLISM

Kropp, Christina[1,2], Kempf, Henning[1,3], Halloin, Caroline[1,2], Robles-Diaz, Diana[1,2], Franke, Annika[1,2], Martin, Ulrich[1,2], Olmer, Ruth[1,2] and Zweigerdt, Robert[1,2], [1]Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover, Germany, [2]REBIRTH Cluster of Excellence, Hannover Medical School, Hannover, Germany, [3]Hannover Medical School, Hannover, Germany

Human pluripotent stem cells (hPSCs), including human induced pluripotent stem cells and embryonic stem cells, are a unique source for the, in principle, unlimited production of functional human cell types in vitro, providing the basis for novel cell therapies and human disease models in vitro. All envisioned applications will require the constant supply of billions of lineage-specific cells generated by robust, economically viable and ultimately Good Manufacturing Practice (GMP) compliant processes. The expansion of hPSCs as cell-only aggregates in 3D suspension culture provides a feasible and potentially superior solution for producing the required cell numbers. We have recently established expansion of single cell inoculated hPSCs as cell-only-aggregates in stirred tank bioreactors. Subsequently, this technology was combined with cardiomyogenic differentiation using chemical Wnt pathway modulators thereby establishing a novel, continuous expansion-differentiation process compatible with controlled mass cell production. Most current media used for hPSC expansion in suspension culture are not chemically defined and relative expensive impeding development of large scale processes. However, "in house production" of previously published "essential 8" (E8), a xeno-free and chemically defined culture medium for hPSC maintenance, facilitates systematic testing of process parameters and upscaling of process dimensions. Using E8 in perfused stirred tank bioreactors we demonstrate a novel, more effective and homogenous hiPSC expansion process yielding up to 3.8×10^6 cells/ mL in a working volume of 100 mL in 7 days, supporting clinical translation of hPSCs at reduced cost. Interestingly, physiological and gene expression assessment indicated distinct changes of the cells' energy metabolism over time suggesting a culture induced switch from glycolysis to oxidative phosphorylation but notably in the absence of hPSC differentiation. Our data highlight the plasticity of hPSCs' energy metabolism and provides clear physiological and molecular targets for process monitoring and further development.

W3017

DEVELOPMENT OF INTEGRATED MULTIPLEX ASSAYS FOR SIMULTANEOUS PROFILING OF RNA AND PROTEIN IN SINGLE CELLS

Liu, Benjamin, Gong, Haibiao, Dakshinamoorthy, Gajalakshmi, Ooi, Aik, Li, Nianzhen, Unger, Marc, Holcomb, Ilona and Ramakrishnan, Ramesh, Fluidigm Corp., South San Francisco, CA, U.S.

Gene expression is a multistep process that includes the transcription, translation and turnover of messenger RNAs (mRNAs) and proteins. Recently, gene expression studies have measured differentiation propensities between different human induced pluripotent stem cell (hiPSC) clones and compared protein coding gene profiles between hiPSCs and embryonic stem cells (ESCs). Indeed, relatively little is known about the expression characteristics of individual RNA transcripts and their corresponding protein within each single hiPSC. In addition, studies of the dynamic interaction between RNA and protein during the cell reprogramming stage remains rare. While a few studies have been conducted primarily by combining fluorescence in situ hybridization for RNA detection and flow cytometry or immunofluorescence staining for protein analysis, these methods have critical limitations, such as low multiplexing levels, low throughput and long turnaround times. We have developed a simple, fast, multiplexed single-cell mRNA and protein co-detection system. Cell capture, cell lysis, antibody binding, oligo extension reaction, cDNA synthesis and multiplexed preamplification were integrated into the C1™ system as a single workflow. Final amplicon products were quantified by qPCR on the Biomark™ HD system. Data was analyzed using the Singular™ Analysis Toolset. A primary dual detection panel — including 52 target proteins and paired 30 RNAs' assays — was developed, which related to cat-

egories of cell cycle (CCNA2, CCNB1, CCND1, etc.), proliferation (*KI67*, etc.), apoptosis (TNFRSF10B, etc.), tumor suppressor (P53, PTEN, etc.), biomarker (ERBB2, BRCA1, EPCAM, etc.), stem cell marker (LIN28A, SOX2, NANOG, etc.) and growth factor (EGFR, TGFß1, VEGFA, etc.). Combined RNA and protein distinct signatures of each hiPSC were identified. We demonstrate that this method is capable of obtaining correlating changes of RNA and protein expression levels during cell transition at a single-cell level, and the current assay system is a suitable modeling approach for providing new insights into cell differentiation, reprogramming, tumor progression, and so on.

W3019

MULTIPRIME: A BACULOVIRUS-BASED MULTIGENE EXPRESSION SYSTEM FOR MAMMALIAN CELLS

Mansouri, Maysam[1], Rizk, Aurelien[1], Xie, Ye[1], Ballmer-Hofer, Kurt[1], Berger, Imre[2] and Berger, Philipp[1], [1]Paul Scherrer Institute, Villigen, Switzerland, [2]EMBL, Grenoble, France

Multigene delivery is emerging as a key technology for many applications in contemporary cell molecular biology. Examples include stem cell engineering, synthetic biology, cellular reprogramming, and genome editing. Infection by viral vectors emerged as the dominant method of choice to deliver genes into primary and stem cells. Current viral delivery system such as retro-, adeno- and lenti viruses suffer from limited DNA capacity. In contrast, transgene capacity of baculovirus, a virus that normally infects specific moth larvae, is very large (more than 100 kb).

We developed a baculovirus-mediated multigene expression system (MultiPrime), allowing simultaneous expression of several genes from a single virus in mammalian cells. MultiPrime can be used for the infection of primary cells or of cell lines that are difficult to transfect. Here, we show that it is possible to express up to five proteins with a modified baculovirus in primary cells, cell lines and stem cells. We also created different intracellular biosensors which can be expressed simultaneously and allow to study receptor trafficking and signaling quantitatively in single cells. Additionally, a wide range of promoters are available in MultiPrime, to regulate and fine-tune individual heterologous target gene expression. Moreover, we utilized MultiPrime for reprogramming of mouse embryonic fibroblast (MEF) cells into induced neurons (iN).Taken together, baculovirus is an efficient vehicle to deliver multiple expression cassettes to mammalian cells and we show multiple applications of our system in cell biology.

W3021

DMSO-FREE CHEMICALLY DEFINED CRYOPRESERVATION OF HUMAN MESENCHYMAL STEM CELLS AND CHONDROCYTES

Onuma, Yasuko, Shimizu, Madoka, Aiki, Yasuhiko and Ito, Yuzuru, AIST, Tsukuba, Japan

A serum- and xeno-free cryopreservation is suitable for cells in clinical usage. In addition, dimethyl sulfoxide (DMSO), which is used for conventional cryoprotectant medium has been concern about toxicity and induction ability on some kinds of cells. We examined the efficiency of a DMSO-free/serum-free/xeno-free chemically defined cryoprotectant media, STEM-CELLBANKER DMSO Free, for human mesenchymal stem cells and chondrocytes. The cells cryopreserved in STEM-CELLBANKER DMSO Free showed similar growth rate, morphology and global gene expression, comparing with that of the conventional freezing medium which is 10% DMSO in 20% fatal bovine serum containing culture medium. We therefore propose that STEM-CELLBANKER DMSO Free may be an effective tool for cell storage in clinical application.

Funding Source: Japan Agency for Medical Research and Development (AMED) and the New Energy and Industrial Technology Development Organization (NEDO)

W3023

A RANDOMIZED TRIAL TO ELUCIDATE EFFECT OF MESENCHYMAL STEM CELLS ON IMMUNE MODULATION IN LIVING RELATED KIDNEY TRANSPLANT PATIENTS

Rakha, Aruna[1], Kaundal, Urvashi[2,3], Ramachandran, Raja[1], Kanwar, Deepesh[1], Sharma, Ratti Ram[1], Bagai, Upma[2], Minz, Mukut[1] and Jha, Vivekanand[1], [1]PGIMER, Chandigarh, India, [2]Panjab University, Chandigarh, India, [3]Panjab University, Chandigarh, Chandigarh, India

Kidney Transplant patient are dependent upon the dosing of immunosuppressive drugs for their lifetime in order to prevent the graft rejection. Knowing the adverse side effects of these immunosuppressive drugs, it is desirable to develop a procedure that preferably avoids or atleast greatly minimizes the use of immunosuppressive drugs. Mesenchymal Stem Cells (MSCs) have emerged as an important component towards cell-based therapeutic immunomodulation as these cells possess low immunogenicity. In this study, we aim to define immune regulation and clinical feasibility of cellular therapy mediated by MSCs in Kidney Transplant (KTx) patients. Patients were divided into two groups, one with MSC infusion and second without the MSC infusion. All patients received standard dose of immunosuppressive drugs. Peripheral

Blood Mononuclear Cells are analyzed in order to characterize the immune profile and proliferative capacity of these cells. Serum creatinine levels, Serum cytokine levels were measured and protocol biopsies were done at various time intervals to monitor the graft function. We found MSCs infusion to be associated with Treg expansion and reduced T-cell proliferation in mixed lymphocyte reactions. The cytokine profile supports the expanded number of regulatory T cells. Memory T cell data also represents a very interesting trend. Serum Creatinine levels normalized in all the patients with the levels being slightly lower in the patients infused with MSCs as compared to the control group. Our study adds to the accumulating literature on the safe use of cell-based therapy for immunomodulation in kidney transplantation and also highlights the effects of MSC dosages at different time intervals.

Funding Source: The funding source for this research is Department of Science and Technology (DST), India.

W3025

GROWTH CHARACTERISTICS FOR HUMAN UMBILICAL CORD DERIVED MESENCHYMAL STROMAL CELLS IN POOLED HUMAN PLATELET LYSATE SERUM

Smith, J. Robert[1], Jimenez, Jake[1], Petry, Florian[2], Leber, Jasmin[2] and Weiss, Mark L[1], [1]Kansas State University, Manhattan, KS, U.S., [2]University of Applied Sciences Mittelhessen, Giessen, Germany

Mesenchymal stromal cells (MSC) have applications in medicine as indicated by clinical trials. MSCs contain several properties that make them ideal for clinical translation, including, immunomodulatory abilities and differentiation into osteoblasts, chondrocytes and adipocytes. Recently, MSCs derived from the umbilical cord have been shown to grow in medium supplemented with pooled human platelet lysate serum (HPL). Translating UC-MSCs from *in-vitro* research to the clinic requires a better understanding of the growth characteristics of the cells in various conditions. Here we tested three HPL media formulations. The variations included, (1) the removal of Glutamax supplementation, (2) the removal of Antibiotic-Antimycotic supplementation, and (3) high glucose DMEM instead of the low glucose. The UC-MSCs were grown in the four conditions on 6 well plates for a week; medium samples were taken daily to test the cell number, and media characteristics. Next, cells were grown on microcarriers in 6 well nonadherent plates. The media characteristics tested were glucose, glutamine, glutamate, lactate, ammonium, and osmolality. UC-MSCs to have similar growth characteristics in all the conditions except for the high glucose. The high glucose DMEM maintain growth in culture for an extra day, even after contact. After four days of culture the glucose would be depleted for the three conditions using low glucose DMEM, growth would decrease and cell death incurred following day five. In the high glucose DMEM condition, the cells did not use all of the glucose in the media, and the media also became more toxic, with a higher lactate level approximately 1 g/L more on the final day. In summary, glucose appears to be the critical component when culturing UC-MSCs; it can lead to increased toxicity over longer culture times suggesting media changes might be important.

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W3027

CRISPR/CAS9-BASED GENE CORRECTION OF ARGINASE-DEFICIENT HUMAN INDUCED PLURIPOTENT STEM CELLS TO RECOVER ENZYME FUNCTION

Truong, Brian, Lee, Patrick, Vega-Crespo, Agustin, Gilmore, William, Hermann, Kip, Kingman, Stephanie, Tang, Jonathan, Chang, Katherine, Byrne, James A. and Lipshutz, Gerald, University of California, Los Angeles, Los Angeles, CA, U.S.

Urea cycle disorders (UCDs) are incurable genetic diseases that affect the body's ability to produce urea due to a deficiency in any of six enzymes in the cycle. For arginase deficiency, a mutation in the ARG1 gene, the final step of the cycle, results in hyperargininemia, developmental delays and disabilities, psychomotor function loss, and in serious cases, death. There is currently no completely effective treatment available. In this study, we applied human induced pluripotent stem cell (hiPSC) research and genome-editing technologies to develop a stem cell-based approach for treating arginase deficiency applicable to all arginase deficient patients regardless of mutation. Fibroblasts from three arginase deficient patients were reprogrammed to hiPSCs by applying a lentiviral STEMCCA-based method and were characterized for pluripotency by immunophenotyping for stemness markers, AP staining, and *in vivo* teratoma formation. Using a site-specific CRISPR/Cas9 nickase-mediated gene transferring system, a selectable, full-length codon optimized human arginase cDNA (coARG) expression cassette was inserted into the HPRT locus under control of constitutive hEF1a promoter (LEAPR); targeting HPRT allowed for secondary clonal selection of on-target integration by 6-thioguanine treatment. After LEAPR modification, hiPSCs were differentiated to hepatocyte-like cells (HLCs) and characterized by immunophenotyping and RNA expression for hepatic markers; HLCs demonstrated more fetal-like characteristics. LEAPR-modified HLCs demonstrated 41% functional arginase activity recovery compared to human fetal liver. In this study, we demonstrated the ability to genetically correct mutated ARG1 gene expression in hiPSCs derived from arginase deficient patients and markedly restore arginase function in HLCs by CRISPR/Cas9-based gene addition. To demonstrate *in vivo* recovery of

arginase deficiency pathogenesis, ongoing studies aim to transplant gene-corrected HLCs into an established arginase deficient immunosuppressed mouse model. Successful restoration of enzyme function in patient-specific hiPSCs and HLCs will highlight hiPSCs as a valuable tool in cell replacement therapies and advance applications of genetically modified hiPSCs to treat UCDs and other single enzyme liver deficiencies.

Funding Source: This research was supported by the grant CIRM TR4-06831 from the California Institute of Regenerative Medicine.

W3029

NEW ANTIBODY REAGENTS TO STUDY STEM CELL-MEDIATED DEVELOPMENT AND REGENERATION OF THE MAMMALIAN INTESTINAL EPITHELIUM

Wood, Antony W, Cotta, Richard, Crosby, Katherine, Viswanathan, Aparna and Simendinger, Jessica, Cell Signaling Technology, Inc., Danvers, MA, U.S.

Growth and regeneration of the mammalian intestinal epithelium is driven primarily by a discrete population of stem cells, known as $LGR5^+$ crypt base columnar (CBC) cells, resident at the base of the intestinal crypt. Despite their fundamental importance for intestinal development and regeneration, there is a lack of validated reagents that can discretely identify $LGR5^+$ CBC cells and their direct progeny. This is due primarily to the low abundance and/or accessibility of the LGR5 epitope. To address this problem, Cell Signaling Technology, Inc. (CST) has developed highly specific rabbit antibodies targeting Olfactomedin-4 , a glycoprotein that exhibits distinct expression in $LGR5^+$ CBC cells in both human and mouse. These antibodies are able to detect endogenous levels of human and mouse Olfactomedin-4 protein, by Western blot in tissue lysates, and by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded tissue samples. Antibody specificity was confirmed by validation on cell types and/or tissues in which the presence/absence or relative abundance of Olfactomedin-4 expression has been described in the published literature. These antibody reagents provide a robust method to visualize CBC cells with exquisite sensitivity and specificity without the need for transgenic models. The availability of these tools will permit greater insights into the role and regulation of CBC cells during development, regeneration and oncogenic transformation of the intestinal epithelium.

W3031

A COST-EFFECTIVE METHOD TO ASSEMBLE BIOMIMETIC 3D STEM CELL CULTURE PLATFORMS

El-Badri, Nagwa[1], Khalil, Sabreen[2], El-Mokhtar, Mohamed[2], Habib, Dina[2], Farghaly, Mohamed[2], **Ayman, Radwa**[1], Al-Mofty, Saif[1], Eldosoky, Mohamed[3] and Mousa, Noha[1], [1]Zewail City of Science and Technology, El Sheikh Zayed District, 6th of October City, Giza, Egypt, [2]Faculty of Medicine, Assiut University, Assiut, Egypt, [3]El Sheikh Zayed Specialized Hospital, El Sheikh Zayed District, 6th of October City, Giza, Egypt

Developing effective stem cell based therapies requires the design of complex in vitro culture systems for more accurate representation of the stem cell niche. Attempts to improve conventional cell culture platforms include the use of biomaterial coated culture plates, sphere culture, microfluidic systems and bioreactors. Most of these platforms are not cost-effective, require industrial technical expertise to fabricate and remain too simplistic compared to the physiological cell niche. The human amniotic membrane (hAM) has been used successfully in clinical grafting applications due to its unique biological and regenerative properties. In this study, we present a combinatorial platform that integrates the hAM with biomolecular, topographic and mechanical cues in one versatile model. We utilized the hAM to provide the biological and the three dimensional (3D) topographic components of the prototype. Institutional research ethics approvals and informed consents were obtained for human samples used in the study. The 3D nano-roughness of the hAM was first characterized using surface electron microscopy and surface image analysis (using Image J and Surface J). Three models of varying complexities of the prototype were assembled. We developed macro-scale and micro-scale versions of the platform which provided shear stress factors to simulate the fluid dynamics of the *in vivo* extracellular fluids.. A well defined 3D surface modulation of the hAM comparable to commercial 3D biomaterial substrates was successfully obtained without complex fabrication and for significantly lower cost. Performance of the prototype for stem cell culture applications was demonstrated using various cells including human umbilical cord mononuclear blood cells (MNCs), human bone marrow mesenchymal stem cells (hMSCs) and human breast cancer tissue explants. Expansion and differentiation (adipogenic and osteogenic) were achieved for mesenchymal stem cells using the platform. In conclusion, this study presents methods of assembling integrated, flexible and low cost biomimetic cell culture platforms for diverse stem cell culture applications.

Funding Source: STDF (Science and Technology Development Fund), Cairo, Egypt. Grant number 5300.

TISSUE ENGINEERING

W3033

STROMAL CELL-DERIVED EXTRACELLULAR MATRIX STIMULATES MESENCHYMAL STEM CELL PROLIFERATION IN VITRO AND BONE FORMATION IN RAT FEMUR SEGMENTAL DEFECTS

Zamilpa, Rogelio[1], Pearson, Joseph[2], Jennings, Sarah[2], Montelongo, Sergio Alejandro[2], Navarro, Mary[1], Lemus, Karina[1], Navara, Christopher[2], **Griffey, Sy**[1] and Guda,Teja[2], [1]StemBioSys Inc, San Antonio, TX, U.S., [2]The University of Texas at San Antonio, San Antonio, TX, U.S.

Bone marrow mesenchymal stem cells (BM-MSCs) are a common treatment of non-union fractures due to their ability to immunomodulate the microenvironment as well as enhancing osteogenesis. One of the issues associated with the use of BM-MSCs for therapeutic treatments is the inherent variability found in patients which most often results in decreased cell yields for clinically relevant doses. To improve the cell yield of BM-MSCs, a cell culture substratum referred to as High Performance Micro Environment (HPME) was generated using bone marrow stromal cells. Mass spectrometry analysis indicated that the HPME is primarily composed of extracellular matrix proteins such as collagen types I, VI, XII, fibronectin, tenascin and transforming growth factor beta inducible protein. When used as a cell expansion substrate, the HPME significantly increased the yield of BM-MSCs, the expression of IL-10, and the expression of stage specific embryonic antigen (SSEA)-4 when compared to tissue culture plastic (all $p<0.05$). When used as a supplement in cell culture medium, an HPME protein extract significantly increased the BM-MSC yield in a dose dependent manner with a concomitant increase in the expression of SSEA-4 (41±6% difference compared to non-supplemented cultures). Interestingly, in combination with hydroxyapatite minigranules and bone marrow, the HPME protein extract stimulated bone regeneration in rat femur segmental defects when compared to empty defects or minigranules combined with bone marrow. These results demonstrate that the HPME increases the proliferation of BM-MSCs in vitro and stimulates bone formation in vivo.

W3035

A THREE-DIMENSIONAL CULTURE MODEL OF THE HUMAN NEUROMUSCULAR JUNCTION

Afshar Bakooshli, Mohsen[1], Lippmann, Ethan[2], Yoo, Paul[1], Tung, Kayee[3], Ahn, Henry[1], Ginsbewr, Howard[1], Ashton, Randolph S.[4] and Gilbert, Penney[1], [1]University of Toronto, Toronto, ON, Canada, [2]University of Wisconsin, Madison, WI, U.S., [3]St. Michael's Hospital, Toronto, ON, Canada, [4]University of Wisconsin-Madison, Madison, WI, U.S.

An in vitro co-culture model of nerve and skeletal muscle cells can serve as a useful platform for studying neuromuscular junction (NMJ). Currently, two-dimensional (2D) cultures are used as the standard platform, and to our knowledge, there is no report of a three-dimensional (3D) culture model of human NMJ. However, 2D cultures fail to recapitulate the 3D cellular microenvironment. Here we report a 3D culture model for co-culturing of skeletal muscle tissues derived from primary human cells together with motor neurons (MNs) derived from human pluripotent stem cells (hPSCs). Our preliminary results show that our MN cluters derived from hPSCs are positive for Hb9 and Islet1 maskers and capable of exocytosis following glutamate stimuli shown by styryl dye FM1-43 experiments. In addition, our 3D muscle cultures generated a tetanus contraction following acetylcholine stimulation demonstrating the organization of the sarcomere units in mature muscle fibers responsive to biochemical stimuli. Results from 3D muscle-neuron co-cultures indicated formation of striated muscle fibers positive for acetylcholine receptor (AchR) in contact with neurites positive for synaptic vesicle glycoprotein synaptophysin. Analysis of AChR cluster size, and synaptic specific proteins revealed that the presence of motor neurons increases muscle fiber maturation. Indeed the higher expression of adult subunit of the AChR, epsilon, in our co-cultures demonstrated a progressive maturation of the NMJ with time in 3D culture. To further assess the functionality of the forming NMJ, muscle cells were lentivirally infected with a genetically encoded calcium indicator, GCAMP6, prior to co-culture. In addtion, hPSCs were infected with ChR2 for optogenetic activation. Light stimulation of muscle neuron co-culutures using GCAMP6+ muscle cells and optogenetically modified MNs indicated an increase in calcium transient of a subpopulation of muscle fibers that were in a functional contact with motor neurons. Our results indicate that our 3D culture platform supports the formation and maturation of muscle fibers and the neuromuscular junction better than current 2D culture models. We envision that our platform will be used as a model to study neuromuscular disorders and as a drug-screening platform for preclinical studies

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W3037

REQUIREMENTS FOR SEEDING AUTOLOGOUS RESPIRATORY EPITHELIAL CELLS ON TISSUE-ENGINEERED AIRWAY CONSTRUCTS

Butler, Colin R[1], Hynds, Robert E.[2], Gowers, Kate H.C.[3], Crowley, Claire[3], Hamilton, Nicholas J.[3], Urbani, Luca[2], De Coppi, Paolo[2], Birchall, Martin A[3] and Janes, Sam M.[3], [1]UCL, London, U.K., [2]University College London, London, U.K., [3]UCL, 5, University Street, U.K.

Tissue-engineered airways hold promise for patients with severe end-stage airway pathology and considerable advances have been made towards clinical translation. Strategies for tracheal replacement have included engrafting both synthetic and decellularised tissue-engineered grafts. One significant hurdle has been the inadequate re-epithelialisation of these grafts. Basal epithelial cells are considered to be the stem/progenitor population that repopulates the airway during homeostasis and following airway injury. We describe the engraftment requirements for three clinical candidate scaffolds (POSS-PCU synthetic; vacuum assisted decellularisation; and standard detergent-enzymatic processing) in a system that replicates cell seeding on full-sized human trachea in vitro with applications towards bioreactors. We show that > 1x10^6 cells/cm^2 cells are required for effective coverage, independent of the scaffold material used. We found that traditional methods for expanding autologous airway epithelial stem cells from biopsies could not meet these requirements. However, using a co-culture method with mitotically inactive 3T3-J2 fibroblasts in the presence of the small molecule Rho-associated protein kinase (ROCK) inhibitor Y-27632 (3T3+Y) generated large numbers of human airway basal stem/progenitor cells in a clinically relevant time-frame. This methodology allowed the delivery of cells in sufficient quantity and with maintained differentiation capacity and function. We show that this method achieved extensive expansion from small endobronchial pinch and brush biopsies derived from human donors, thereby making autologous delivery of cells possible. Our finding that a significant quantity of airway basal stem cells are required for tracheobronchial tissue engineering suggests that delayed epithelial regeneration in previous clinical cases may be due to reliance upon host epithelial cell migration into the scaffold post-implantation. Adoption of 3T3+Y could allow the generation of large numbers of basal cells to expedite mucosal regeneration in these patients.

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W3039

A TISSUE ENGINEERED CNS MODEL WITH INTEGRATED BLOOD BRAIN BARRIER FOR PROBING MULTI-ORGAN INTERACTIONS AND DRUG TOXICITY

Edington, Collin[1], Lee, Iris[1], Phabmixay, Pierre[1], Brown, Alex[1], Schwartz, Michael[2], Hou, Zhonggang[3], Murphy, William[2], Thomson, James[4] and Griffith, Linda[1], [1]Massachachusetts Insitute of Technology, Cambridge, MA, U.S., [2]University of Wisconsin-Madison, Madison, WI, U.S., [3]Harvard, Boston, MA, U.S., [4]Morgridge Institute for Research, Madison, WI, U.S.

Stem cell-derived models of the human central nervous system have dramatic potential to improve our understanding of the developmental and mature brain. Integrating them into biologically relevant models are also an important step towards reducing industry reliance on small animal and primate testing, enabling improved disease models and earlier detection of neurotoxicity and efficacy in the drug pipeline. Towards this end we have incorporated a blood-brain barrier component into a previously developed 3D neural construct for inclusion in a human-on-a-chip platform. Evaluation of the gene expression patterns in these ESC-derived neural constructs has been shown to be predictive of developmental neurotoxicity when perturbed with known and blinded toxins (*1*). Human cerebral microvascular endothelial cells (hCMEC/D3 line) were cocultured with human brain vascular pericytes in a transwell system, using the porous membrane to maintain barrier polarity while allowing cell-cell contact through the pores. Following 6-12 days of culture, monolayer integrity was evaluated with trans-epithelial electrical resistance (TEER) and FITC-dextran permeability. We recorded TEER values of up to 41.5 ± 2.4 (Ohms*cm^2), similar to previously reported values for the hCMEC/D3 cell line (*2*). Permeability coefficients of FITC-dextrans (4, 10, and 70 kDa) were found to decrease with increasing molecular weight, and were as low as 8.5x10^-8 cm/s for the 70 kDa molecules. Endothelial monolayers stained positive for ZO-1 and Claudin-5 tight junction proteins, and maintained TEER values under coculture conditions with the neural progenitor population.

This CNS model will be used for biomarker development and the interrogation of multi-organ interactions, particularly along the gut-brain axis (CNS, small intestine, liver, and pancreas).

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W3041

A COMPARISON OF HUMAN ADIPOSE DERIVED STEM CELLS (ASCS) IN 2-D AND 3-D CULTURE FOR WOUND HEALING APPLICATIONS

Houlihan, Alison, Bell, David and LoGuidice, Amanda, RTI Surgical, Alachua, FL, U.S.

Adipose derived mesenchymal stem cells (ASCs) are a multipotent stem cell abundant in native adipose tissue. ASCs are highly proliferative and have been shown to participate in the regulation of major cell types involved in wound healing, including fibroblasts, macrophages and endothelial cells. Much of this regulation takes place via chemokine and cytokine secretion. This study investigates the phenotypic characteristics of primary ASCs isolated from human cadaveric donors; specifically focusing on their ability to influence their immediate environment through chemical signaling. ASCs were cultured in normal 2-D cell culture, as 2-D cell sheets, and as 3-D cell aggregates. Using cytokine arrays, ELISAs and western blotting, the protein expression and secretion profiles were evaluated for differences in relevant proteins such as vascular endothelial growth factor (VEGF), Transforming Growth Factor (TGF-b), Platelet Derived Growth Factor (PDGF) and Fibroblast Growth Factor (FGF).The culture groups resulted in varied levels of extracellular matrix (ECM) protein deposition, proliferation rate, protein expression and soluble factor secretion. The culture groups were then seeded onto a dermal collagen scaffold produced from decellularized human tissue. The interaction with the dermal scaffold resulted in increased protein expression and soluble factor secretion of the seeded ASCs. This study measured the impact of cell-cell contact, culture dimensionality, and interaction with the decellularized dermal scaffold on the functional characteristics of primary ASCs. ASCs are key regulators in the wound healing environment, making them strong candidates for therapeutic use in tissue engineering applications, and particularly relevant as a treatment for burns and deep cutaneous wounds. An understanding of the impact of culture morphology on regulatory efficacy will allow for more specific manipulation of the *in vivo* wound environment, leading to the development of improved surgical interventions.

W3043

NEURAL STEM CELLS BENEFIT A MOUSE MODEL OF PARKINSON'S DISEASE CAUSED BY SINGLE INTRANASAL ADMINISTRATION OF THE NEUROTOXIN MPTP

Leite Pereira, Marcia Cristina[1,2], Vallim, Gustavo B.[1,2], Hamblin, Milton H.[3] and **Lee, Jean-Pyo**[1,2], [1]Tulane University School of Medicine, Neurology, New Orleans, LA, U.S., [2]Tulane University School of Medicine, Center for Stem Cell Research and Regenerative Medicine, New Orleans, LA, U.S., [3]Tulane University School of Medicine, Pharmacology, New Orleans, LA, U.S.

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder affecting up to 2% of those 55 years and older. Less than 5% of PD cases are inherited, with exposure to environmental toxins a possible risk factor. We investigated the therapeutic effects of transplanting human neural stem cells (hNSCs) to ameliorate the pathophysiology of pre-clinical PD in a unique MPTP-exposed PD model, which mimics PD's both early and late stages (other models mainly mimic late stages). Damage to the nigrostriatal dopaminergic system in mice was uniquely induced with the neurotoxin MPTP. Specifically, MPTP was infused intranasally with a peristaltic pump, and a catheter was inserted into the nasal cavity. Transplantation was 7 days post-MPTP. The time frame for hNSC transplantation corresponded to upregulation of endogenous proinflammatory cytokines, which play a role in stem cell migration. We administered a single unilateral intracranial injection of hNSCs. Three weeks post-transplantation (4 weeks post-MPTP), behavioral deficits were significantly improved as assessed by an open field test for hypokinesia (decreased body movement) and a tail suspension test for catalepsy (muscular rigidity). Nontransplanted MPTP control mice had about 78% (tyrosine hydroxylase) TH^+ neuronal death (i.e., only 22% neurons survived) in the substantia nigra (SN). However, hNSC-engrafted mice significantly protected host neurons from degeneration (60% TH^+ neurons). Surviving neurons (TH^+ host neurons) were not donor-derived, showing a "chaperone" effect of donor cells. Preserving dopaminergic neurons in the SN and improving behavioral deficits are therapeutic end goals of PD and we are the first to show that hNSCs attenuated the degeneration of dopaminergic neurons following intranasally-infused MPTP.

W3045

THERAPEUTIC POTENTIAL OF HUMAN IPS CELL-DERIVED ENGINEERED CARDIAC TISSUE INCLUDING CARDIOMYOCYTES AND MULTIPLE VASCULAR LINEAGES FOR MYOCARDIAL INFARCTION

Masumoto, Hidetoshi[1,2], Nakane, Takeichiro[1,2], Tinney, Joseph[1], Yuan, Fangping[1], Ye, Fei[1], Kowalski, William[1], Minakata, Kenji[3], Sakata, Ryuzo[3], Yamashita, Jun[2] and Keller, Bradley[1], [1]Cardiovascular Innovation Institute, University of Louisville, Louisville, KY, U.S., [2]Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan, [3]Kyoto University Graduate School of Medicine, Kyoto, Japan

Three-dimensional (3D) engineered cardiac tissues (ECTs) are a robust platform technology to investigate cardiovascular (CV) cell function and provide an excellent microenvironment for tissue implantation and cardiac repair. We generated 3D ECTs using cardiomyocytes (CMs), endothelial cells (ECs), and vascular mural cells (MCs) efficiently differentiated from human iPS cells (hiPSCs). We employed 3 different monolayer culture-based differentiation protocols: 1) CM+EC protocol: mesoderm induction followed by VEGF (mes+VEGF) produced a distribution of 61.8±8.0% cTnT^{+}-CMs, 19.4±9.1% VE-cadherin^{+}-ECs, and 1.7±2.0% PDGFRβ^{+}-MCs, (n=26, day15); 2) CM+MC protocol: mes+Dkk1 to induce CMs and MCs; and 3) MC protocol: exclusive induction of MCs. We collected the cells on differentiation day 15 and mixed them to generate 3 classes of ECTs composed of different CV cell population patterns: 1). CM+EC; 2). CM+MC, and 3). CM+EC+MC. We seeded the each cell mixture in a collagen/Matrigel mixture to form spontaneously beating ECTs. In vitro force measurement analysis showed that CM+EC+MC ECTs possessed the highest maximum capture rate (4.6±0.6 Hz, $P<0.0001$) and lowest excitation threshold (1.2±0.3 V/cm, $P<0.01$) indicating that incorporation of vascular cells augmented tissue maturation and function. We confirmed more preferential alignment of CMs to the ECT long axis in ECTs with MCs. Transmission electron microscopy revealed that incorporation of MCs increased CM sarcomeric structure. Incorporation of both ECs and MCs led to vasculature formation within ECTs during 14 days of in vitro culture and activated multiple tissue maturation pathways. These results indicate that incorporation of vascular cells accelerated tissue structural maturation. Next CM+EC+MC ECTs were implanted onto infarcted athymic rat hearts. Echocardiogram revealed a significantly higher cardiac output at 4 weeks after implantation compared to that of sham-operated rats (137±23 vs 95±23 mL/min, $P<0.05$). Immunostaining 4 weeks after implantation showed epicardial engraftment of human cells as a regenerated myocardium vascularized both by graft and host-derived vasculature. HiPSC-derived ECTs including vascular cells showed novel properties relevant for clinical translation.

Funding Source: J.K.Y. is a founder, equity holder, and scientific adviser of iHeart Japan Corporation, and a co-inventor on multiple pluripotent stem cell-related patents.

W3047

SUSTAINED RELEASE OF BFGF FROM MULTILAYER NANOFILM TO SUPPORT HUMAN STEM CELL CULTURES WITHOUT DAILY FEEDING

Park, Juhyun, Kangwon National University, Chuncheon-si, Korea, South

The successful maintenance of undifferentiated state is an essential aspect of stem cell culture. In the case of human pluripotent stem cells including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, basic fibroblast growth factor (bFGF) plays a crucial role in promoting the undifferentiated state while minimizing spontaneous differentiation into other cell types. However, bFGF has been known to be very labile under normal culture conditions. Therefore, pluripotent stem cell cultures require a daily replacement of culture medium, making the culture costly and labor intensive. Recently, it has been reported that bFGF levels in human pluripotent stem cell culture significantly fluctuate even with daily feeding. Here, a layer-by-layer (LbL) technique-based nanofilm system consisting of charged polymeric materials was established for the sustained release of bFGF. The release of bFGF was continued for over 10 days. Then, the bFGF-releasing nanofilm was constructed on the membrane of transwell permeable support and human iPS cells were cultured with the transwell. As a result, human iPS cells maintained their undifferentiated morphology and expression of pluripotency markers including SSEA-4, Nanog and Oct4 even with less frequent media changes. Furthermore, it was demonstrated that undifferentiated state of iPS cells was preserved during several passages and the differentiation potential was not altered. The bFGF-releasing nanofilm provides a useful method to maintain the undifferentiated state of human pluripotent stem cells. Especially, it is anticipated that the controlled release of bFGF from the biocompatible nanofilm reduces the frequency of media replacement needed to maintain stem cell cultures.

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W3049

A THREE DIMENSIONAL IDENTIFICATION BETWEEN GRAFT CELLS AND THE HOST THROUGH INTACT TISSUE CLEARING IN RAT PARKINSON'S DISEASE

Song, Byeong-Wook, EIT/LOFUS R&D Center, Institute for Integrative Medicine, Catholic Kwandong University, Gangneung, Korea and Hwang, Ki-chul, Institute for Bio-Medical Convergence, Catholic Kwandong University, Gangneung, Korea

Stem cell therapy is an emerging strategy for restoring neural function in neurodegenerative disease. In Parkinson's disease (PD), stem cells have an effect on functional enhancement in a profound and selective loss of nigrostriatal dopaminergic neurons. How grafted stem cells integrate into brain tissue and functionally interact with the host is as yet unanswered. A three dimensional analysis of brain is indispensable for measuring the structure-function relationships about therapeutic safety and efficacy of stem cells. A few years ago, hydrogel-based structure technique was developed with potential relevance for brain-mapping study. This process creates physically stable and permeable to both visible-spectrum photons and exogenous molecules in the brain tissue. Here, we examined a three dimensional structure of intact brain tissue after bone marrow mesenchymal stem cell (MSC) treatment in PD. Rat PD model was unilaterally induced by injecting 6-hydroxydopamine (6-OHDA) hydrobromide into the medial forebrain. Three weeks after surgery, amphetamine-induced rotation test was performed to evaluate the degree of the dopaminergic lesion. For transplantation, MSCs (1×10^7 cells/animal) were injected into the femoral vein. One day after the final behavior test (day 29), the rats were anaesthetized, and perfused with a mixture of 4% paraformaldehyde, 4% acrylamaide, 0.05% bis-acrylamide, 0.25 VA044 in PBS. Isolated brains were incubated in 4°C for 3 days, and then temperature was increased to 37°C to induce polymerization for 3 hours. Hydrogel-embedded brains were plated in 10~60V organ-electrophoresis system, circulating 200 mM sodium borate buffer with 4% SDS for 2 days. We showed three consecutive rounds, including PKH26-labeled MSCs with doparminergic neurons stained with tyrosine hydroxylase. The graft-host 3D interface, i.e. brain microenvironment, was visualized by immunofluorescence to MSC-positive cells, polysialylated neural cell adhesion molecule, and/or neuron-specific class III beta-tubulin marker (NRF-2015R1C1A1A02037693).

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W3051

PRE-CLINICAL MODEL OF LIVER REGENERATIVE THERAPY USING ORGAN DERIVED SCAFFOLD WITH/WITHOUT HUMAN IPS DERIVED CELLS

Yagi, Hiroshi, Tajima, Kazuki, Makiko, Hieda, Hibi, Taizo, Abe, Yuta, Kitago, Minoru, Shinoda, Masahiro, Itano, Osamu and Kitagawa, Yuko, Keio University, School of Medicine, Tokyo, Japan

To establish 3D organ structure, we have been investigated a tissue engineering approach "decellularized organ frame" by discarding viable cells from native tissues, which can be used as a extra cellular matrix scaffold for regenerative cells. The aim of this study was to generate native tissue derived organ frame for the development of liver regenerative therapy, with or without primary and human iPS derived cells. Rat/ Porcine livers were successfully decellularized and sutured onto the surface after radical liver resection, reducing the size to fit for the surface area. Interestingly, not only endothelial cells but also bile duct epithelial cells were migrated into the luminal surface and formulated small vessels and bile ducts 7 days after the surgery. Hepatocytes were also repopulated in the parenchymal space reconstructing hepatic lobule like structures. These results suggested that this organ derived frames have strong affinity to the liver cells and would be applicable for organ regeneration. Therefore, we standardized each protocol for organ harvest, de/re-cellularization and transplantation to establish pre-clinical large animal model for organ regenerative therapy. Especially, standardization of the procedure for introduction of a large number of the different types of the cells into the expected location of the parenchymal/non-parenchymal space was critical and was finally established by using pressure monitoring system. In addition, angiography after transplantation of the liver graft revealed that continuous blood flow was observed in the graft which was connected by surgical anastomosis to portal vein and inferior vena cava. Taken together, we could have a solid large animal model to systematically evaluate hepatic function of regenerative liver graft in vivo. Finally, a large number of human iPS derived hepatic progenitor cells and mesenchymal stem cells were introduced into the porcine scaffold and successfully transplanted into the porcine body under immune suppression. The liver graft with iPS derived cells were evaluated 7 days after the surgery, which showed clusters of hepatocyte like cells with hepatic markers. We believe that this large animal model could help developing a novel therapeutic strategy using regenerative approach with an attractive tissue engineering technology.

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REGENERATION MECHANISMS

W3055

DIRECTLY REPROGRAMMED HUMAN NEURAL PRECURSOR CELLS RESPOND TO ENVIRONMENTAL CUES IN THE ADULT MURINE BRAIN FOLLOWING TRANSPLANTATION

Azimi, Ashkan[1], Vonderwalde, Ilan[1], Sachewsky, Nadia[1], Ahlfors, Jan-Eric[2] and Morshead, Cindi M.[1],
[1]University of Toronto, Toronto, ON, Canada, [2]New World Laboratories, Laval, QC, Canada

The use of neural precursor cells (NPCs) to repair the injured CNS holds much promise. One regenerative approach is NPC transplantation to promote tissue repair. Although some strategies have demonstrated limited success, a major concern still to be addressed is the identification of an ideal cell source that is easily accessible, immunologically privileged to avoid rejection, and outside ethical concerns. The practicality of isolating brain-derived NPCs from patients is not obvious; hence, the ability to generate NPCs from somatic cells, thereby permitting patient-specific cell sources to be used for regenerative strategies is a necessary goal. Herein we used in vitro and in vivo assays to explore the fundamental biology of a novel population of human neural precursor cells that have been directly reprogrammed from somatic cells (termed drNPCs) without the use of viral vectors and without passing through a pluripotent state. We have shown that drNPCs display the two cardinal properties of stem cells, self-renewal and multipotentiality. RT-PCR and immunocytochemistry revealed expression of NPC markers (nestin, sox2, ascl1, pax6, MAP2) and absence of pluripotency markers (nanog and oct4). Further, we asked if the drNPCs are capable of responding to environmental cues similar to endogenous NPCs, in 2 mouse models. First, drNPCs were transplanted into the periventricular region of SCID-beige mice and examined at various times post-transplantation to evaluate cell survival, location, and differentiation profile. Similar to endogenous NPC behaviour, a subpopulation of drNPCs survived and migrated to the olfactory bulb. In separate experiments we transplanted drNPCs in a mouse model of dysmyelination (shiverer mice) to examine the oligodendroglial potential of the cells. Again, similar to what is observed when adult mouse NPCs are transplanted on the corpus callosum of shiverer mice, drNPCs generated mature oligodendrocytes producing myelin basic protein at both 1-week and 1-month post-transplant. In summary, we have demonstrated drNPCs respond to environmental cues similar to transplanted NPCs, supporting the hypothesis that drNPCs are an ideal cell source for neural regenerative strategies.

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W3057

DIRECTLY REPROGRAMMED NEURAL PRECURSOR CELLS VIA TRANSIENT EXPRESSION OF SYNTHETICALLY OPTIMIZED TRANSCRIPTION FACTORS IN HUMAN SOMATIC CELLS

Ahlfors, Jan-Eric, El-Ayoubi, Rody, Mani, Sarathi, Abraham, Suraj, Mihai, Oana and Thomson, Alison, New World Laboratories, Laval, QC, Canada

Direct somatic cell reprogramming into a multipotent stem cell provides an attractive venue for clinical translation of autologous stem cell therapy, especially for difficult to obtain autologous stem cells such as neural stem cells. Transient expression of JA1, a synthetically optimized plasmid containing three synthesized transcription factors, Musashi-1 (Msi1), Neurogenin-2 (Ngn2) and Methyl-CpG Binding Domain Protein 2 (MBD2), results in robust reprogramming of human fibroblasts, keratinocytes, $CD34^+$ cells, MSCs and ADSCs into neural stem cells, termed directly reprogrammed neural precursor cells (drNPCs), within 1-2 weeks at high efficiency. RT-PCR and Southern blot analysis showed no genome integration of any part of the synthetic plasmid into the cell population. These directly reprogrammed neural precursor cells (drNPCs) are characterized by more robust proliferation and a higher proportion of neurons upon differentiation than human fetal neural precursor cells (hNPCs), and display a greater degree of functional recovery in rodent models of traumatic brain injury compared to hNPC implants. Implantation of the drNPCs into SCID/beige mice models for detecting tumour or teratoma forming cells resulted in no tumour formation even at high concentrations. drNPCs express neural stem cell markers but no markers specific to the starting cell type, and are able to form neurospheres from single cell clones and differentiate into glutamatergic, acetylcholinergic, dopaminergic, serotonergic and GABAergic neurons as well as astrocytes and oligodendrocytes. drNPCs are characterized by high telomerase expression that disappears during differentiation, expression of numerous neural associated cytokines, nestin activation by demethylation of its second intron region, functional GAP junctions, and continued maintenance of normal karyotype at high passage numbers. These characteristics along with rapid reprogramming and proliferation of the drNPCs with no genome integration and no use of animal components or viruses, present an attractive avenue for providing autologous neural stem cells at mass scale for personalized and regenerative medicine applications.

W3059

NON-PLATELET RNA-CONTAINING PARTICLES ARE THE REGENERATIVE SUBCELLULAR MATERIALS

Kong, Wuyi[1], Sun, Lei[2], Zhu, Xiaoping[1], Nuo, Mu[1] and Wang, Hong[1], [1]Khasar Medical Technology Co., Beijing, China, [2]Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

Recently, the rejuvenation and regeneration of young blood plasma has been described. However, the juvenile factor or factors involved need to be elucidated. In the previous studies, we have found that tissue regeneration is by a group of short-rod shaped particles with regenerative activities that locate in blood plasma and named them non-platelet RNA-containing particles (NPRCPs). We have found that NPRCPs can regenerate cardiomyocytes, brain cells, and improve wound healing. However, the morphological differences between the platelets and NPRCPs and the origins of the NPRCPs need to be clarified. In the present studies, we examined the insoluble platelet-sized particles and the large precipitated cellular clumps in mouse blood. We found that NPRCPs have distinct morphology from platelets. NPRCPs are in dimer, trimmer or polymer shapes multiples of that can further fuse together. The fused NPRCPs further differentiate into epithelial cell and other cell types, however, not blood cells. In addition, we found that NPRCPs are released from giant cells that had distinct morphology from megakaryocytes and have never been described. Our data provide evidence of a new blood subcellular population, NPRCPs, and their releasing cells, the giant cells in the blood.

W3061

THE MECHANISM OF PERIODONTAL TISSUE REGENERATION BY TRANSPLANTATION OF ADIPOSE TISSUE-DERIVED MULTI-LINEAGE PROGENITOR CELLS

Sawada, Keigo[1], Takedachi, Masahide[1], Iwayama, Tomoaki[1], Yamamoto, Satomi[1], Morimoto, Chiaki[1], Hirai, Asae[1], Lee, Chunman[2], Matsuyama, Akifumi[3], Okura, Hanayuki[3], Sano, Yuko[1], Kitamura, Masahiro[1] and Murakami, Shinya[1], [1]Osaka University Graduate School of Dentistry, Suita, Japan, [2]Osaka University Hospital, Suita, Japan, [3]National Institute of Biomedical Innovation Health and Nutrition, Amagasaki, Japan

The reconstruction of periodontal tissue destroyed by periodontal diseases is a major goal of periodontal regenerative therapy. Due to unsatisfied efficacy of current periodontal regenerative therapies, development of new cell-based therapies has been hoped for. We have been revealed the efficacy of periodontal tissue regeneration by transplantation of adipose tissue-derived multi-lineage progenitor cells (ADMPCs) with experimental periodontitis models on beagle dogs. The safety and efficacy of this new periodontal regenerative therapy is currently under investigation in the First-in-Man clinical study. So far, autologous transplantation of ADMPCs resulted in regeneration of tooth-supporting alveolar bone and no transplantation-related adverse events were observed. In this study, we examined the trophic effects of ADMPCs on the periodontal tissue regeneration. All human subjects provided informed consent according to a protocol that was reviewed and approved by the Institutional Review Board of the Osaka University Graduate School of Dentistry. We harvested subcutaneous adipose tissue from healthy volunteers and isolated ADMPCs. We cultured the ADMPCs in D-MEM for 3 days and collected the supernatant as an ADMPC conditioned medium (ADMPCs-CM). Human Growth Factor Array® revealed that ADMPCs-CM contained considerable amounts of various growth factors. Then, we evaluated whether ADMPCs-CM have effects on osteogenic differentiation of human periodontal ligament cells (HPDLs). HPDLs were cultured in mineralization-inducing medium with or without ADMPCs-CM. Addition of ADMPCs-CM resulted in the upregulation of osteogenic gene expression, alkaline phosphatase activity and calcified nodule formation in HPDLs. Interestingly, it was demonstrated that the effects of ADMPCs-CM on HPDLs differentiation were partially regulated by insulin-like growth factor binding protein 6 (IGFBP6) in ADMPCs-CM. These results suggest that ADMPCs transplanted into a defect in periodontal tissue release trophic factors which can stimulate the differentiation of HPDLs to mineralized tissue-forming cells, such as osteoblasts and cementoblasts. IGFBP6 may play important roles in ADMPC-induced periodontal tissue regeneration.

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W3063

ADIPOCYTES UNDERGO MACROPHAGE-MEDIATED LIPID FILLING TO CONTROL MAMMARY GLAND REGENERATION AFTER LACTATION

Zwick, Rachel K.[1], Shook, Brett[1], Wysolmerski, M.D., John[1], Rodeheffer, Matthew S.[2] and Horsley, Valerie[1], [1]Yale University, New Haven, CT, U.S., [2]Yale Univ, New Haven, CT, U.S.

Adipocytes comprise a significant portion of the mammary gland stroma and are dynamic during reproductive cycles. During lactation, the gland fills with milk- producing epithelial cells and adipocytes regress. After lactation, milk fat is resorbed into epithelial cells, promoting epithe-

lial cell death and mammary gland involution. Involution serves as a natural model of wound healing, involving a similar inflammatory response, and the regeneration of the mammary stroma - most prominently the massive expanse of adipose tissue. It has been suggested that adipocytes regenerate during involution through transdifferentiation of epithelial cells. Here, we use genetic lineage tracing in mice to show that epithelial cell transdifferentiation is not a major mechanism of adipocyte repopulation. We identified a population of adipocyte stem cells in the mammary gland that expands in cell number during involution. The predominate mechanism contributing to adipocyte repopulation during involution, however, is lipid filling of long-lived mature adipocytes that reside in the mammary gland prior to pregnancy. We find that this increase in adipocyte size during involution is not directly regulated hormonally, but rather by local signals within the gland, including macrophages. Functional studies reveal that adipocytes support proper epithelial regression during mammary gland involution. These results demonstrate that interactions between mammary epithelial cells, macrophages and adipocytes are critical for proper coordination of epithelial regression and subsequent tissue regeneration and have implications for cross talk between these cell types in tissue regeneration.

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ETHICS AND PUBLIC POLICY; HISTORY OF STEM CELL RESEARCH; SOCIETY ISSUES; EDUCATION AND OUTREACH

W3067

STEM CELLS ACROSS THE CURRICULUM: EDUCATIONAL MODULES THAT INTEGRATE ETHICAL REASONING AND BIOLOGY LEARNING USING INFOGRAPHIC THINKING & CASE-BASED TEACHING

Chamany, Katayoun, Eugene Lang College, The New School for Liberal Arts, New York, NY, U.S., Riggs, Alexa, CUNY Graduate Center, New York City, NY, U.S., Schwartz-Orbach, Lianna, Memorial Sloan Kettering Cancer Center, New York City, NY, U.S. and Wargaski, Julia, Parsons School of Design at The New School, New York City, NY, U.S.

STEM education must integrate the biological and social dimensions of emerging technologies if we intend to promote scientific innovation and socially just practices and policies. Using infographic thinking and case-based teaching methods, we developed Stem Cells Across the Curriculum (SCAC*),* a modular collection of educational resources designed to integrate the biological and the social dimensions of stem cell research (SCR). Our project was informed by common student misconceptions about the temporal and spatial relationships of scientific experimentation involved in SCR including: intracellular v. extracellular interactions; in vivo v. in vitro experimentation; characterization of embryonic v. adult stem cell characteristics; and location of experimentation (animal, human, cell culture, industry, research, etc.). Additionally, we sought to broaden students' views of ethical issues related to SCR using a social justice framework that highlights the emergence of a bioeconomy, differential funding and regulation in the public and private sectors, and differential access to stem cells and therapies. Early stage renditions of these learning tools were used in undergraduate seminars and then scaled up for a university lecture course serving design and liberal arts students. Directed content analysis of student exams revealed that 100% were able to identify multiple ethical issues regarding SCR. Additionally, to varying degrees, students applied case based analysis to categorize stem cell types, describe experimental manipulations, propose next steps, and comment on the ethical and legal issues of translational research. Qualitative data using student essays suggest that the case studies were effective in promoting empathetic thinking and ethical reasoning. These data were used to refine the SCAC resources (animated slide sets, timelines, and information designs) that integrate the biological and the social. As an example, a downloadable interactive pdf depicting the provenance, access, and use of various stem cell types will be on display. We present this open access online curriculum for others to adopt and adapt, and reflect on the use of infographic thinking in the design of curricula to improve undergraduate science education in a variety of courses across the curriculum.

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W3069

SKIP (Stemcell Knowledge and Information Portal): One stop database for researchers, commercial entities and citizens

Kawase, Satoshi[1,2], Shimura, Takako[2], Tsuyama, Jun[1,2], Fujimori, Kouki[1,2], Suzuki, Sadafumi[1,2], Yoshimatsu, Sho[1,2], Ito, Shougo[2,3], Hasegawa, Youko[2], Tsujimoto, Michiko[2], Kosaki, Kenjiro[2,4] and Masui, Tohru[2,4], [1]Keio University School of Medicine, Tokyo, Japan, [2]The Human Stem Cells Informatization Project of the Ministry of Health, Labour and Welfare, Tokyo, Japan, [3]Department of Internal Medicine Keio University School of Medicine, Tokyo, Japan, [4]Center for Medical Genetics, Keio University School of Medicine, Tokyo, Japan

In 2014 Japanese government launched three legislation which acts to promote and secure regenerative medicine. Therefore, the 2014 is called the first year of regenerative medicine in Japan. Acceleration of stem cell sciences and realizaiton of their outcomes to benefit patients, efficient exchange of stem cells information and inspiring database for science are indispensable. This database also aims to bridge science and patients and citizens to facilitate their participation in clinical research. SKIP is an initiative to promote the exchange of information and facilitate joint research between researchers by providing one stop database of information of stem cells (iPS cells, original diseased fibroblasts, lymphoblast cells, etc.), data include cell types, ownership, characters, culture and preservation conditions, literature etc..SKIP also aims to offer information on stem cells to the general public, including patients, in order to promote societal understanding and enhance participation of medical research using stem cells. SKIP is administrated by an operating committee ("SKIP Operating Committee") of Keio University as part of the Ministry of Health, Labour and Welfare, Human Stem Cells Informatization Project. To date, we have had more than 1250 cell information and about 800 can be accessed form our homepage for free. The information is registered from published papers and open resources, and also from original establishers of their culture. We also create and provide secure database to share stem cell information between different institutions at collaborations. We are calling you to register your own cell lines to SKIP to promote your collaboration. Accession reached more than about 300 per day and is increasing. Researchers can reach objective disease iPS cells from the name of disease, ICD-10 code and publications. Visibility of the database increases its visibility and we are trying to add detailed relational information of cells and make a database more fruitful. SKIP is developing to be a powerful tool for researchers to liberate their idea and promote stem cell sciences involving public and commercial entities.

W3071

ETHICAL IMPLICATIONS FOR STEM CELL RESEARCH OF OPHTHALMOLOGY TREATMENT

Takashima, Kayo[1], Tashiro, Shimon[2] and Muto, Kaori[1], [1]The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [2]National Cancer Center, Tokyo, Japan

The implementation of translational research tends to focus on peculiar issues and their novelty. Ophthalmic clinical research can reasonably be considered the first verification step for the safety of the entire translational evaluation in clinical trials of stem cell therapies. Besides lacking effective treatment for age-related macular degeneration (AMD) or donors for corneal transplantation, implementation of ocular diseases has advantages to take a position conducting the first-in-human (FIH) study based on ease of observation, a limited number of required cells and a nonfatal condition. This paper analyzes fundamental ethical issues for clinical trials on ophthalmic diseases using stem cells, reflecting the FIH trial using induced pluripotent stem cell (iPSC) for AMD in 2014 and other planned trials in the future. In this evaluation, we conducted a literature survey on the ethical challenges of ophthalmic disorders and analyzed the issues extracted from the research ethics support services that we have been involved. We then examined the ethical issues affecting stem cell research on eye disease patients. Ophthalmic research is sometimes required a thorough evaluation of translational research of cell therapies in the first place. For example, discussions on safety measurements for tumorigenicity affected the FIH for AMD. However, the following matters should be considered with regard to the ethical conduct of research: 1) information support for people with vision loss regarding informed consent and participation in clinical trials; 2) risk and benefit analysis concerning stem cell therapies and other ophthalmic standards of care; 3) scientifically and ethically valid selection of participants with various vision impairment,; 4) low vision care after the completion of clinical trials; and 5) social benefits of the research, such as solutions for the lack of donors. Additionally, it would be necessary to carefully consider the effects on not only the target population but also other disables.

Funding Source: This work was supported by Highway Program for Realization of Regenerative Medicine of Japan Agency for Medical Research and Development.

LATE BREAKING ABSTRACT

W4001

GENERATION OF CLINICAL-GRADE FUNCTIONAL CARDIOMYOCTES FROM HUMAN EMBRYONIC STEM CELLS IN CHEMICALLY DEFINED CONDITIONS

Hao, Jie, Tan, Yuanqing and Zhou, Qi, Institute of Zoology ,Chinese Academy of Sciences, Beijing, China

Highly efficient cardiac differentiation from human pluripotent stem cells (hPSCs) can be achieved with existing methods, especially the generally accepted B27 induction system. However, bovine serum albumin (BSA), derived from animal products and being one of the essential ingredients in B27, has potential risks of virus contamination which will greatly complicate the clinical studies and applications of hPSC-derived cardiomyocytes and cause high cost. Here, we report to have developed a BSA-free and chemically defined medium (named VN) for differentiating hPSCs to clinical-grade cardiomyocytes. Using this xeno-free differentiation system we generated more than 80% cTNT positive cardiomyocytes with contractile stretch, at a yield of 3.3×10^5 cells per square centimeter. When engrafting the cardiomyocytes into the hearts of myocardial infarction model rats, the rats survived with statistically significant improved heart functions in ¢EF and ¢FS. Importantly, the human embryonic stem cell line (Q-CTS-hESC-2) chosen for differentiation was a clinical-grade one maintained in defined xeno-free conditions in our lab. Compliant with the biological safety requirements, the Q-CTS-hESC-2-derived cardiomyocytes passed sterility and pathogen criteria tests of NIFDC for clinical applications. In this study, we report for the first time the clinical-grade and functional cardiomyocytes differentiated from hPSCs at BSA-free and chemically defined conditions throughout the whole process. This provides the possibility of future therapeutic use of clinical grade hPSCs-derived cardiomyocytes in treating heart diseases.

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W4003

DETERMINISTIC RESTRICTION ON PLURIPOTENT STATE DISSOLUTION BY CELL CYCLE PATHWAYS

Gonzales, Kevin Andrew Uy, Liang, Hongqing and Ng, Huck Hui, Genome Institute of Singapore, Singapore, Singapore

During differentiation, human embryonic stem cells (hESCs) have to shut down the regulatory network conferring pluripotency in a process we designated as pluripotent state dissolution (PSD). In a high-throughput RNAi screen using multiple differentiation conditions, we identify centrally important and context-dependent processes regulating PSD in hESCs. Strikingly, we unexpectedly detected a strong and specific enrichment of cell cycle genes involved in DNA replication and G2 phase progression. Genetic and chemical evidences demonstrate that the S and G2 phases possess an intrinsic propensity towards the pluripotent state that is independent of the G1 phase. Our study thus functionally establishes that pluripotency control is hardwired to the cell cycle machinery, where S and G2 phase-specific pathways deterministically restrict PSD while the absence of such pathways in the G1 phase potentially contributes to its amenability to PSD.

W4005

SPC25 PLAYS IMPORTANT ROLES IN THE ONCOGENESIS OF NEUROBLASTOMA

LI, Tianfeng, CUHK, HONG KONG, Hong Kong and Zhao, Hui, The Chinese University of Hong Kong, Hong Kong, China

Neuroblastoma is the third most common childhood cancer after leukemia and brain cancers, and accounts for 8% to 10% of all childhood cancers and for approximately 15% of cancer deaths in children. Although treatment for children with high-risk metastatic neuroblastoma has improved significantly in the past 20 years, only 45% of these patients become long-term, disease-free survivors. Mechanism studies are still urgent needed to illustrate the oncogenesis of neuroblastoma. In an attempt to identify genes involved in the oncogenesis of neuroblastoma, we found SPC25, one of the four subunits of NDC80 complex which is the key microtubule-binding complex at kinetochores during mitosis, may play important roles in neuroblastoma. In this study, we showed that high expression of SPC25 is related with poor prognosis of neuroblastoma. The survival of neuroblastoma patients with high SPC25 expression is significantly lower than those with low SPC25 expression. MYCN amplification and ALK mutations are the two most often identified genetic lesions in neuroblastoma. SPC25 is significantly up-regulated in neuroblastoma with MYCN amplication. ALK mutations can also up-regulate SPC25 expression. All these results

suggest an important role of SPC25 in the oncogenesis of neuroblastoma. SPC25 could be a useful diagnostic marker and therapy target of neuroblastoma. More details of SPC25 in neuroblastoma will be further investigated.

W4007

SINGLE-CELL ANALYSIS OF X-CHROMOSOME INACTIVATION DYNAMICS AND PLURIPOTENCY DURING DIFFERENTIATION

Chen, Geng[1], **Schell, John Paul**[1], Benitez, Julio Aguila[1,2], Reinius, Björn[1], Yilmaz, Marlene[1,3], Petropoulos, Sophie[1,2], Alekseenko, Zhanna[4], Shi, Leming[5], Hedlund, Eva M.[1], Lanner, Fredrik[2], Sandberg, Rickard[1] and Deng, Qiaolin[2], [1]Karolinska Institutet, Stockholm, Sweden, [2]Karolinska Institute, Stockholm, Sweden, [3]Karolinska Institute, Solna, Sweden, [4]Karolinska Institutet, CMB, Stockholm, Sweden, [5]Center for Pharmacogenomics, Fundan University, Shanghai, China

Pluripotency, differentiation and X-chromosome inactivation (XCI) are key aspects of embryonic development; however, the relationship and underlying mechanisms amongst these processes remains unclear. Here we systematically dissected these features of developmental progression by modeling them with different states of mouse embryonic stem cells (mESCs), and investigating global transcriptomic variances using single-cell RNA-seq with allelic resolution. While preimplantation mouse epiblast cells displayed profiles distinct from ground state 2i grown mESCs, endogenous postimplantaion epiblast closely resembled in vitro EpiStem cells and the two clustered similarly. Sex-related gene expression differences vary greatly across distinct developmental states. We identified many potential novel markers highly enriched in each developmental state. Moreover, we revealed that novel pathways including PluriNetWork and Focal Adhesion were responsible for the delayed progression of female EpiStem cells. Unexpectedly, XCI exhibited variability in each developmental state including 2i conditions. Intriguingly, XCI progression was globally correlated with the loss of pluripotency and initiation of differentiation; however, these processes were not tightly synchronized at the single-cell level. Transcription factors that were highly significantly correlated with XCI progression mainly belonged to zf−C2H2 (zinc finger, C2H2 type) family. Additionally, highly expressed genes, including core pluripotency factors, were in general biallelically expressed whereas monoallelic expression became more prevalent as expression levels decreased. Collectively, our study sheds light on the dynamics of XCI progression and the asynchronicity between pluripotency, differentiation and XCI during early development.

Funding Source: This work was supported by grants from the Swedish Research Council (2014-2870, E0287001), Svenska Sällskapet för Medicinska Forskning. SP is supported by the Mats Sundin Fellowship in Developmental Health.

W4009

SYNAPTIC INPUT ALTERATION OF NEURON NETWORK WITH FUNCTIONAL GABAERGIC AND GLUTAMATERGIC CELL TYPE DERIVED FROM HUMAN EPILEPTIC iPSCs

Liu, Jingxin[1], Hang, Rongqi[1] and Li, Zhiyuan[2], [1]Guangzhou Institutes of Biomedicine and Health,Chinese Academy of Sciences, Guangzhou, China, [2]Guangzhou Institutes of Biomedicine & Health, Chinese Academy of Sciences, Guangzhou, China

Although iPSCs and neuronal differentiation have provided a new method to study genetic epilepsy caused by SCN1A mutations, this technology still has some inherent limitations that have limited our understanding of obtained data related to epilepsies. In the present project, we reported a work combining the technology of CRISPR Cas9- and TALEN-mediated gene editing with neuronal differentiation of patient iPSCs to make study of epilepsy can be conducted at the level of not only neuronal subtype but also neuronal networks based on the comparison between patient neurons and itsisogenic controls. Using this approach, we for the first time performed patch clamp recording on a Nav1.1-expressing neuronal subtype and monitored postsynaptic activity of both inhibitory and excitatory types in a system composed of GABAergic and glutamatergic neurons. We found the mutation c.A5768G, which led to no current of Nav1.1 in exogenously transfected system, influenced the properties of not only Nav current density, but also Nav activation in patient-derived Nav1.1-expressing GABAergic neurons. And alterations in Nav further influenced the AP firing ability in patient-derived GABAergic neurons and led to a weakened spontaneous inhibitory postsynaptic currents (sIPSCs), as well as the shift of spontaneous postsynaptic input from the inhibition to excitation dominated state in patient-derived neuron network. These results suggested that although the spontaneous excitatory postsynaptic currents (sEPSCs) did not alter obviously, changes in the sIPSCs alone was sufficient to significantly affect the whole condition of spontaneous postsynaptic currents and increase the risk of occurrence of epileptic seizure. Our findings fill the gap of our knowledge regarding the relationship between *SCN1A* mutation effect recorded on exogenously transfected cells and on Nav1.1-expressing neurons, and reveals the physiological basis underlying epileptogenesis that is caused by *SCN1A* loss-of-function mutation. These findings provide practical instructions for clinical drug administration.

W4011

REPROGRAMMING OF ADULT MAMMALIAN CARDIOMYOCYTES TO A PROGENITOR STATE BY CCNA2

Ranjan, Amaresh, Icahn School of Medicine at Mount Sinai, New York, NY, U.S., Vadakke Madathil, Sangeetha, Ichan School of Medicine at Mount Sinai, New York, NY, U.S. and Chaudhry, Hina W., Mount Sinai School of Medicine- Cardiology, New York, NY, U.S.

Adult mammalian heart is known as one of the organs with a very low abundance of progenitor cells, which can take part in active cycling and regeneration after damage. Cardiomyocytes exit the cell cycle soon after birth coincident with the silencing of cyclin A2 (CCNA2). In our previous studies, we demonstrated that viral delivery of *Ccna2* induces cardiac regeneration in infarcted hearts of small and large animal models. We have now optimized culture methods for adult human and mouse cardiomyocytes to investigate the molecular mechanisms of cell division in these cells. The isolated cardiomyocytes start to adhere and spread after 1 week of culture. We induced expression of CCNA2 using adenovirus encoding human CCNA2 cDNA driven by the cardiac specific chicken troponin T (cTnT) promoter. Cytokinesis was visualized using live cell epifluorescence imaging with time lapse microscopy after cotransfecting with adenovirus encoding a GFP reporter driven by cTnT (cTnT-GFP). Approximately 3 fold higher cytokinesis in test samples was observed as compared with controls (cTnT-GFP virus only). We then examined the effect of CCNA2 expression on dedifferentiation of these cells. Isl1 has been shown to be expressed in cardiac precursors and is downregulated upon differentiation. We detected expression of Isl1 in cultured cardiomyocytes transfected with CCNA2-adenovirus while it was absent in null-adenovirus transfected cells. We observed appearance of cardiac progenitor marker "non-muscle myosin IIB" and epithelial to mesenchymal transition markers (Vimentin and FSP1) in these cells along with cardiac marker "troponin Tc". We also examined the gene expression of cardiac markers by qPCR. This was normalized with GAPDH and expression of genes at 3 weeks of culture compared to day 0 and fold ± SEM was determined. We observed decreased expression of adult cardiac markers a-MHC (0.24 ± 0.12), Ckmt2 (0.08 ± 0.03) and Troponin Tc (0.50 ± 0.02). These observations imply that CCNA2 mediates dedifferentiation of adult cardiomyocytes in vitro to a cardiac stem/progenitor cell phenotype which may then re-enter the cell cycle. We are further investigating the potential for these resultant cells to differentiate into functional adult cardiomyocytes in vitro and in vivo.

W4013

CREST-SEQ REVEALS DISTAL PROMOTERS REGULATING POU5F1 EXPRESSION AS CIS-REGULATORY ELEMENTS VIA CHROMATIN LOOPING IN HUMAN EMBRYONIC STEM CELLS

Diao, Yarui[1], Fang, Rongxin[1], Li, Bin[1], Meng, Zhipeng[2], Guan, Kun-Liang[2] and Ren, Bing[3], [1]Ludwig Institute for Cancer Research/UCSD, San Diego, CA, U.S., [2]University of California, San Diego, La Jolla, CA, U.S., [3]Ludwig Institute for Cancer Research, La Jolla, CA, U.S.

Millions of cis-regulatory elements have been predicted through analysis of chromatin biochemical features in the human genome. However, knowledge of their biological function in relevant cell types and in native chromatin context has been lacking. Here we developed CREST-seq for cis regulatory element scan with tiling deletion and sequencing, to identify regulatory sequences in an unbiased, high-throughput manner. CREST-seq is a dual CRISPR mediated, tiling deletion based high throughput genetic screen method. By applying this method to a 2Mbp locus containing POU5F1, we identified 45 cis-regulatory elements controlling POU5F1 expression in human embryonic stem cell (hESC), including POU5F1 promoter, proximal enhancer, and previously characterized TEMP enhancers. The cis-regulatory elements being identified are significantly enriched with active epigenetic marks, like RNA polymerase II, H3K27ac, H3K4me3, H3K4me1, DNase I hypersensitivity, RAD21, CTCF, and multiple transcription factors. Surprisingly, we uncovered many distal promoters function as cis-acting elements for optimal POU5F1 expression in hESC, and these promoters exhibit high interaction frequency with POU5F1 promoter. These results demonstrate that CREST-seq is a robust way for functional characterization of non-coding genomic sequence in large genomic region, and for the first time, we provide in vivo evidence showing that promoters may function as cis-regulatory elements controlling distal gene expression via high order chromatin organization, and provide insights into transcriptional regulation by three-dimensional chromatin structure.

Funding Source: Human Frontier Science Program Long-term Fellowship

W4015

NOVEL DNA MODIFICATION IN MOUSE ESC

Wu, Tao, Wang, Tao, Lin, Kaixuan, Liu, Yifei and Xiao, Andrew, Yale Stem Cell Center, New Haven, CT, U.S.

It has been widely accepted that 5-methylcytosine is the only form of DNA methylation in mammalian genomes. Herein, we discover the presence of N^6-methyladenine in mammalian genomes, especially, in mouse embryonic stem cells. Our work also identifies *Alkbh1* as a demethy-

lase for N^6-methyladenine. Increase of N^6-methyladenine levels in *Alkbh1* deficient cells leads to silencing. Interestingly, N^6-methyladenine deposition is inversely correlated with the evolutionary age of LINE-1s; its deposition is strongly enriched at young but not old L1s. The deposition of N^6-methyladenine correlates with epigenetic silencing of such LINE-1s, together with their neighboring enhancers and genes, thereby resisting the gene activation signals during embryonic stem cell differentiation. As young full-length LINE-1s are strongly enriched on the X-chromosome, genes located on the X-chromosome are also silenced. Thus, N^6-methyladenine adopts a new function of epigenetic silencing in mammalian evolution, distinct from its role in gene activation in other organisms. In summary, our results demonstrate that N^6-methyladenine constitutes a crucial component of the epigenetic regulation repertoire in mammalian genomes. In summary, the discovery of N^6-methyladenine in mammalian ESCs sheds new light on epigenetic regulation during early embryogenesis. This work will have far-reaching impacts in the fields of epigenetics, stem cell and developmental biology.

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W4017

AN OVOL2-ZEB1 CIRCUIT REGULATES MAMMARY BASAL-LUMINAL BINARY DIFFERENTIATION

Villarreal-Ponce, Alvaro[1], Watanabe, Kazuhide[1,2] and Dai, Xing[1], [1]University of California School of Medicine, Irvine, CA, U.S., [2]RIKEN, Tokyo, Japan

Epithelial-to-mesenchymal transition (EMT) is a recognized form of cell fate plasticity that converts epithelial cells (e.g., epiblast) into mesenchymal cell types (e.g., mesoderm) during embryogenesis. EMT-like processes are also strongly implicated in cancer metastasis and wound healing. Recent studies, mostly utilizing cultured cell lines, uncover an interesting link between EMT and stemness: differentiated epithelial cells that have undergone transient EMT acquire stem cell (SC)-like traits such as multipotency and self-renewal. Despite emerging data suggesting the physiological relevance of this finding, whether a dynamic interplay of EMT-promoting and -inhibiting factors influences stem cell fates in vivo during epithelial morphogenesis and regeneration is yet to be elucidated. We previously identified Ovol2 as a master gatekeeper of epithelial identity in the mammary gland through its direct inhibition of EMT genes, particularly *Zeb1* that encodes an EMT-inducing transcription factor. Using a systems biology approach, we delineated the importance of cross repression between Ovol2 and Zeb1 for the robust production of partial EMT states with differential plasticity and propensity to differentiate. Here, we report that the mammary stem cell (MaSC)-containing basal compartment, but not the luminal compartment, exists in a partial EMT state that is regulated by both Ovol2 and Zeb1. Utilizing gene knockout models and lentiviral expression systems, we provide data suggesting that Ovol2 promotes mammary epithelial cells to adopt a luminal fate, whereas Zeb1 promotes a basal/myoepithelial cell fate. We further describe an important role for Zeb1 in ductal branching during mammary regenerative/morphogenic processes. Together, our results support a model where positive and negative regulation of EMT controls the binary basal-luminal decision within the differentiating mammary stem/progenitor cell populations.

Funding Source: NIH

W4019

MODULATING THE SELECTIVE ADVANTAGE OF GENETICALLY VARIANT HUMAN PLURIPOTENT STEM CELLS

Thompson, Oliver, University of Sheffield, CSCB, Sheffield, U.K.

Human pluripotent stem cells (hPSC) have the capacity to become any specialised cell type; harnessing this ability is key to developing stem cell based therapies and improving regenerative medicine. If hPSCs are to be exploited as tools for regenerative medicine we must understand their behaviour and growth characteristics, and translate this knowledge into the clinic. Current data indicate that hPSCs commonly gain of extra copies of chromosomes in culture, which may provide a selective advantage to variant cells and drive further genetic instability and mutation. These mutations also cause cells to become cancer-like in their growth and can impair their capacity to differentiate into useful tissues, compromising their safety and efficacy in downstream clinical use. Therefore, it should be a priority to refine culture conditions to suppress or eliminate variants once they arise. Determining the causes, and understanding the effects of these chromosomal abnormalities on hPSC growth and behaviour will also help the screening and production of safe, effective stem cells for clinical application. A chromosome 20q11.21 CNV arises frequently in cultured hPSCs. This duplication is too small to be detected by traditional karyotyping, yet allows variants to outcompete their normal counterparts and may render variant cells prone to acquiring other abnormalities. We show that modulating basic passage methods can influence the selective advantage of genetically variant hPSC lines; in particular we demonstrate suppression of the commonly occurring and potentially clinically relevant 20q11.21 variant.

Funding Source: MRC

W4021

ADULT MOUSE OVARIES CONTAINS PUTATIVE GERMLINE AND PLURIPOTENT STEM CELLS WITH THE CAPACITY OF DIFFERENTIATION INTO GERM AND SOMATIC CELLS

Esmaeilian, Yashar[1], Atalay, Arzu[1] and Erdemli, Esra[2], [1]Biotechnology Inst-Ankara University, Ankara, Turkey, [2]Department of Histology and Embryology, School of Medicine-Ankara University, Ankara, Turkey

For decades, scientists have believed that ovary of female mammals contains a restricted number of oocytes and there is no possibility of replenishing if oocytes are lost due to disease or injury. However, in the last decade, controversy about the possible presence of oocytes and granulosa cells that derived from stem cells in the adult mammalian ovaries, were intensified. We investigated the presence of putative germline and pluripotent stem cells in the adult mouse ovary and their differentiation potential into germ and somatic cells. In the undifferentiated cells, which were harvested from ovarian cell culture, presence of pluripotent stem cell markers (Oct-4, Nanog, Sox2 and SSEA1) and germline stem cell markers (DAZL and DDX4/VASA) were detected by immunofluorescence staining and western blot analysis. In the ovarian tissue and undifferentiated cells, expression of Oct-4, Sox2*,* Nano*g,* SSEA1*,* DDX4, DAZL, SCP3, DMC1, PRDM1 and STRA8 genes were also assessed by RT-PCR and further gene expression analysis were performed by qRT-PCR method in order to determine the significance of difference between different samples. Overall, in vitro differentiation of ovarian stem cells led to formation of new oocytes, neural cells, chondrocytes and osteoblasts. In the differentiated cells, expression of related markers were observed both in mRNA and protein levels. The outputs of this study, revealed the presence of pluripotent and germline stem cells in the adult mouse ovary and their differentiation potential into somatic and germ cells.

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W4023

HYDROGEL LOADING WITH CATECHINS TO PROTECT RAT MICROGLIA CELLS FROM LIPOPOLYSACCHARIDE-INDUCED ACTIVATION

Yu, Wu Xiao, Chung Yuan Christian University/ Department of Biomedical Engineering, Taoyuan/ ChungLi, Taiwan

It has clinically documented that inflammation in brains is involved in the neurodegenerative disorders such as Parkinson's disease (PD). A variety of insults in the brain can activate microglial cells which produce proinflammatory factors and further trigger the death of neurons. Among various nutraceutical agents, polyphenol molecules such as catechins extracted from green tea has been reported to exhibit inhibitory effect on microglial activation.(-) epigallocatechin gallate (EGCG) is one of green tea extract (GTE) that possess highest potential of antioxidation. Microglial cells were down regulated to decrease the inducible NO synthase and TNF-α upon the addition of EGCG. These findings confirm that the administration of GTE to the central nerve system could be a potential treatment for neurodegenerative diseases. The present study aims to develop an injectable hydrogel to sustain release GTE in the brain tissue. Among various polymeric biomaterials, chitosan and hyaluronic acid are widely used because of good biocompatibility and versatile biological functions were performed in this study.

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W4025

REVERSAL OF NATURALISTIC DEMENTIA-LIKE DISORDER IN OLDER DOG WITH PATIENT SPECIFIC CELL THERAPY

Valenzuela, Michael[1], Duncan, Thomas[2], Lowe, Aileen[2], Toole, Sarah[3], Voss, Katja[4], Brunel, Laurencie[4], Jacobsen, Erica[5] and McGreevy, Paul[4], [1]School of Psychiatry, UNSW, Sydney NSW, Australia, [2]The University of Sydney, Sydney, Australia, [3]University of Wollongong, Wollongong, Australia, [4]University of Sydney, Sydney, Australia, [5]Prince of Wales Hospital, Sydney, Australia

Dementia affects 32 million individuals with projections to reach 130 million by 2050. Medical management has not substantively progressed over the last 20 years; one reason for this is that dementia is not adequately modelled in transgenic mice. It remains an incurable, progressive and fatal disorder. Accordingly, we have studied Canine Cognitive Dysfunction (CCD) in older pet dogs. Dogs with CCD: display amnesia, disorientation, anhedonia, sleep-wake disturbance and agitation and express both Alzheimer and non-Alzheimer pathology as well as neurodegeneration. Prevalence doubles every 2-years after 10 years of age and veterinary trials recapitulate the poor outcomes seen in humans. CCD is therefore an important translational model of human dementia. This case reports on Timmy, a 13-year old Cocker spaniel with a 2-year history of progressive CCD signs as well as multiple age-related co-morbidities. His principal clinical problem was nocturnal wakefulness, barking and agitation, so severe that his owners were considering euthanasia. Before intervention his total CCDR score was 57 (out of hypothetical 80 maximum), where >50 is diagnostic. In Au-

gust 2015 we treated Timmy with intrahippocampal cell therapy in the context of a Phase I veterinary clinical trial. Using MRI guidance, 500,000 patient-specific genetically non-modified skin-derived neuroprecursors, produced using our published protocol, were stereotaxically injected under general anaesthesia.Timmy showed marked improvement 3 months post treatment. His CCDR score reduced by 50% and is now normal. Moreover, Timmy's sleep behavior improved in a clinically meaningful way. At night he can now orientate himself to the doggie-door, will urinate outside and return to his bed. His owners are delighted, commenting, "It's amazing...it's like having our old friend back". This clinical improvement was paralleled on our objective spatial memory test (Canine Sand Maze), where Timmy could successfully learn the position of a hidden food treat following treatment. Overall, this unprecedented case study demonstrates in-principle the reversibility of a naturalistic dementia-like syndrome using patient-specific cell therapy.

W4027

REGULATION OF DNA METHYLATION LANDSCAPE IN HUMAN SOMATIC CELL REPROGRAMMING BY MIR-29 FAMILY

Hysolli, Eriona[1], Tanaka, Yoshiaki[1], Kim, Kun-yong[2], Weissman, Sherman [3] and **Park, In-Hyun**[2], [1]Yale University, New Haven, CT, U.S., [2]Yale School of Medicine, New Haven, CT, U.S., [3]Yale University School of Medicine, New Haven, CT, U.S.

Reprogramming to pluripotency after overexpression of OCT4, SOX2, KLF4 and MYC is accompanied by global genomic and epigenomic changes. Histone modification and DNA methylation states in iPSCs have been shown to be highly similar with embryonic stem cells (ESCs). However, epigenetic differences still 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 to find factors that regulate DNA methylation states in reprogramming. Here, we found that the miR-29 family is an important epigenetic regulator during human somatic cell reprogramming. Our global DNA methylation and hydroxymethylation analysis shows that DNA demethylation is a major event mediated by miR-29a depletion during early reprogramming, and that iPSCs derived from miR-29a depletion are epigenetically closer to ESCs and contribute to a more intricate three germ layer pattern upon teratoma differentiation than control clones. Our findings uncover an important miRNA-based approach to generate clinically robust iPSCs.

W4029

SUPPRESSION OF SWI/SNF COMPONENT Arid1a PROMOTES MAMMALIAN REGENERATION THROUGH CHROMATIN REMODELING

Sun, Xuxu, University of Texas Southwestern Medical Center, Dallas, TX, U.S.

The astounding regenerative capabilities of some vertebrates have been partially lost in mammals, possibly due to chromatin-remodeling mechanisms that enforce terminal differentiation. We show that the SWI/SNF component Arid1a is suppressed in regenerating tissues, and that genetic deletion of Arid1a substantially improved tissue repair in mice after an array of injuries. Arid1a deficient livers exhibited increased regeneration, reduced tissue damage, and improved organ function in response to surgical resection and chemical injury, while whole-body Arid1a loss potentiated soft tissue healing in the ear. The chromatin state as reprogrammed by Arid1a loss restricted access to promoters by transcription factors that ordinarily enforce differentiation and suppress cell cycle reentry, thus increasing regeneration after injury. Epigenetic reprogramming mediated by the deletion of a single gene improves mammalian regeneration in multiple contexts.

W4031

THE ROLES OF SALL4 IN THE NEURAL CREST AND IN MELANOMA

Baggiolini, Arianna[1], Diener, Johanna[2], Varum, Sandra[2], Häusel, Jessica[2], Cheng, Phil[3], Levesque, Mitchell[3], Treier, Mathias[4], Dummer, Reinhard[3] and Sommer, Lukas[2], [1]Universität Zürich, Zürich, Switzerland, [2]Univeristy of Zurich, Zurich, Switzerland, [3]University Hospital Zurich, Zurich, Switzerland, [4]Max-Delbrück Center for Molecular Medicine (MDC), Berlin, Germany

Melanoma is a malignancy of the melanocytes, which are the pigment-producing cells of neural crest (NC)-origin. Cutaneous melanoma, which originates from the melanocytes of the skin, is one of the most aggressive cancers and it is responsible for 75% of deaths related to skin cancer. Melanoma cells can in fact often metastasize to proximal and distant organs and their invasive capacity is reminiscent to the great migratory properties of the NC cells. This has contributed to the idea that NC-derived malignant cells may exploit developmental regulatory programs to induce melanoma formation and progression. To investigate whether malignant cells may exploit genes responsible for the multipotency of the NC during melanoma progression, we performed a microarray analysis comparing migratory NC stem cells and their primed counterparts. Among the genes that were specifically expressed in the NC and that were downregulated in the

primed NC counterparts we further investigated Sall4 and studied its function in the premigratory NC. Preliminary analyses upon Sall4 loss at the premigratory stage suggest a reduction of the melanocytic lineage in the embryo. Moreover, Sall4 loss in the Tyr::NRas^{Q61K} Ink4a$^{-/-}$ melanoma mouse model impairs tumor growth, suggesting that Sall4 is necessary for tumor formation.

W4033

BIS-MEDIATED STAT3 STABILIZATION REGULATES GLIOBLASTOMA STEM CELL-LIKE PHENOTYPES

Im, Chang-Nim[1], Yun, Hye Hyeon[1], Song, Byunghoo[1], Youn, Dong-Ye[1], Cui, Mei Nu[1], Kim, Hong Sug[2], Park, Gyeong Sin[1] and Lee, Jeong-Hwa[1], [1]The Catholic University of Korea, Seoul, Korea, [2]Macrogen Inc, Seul, Korea

Glioblastoma stem cells (GSCs) are a subpopulation of highly tumorigenic and stem-like cells that are responsible for resistance to conventional therapy. Bcl-2-intreacting cell death suppressor (BIS; also known as BAG3) is an anti-apoptotic protein that is highly expressed in human cancers with various origins, including glioblastoma. However, the role of BIS in GSC subpopulations remains unknown. Therefore, in the present study, we examined the expression profile of BIS in glioblastoma cell lines A172 and U87-MG under specific in vitro culture conditions that enrich GSC-like cells in spheres. Both BIS mRNA and protein levels significantly increased under the sphere-forming condition as compared with standard culture conditions. BIS depletion resulted in notable decreases in sphere-forming activity and was accompanied with decreases in SOX-2 expression. The expression of STAT3, a master regulator of stemness, also decreased following BIS depletion concomitant with decreases in the nuclear levels of active phosphorylated STAT3; however, ectopic STAT3 overexpression resulted in recovery of sphere-forming activity in BIS-knockdown glioblastoma cells. Additionally, immunoprecipitation and confocal microscopy revealed that BIS physically interacts with STAT3. Furthermore, BIS depletion increased STAT3 ubiquitination, suggesting that BIS is necessary for STAT3 stabilization in GSC-like cells. BIS depletion also affected epithelial-to-mesenchymal transition-related genes by suppressing SNAIL and MMP-2 expression and increasing E-cadherin expression in GSC-like cells. Our findings showed that high levels of BIS expression might confer stem-cell-like properties on cancer cells through STAT3 stabilization, indicating that BIS is a potential target in cancer therapy.

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W4035

DISTINCT TRANSCRIPTIONAL PROFILES CONTROLLED BY CHEMISTRY AND SURFACE TOPOGRAPHY IN HUMAN OSTEOBLASTS AND MESENCHYMAL STEM CELLS GROWN ON CALCIUM PHOSPHATE CERAMICS

Hebels, Dennie and de Boer, Jan, Maastricht University, Maastricht, Netherlands

Calcium phosphate (CaP) ceramics possess bone-resembling mineral phases and are biocompatible, osteoconductive, and in some specific cases also osteoinductive. This special type of ceramics has been used and studied within the context of repair of large bone defects for these reasons. However, the molecular mechanisms underlying material-induced osteoinductive behavior and knowledge of the responsible material properties remain largely undefined. We therefore used microarray-based transcriptomics to investigate the cellular and molecular mechanisms induced by various CaP ceramics in MG-63 osteosarcoma cells and human mesenchymal stem cells in order to determine the responsible material properties underlying material-induced bone formation. Following analysis, 12 genes were found to correlate with the in vivo osteoinductive capacity and microstructural properties of CaP materials. Their expression was further verified using MG-63 cells cultured on osteoinductive tricalcium phosphate ceramic discs with tailored microstructural properties, to determine the effect of surface structure. In order to independently study ion dissolution properties, we used microfabrication protocols to replicate the surface of non- and osteoinductive ceramics into polystyrene films and measure the effect of elevated medium levels of Ca/Pi ions. Gene network analysis was used to visualize the molecular interactions among genes and identify other possible key players in the osteogenic process. We were able to attribute the expression of tenascin C (TNC) and hyaluronan synthase 2 (HAS2) to surface structure effects, whereas CEMIP (cell migration inducing protein, hyaluronan binding) and TNC were affected by extracellular calcium and phosphate. This study points towards the important role of the extracellular matrix (ECM) in response to properties presented by osteoinductive materials. We propose a mechanism by which the surface structure and ceramic-mediated ion release induce expression of HAS2 and TNC and repression of CEMIP, thereby modulating the molecular weight of hyaluronic acid secreted in the ECM. The upregulation of TNC also suggests its presence in the ECM of cells cultured on osteoinductive ceramics.

Funding Source: This research has been made possible with the support of the Dutch Province of Limburg.

W4037

Lin28a FUNCTIONS AS AN INJURY-RESPONSE GENE TO PROMOTE ZEBRAFISH LATERAL LINE HAIR CELL REGENERATION VIA WNT ACTIVATION

Jiang, Linjia, Stowers Institute for Medical Research, Kansas City, MO, U.S.

The zebrafish lateral line hair cells, which are localized in the center of neuromast and surround by support cells, are similar to sensory hair cells in the mammalian inner ear. Investigating how zebrafish regenerate hair cells will contribute to our understanding and treatment of human hearing loss. By performing RNA-Seq analyses at different time points of hair cell regeneration we identified lin28*a* as one of the early response genes after hair cell ablation. The RNA-Seq data also reveals that Wnt pathway genes, such as wnt10a, β-catenin and c-myc, are increased after lin28a induction during hair cell regeneration. It is known that Wnt pathway is both sufficient and necessary for hair cell regeneration. However, how Wnt signaling is activated during hair cell regeneration remains elusive. *l*in28a and its homologue lin28b are important regulators of stem cell maintenance and tissue regeneration, and therefore could be candidates to activate Wnt signaling and promotes hair cell regeneration. By in situ hybridization lin28b*,* but not lin28a is expressed in homeostatic neuromasts. Immediately after hair cell death lin28*a* is dramatically induced in one or two neuromast cells, whereas lin28b expression is unchanged. Furthermore after severe injury, when both hair cells and support cells are depleted, lin28a is even more highly upregulated. This indicates that in the zebrafish lateral line lin28a and *l*in28b may have different functions in homeostasis and regeneration. To further investigate the role of lin28a in hair cell regeneration we have created heat shock-inducible lin28a transgenic fish and lin28a mutant fish. In both homeostasis and regeneration support cell proliferation is increased whereas hair cell differentiation is not affected when lin28a is overexpressed. Conversely lin28a mutant fish have less proliferating support cells during severe injury-induced regeneration. We have also shown that increased proliferation in *lin28a*-overexpressed larvae is Wnt-dependent. Inhibition of Wnt signaling by overexpression of dkk1 significantly reduced proliferation in hs:lin28a fish. These results suggest that lin28a functions as an injury-response gene to facilitate Wnt activation and promote cell amplification during hair cell regeneration.

W4039

THREE-DIMENSIONAL CULTURE OF SINGLE EMBRYONIC STEM-DERIVED NEURAL/STEM PROGENITOR CELLS IN FIBRIN HYDROGELS: NEURONAL NETWORK FORMATION AND MATRIX REMODELLING

Bento, Ana Rita[1], Oliveira, Maria José[1], Quelhas, Pedro[2], Pêgo, Ana Paula[1,3] and Amaral, Isabel[1,3], [1]i3S - Instituto de Investigacao e Inovacao em Saude, Universidade do Porto, Porto, Portugal, [2]INEB - Instituto de Engenharia Biomedica, Universidade do Porto, Porto, Portugal, [3]Faculdade de Engenharia, Universidade do Porto, Porto, Portugal

In an attempt to improve the efficacy of neural stem/progenitor cell (NSPC) based therapies, biodegradable hydrogels such as fibrin (Fb) are being explored to provide a more permissive microenvironment for cell survival and differentiation upon transplantation. Herein we explored the ability of Fb to support the survival and neuronal differentiation of NSPCs derived from mouse embryonic stem (ES) cells under adherent monoculture. Single ES-NSPCs were cultured within Fb (fibrinogen concentration: 6 mg/mL) under neuronal differentiation conditions up to 14 days. ES-NSPCs within Fb retained high cell viability and proliferated within small-sized spheroids. With time, an increase in the levels of βIII-tubulin and NF200 was observed, showing that ES-NSPCs are able to differentiate along the neuronal lineage in Fb. At day 14, flow cytometry analysis further revealed a population mainly comprised of NSPCs and neurons, with 46.5% being βIII-tubulin+ cells. GABAergic and dopaminergic/noradrenergic neurons were also observed along with a network of synaptic proteins. Throughout the cell culture period, ES-NSPCs in Fb deposited extracelullar matrix (ECM) proteins, specifically fibronectin, laminin, and collagen type IV. Expression of matriptase and secretion of MMP-2 and MMP-9 were also detected, MMP-2 activity increasing overtime. Varying the fibrinogen concentration revealed that gels prepared with 8 or 10 mg/mL of fibrinogen are less permissive to neurite extension and neuronal differentiation. Furthermore, the culture of human ES-NSPCs in this 3-D culture system led to similar results, namely in terms of cell viability, expression of neural markers, and deposition of ECM proteins. Overall, we show that ES-NSPCs obtained in monolayer culture are able to proliferate and establish neuronal networks within Fb gels, and to remodel Fb through MMP secretion and ECM deposition. The settled 3-D platform is expected to constitute a valuable tool for the development of Fb-based hydrogels designed for ES-NSPC delivery into the injured human central nervous system.

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W4041

POMA SCAFFOLD WITH BIOMIMETIC HIERARCHICAL STRUCTURES FOR THE GROWTH OF NEURONAL STEM CELL

Yeh, Juiming, Chung Yuan Christian University, Chung Li, Taiwan

A three-dimensional (3D) bio-printing technique was first used to fabricate the high surface roughness scaffold for tissue engineering. First, poly(*ortho*-methoxyaniline) (POMA) was synthesized by conventional oxidative polymerization. Moreover, the POMA scaffold with 3D bio-mimetic morphology was fabricated by using poly(dimethylsiloxane) (PDMS) as negative soft template from natural leaf surfaces of *Xanthosoma sagittifolium*, transferring the pattern to POMA. Subsequently, cell–scaffold interactions were carried out by culturing the rat neural stem cells (rNSCs) on the POMA scaffolds in vitro. It should be noted that bio-mimetic POMA scaffold showed better rNSCs attachment but induced growth arrest that compared with the PDL-coated substrate. In addition, the cells were found to elongate the neuritis resulting from rNSCs were cultured and differentiated on the bio-mimetic scaffold for 19 days. In summary, the bio-mimetic POMA scaffold was found to promote the rNSCs differentiation efficiency and maintenance neurite outgrowth for long-term studies on nerve regenerative medicine.

W4043

NON-CANONICAL WNT AND NOTCH3 SIGNALLING COOPERATE TO REGULATE LUMINAL CELL FATE IN THE BIPOTENT BREAST EPITHELIAL PROGENITORS

Bhat, Vasudeva and Raouf, Afshin, University of Manitoba, Winnipeg, MB, Canada

Breast cancer is the second leading cause of cancer-related death among women. Majority of breast cancer tumours are of luminal type. The proliferation and differentiation potentials of Breast Epithelial Stem Cells (BESCs) or progenitors could be hijacked to produce malignant luminal type tumors. Current evidence suggests that the evolutionarily conserved Notch and Wnt signaling pathways play an important role in regulating Self-renewal, proliferation and differentiation of BESCs. Interestingly, these same signalling pathways also play a vital role in regulating Breast Cancer Stem Cells function. Therefore, understanding the molecular mechanisms that regulate the proliferation and differentiation of the stem cells and progenitors could provide new insights into how their altered functions could lead to breast carcinogenesis, especially luminal type of breast cancers. We previously demonstrated that NOTCH3 receptor (NR3) signalling specifically commits the undifferentiated bipotential progenitors to the luminal cell fate. Here, we investigated how NR3 signaling promotes luminal cell fate. From the unbiased transcriptome profiling approach, we discovered that unlike other Notch receptors, NR3 signaling uniquely increased expression of 10 genes that are involved in the Non-canonical Wnt signaling. One such gene was the Wnt receptor FRIZZLED7 (FZD7), and we recently demonstrated that FZD7 was uniquely regulated by NR3 in a non-canonical fashion. Furthermore, we found that the non-canonical Wnt ligand, Wnt7A, enhanced the commitment of human breast epithelial bipotent progenitors to the luminal lineage. However, canonical Wnt ligand, Wnt3A did not have any effect on the breast epithelial bipotent progenitors, suggesting the non-canonical Wnt signaling is important in luminal fate determination. Interestingly, we observed that loss of FZD7 signaling enhances the luminal cell fate of bipotent progenitors. Similar observation was made in the Fzd7-null mouse mammary glands where loss of Fzd7 resulted in increased luminal progenitors and enhanced branch morphogenesis. These observations suggest that *FZD7* acts to regulate the size of luminal progenitor pool. Our data indicates that signaling through NR3-FZD7 regulates the balance between luminal progenitor proliferation and differentiation.

W4045

TARGETING SELF-RENEWAL IN HIGH-GRADE BRAIN TUMORS LEADS TO LOSS OF BRAIN TUMOR STEM CELLS AND PROLONGED SURVIVAL

Zhu, Zhe[1], Liu, Haikun[2], Feng, Weijun[2], Gronych, Jan[2], Chai, Jiani[3], Rich, Jeremy N.[1,4], Wang, Xiuxing[1] and Mack, Stephen[1], [1]Cleveland Clinic Foundation, Cleveland, OH, U.S., [2]DKFZ, Heidelberg, Germany, [3]Washington University, St.Louis, MO, U.S., [4]Cleveland Clinic, Cleveland, OH, U.S.

Cancer stem cells (CSCs) have been suggested as potential therapeutic targets for treating malignant tumors, but the in vivo supporting evidence is still missing. Using a GFP reporter driven by the promoter of the nuclear receptor tailless (Tlx), we demonstrate that Tlx(+) cells in primary brain tumors are mostly quiescent. Lineage tracing demonstrates that single Tlx(+) cells can self-renew and generate Tlx(-) tumor cells in primary tumors, suggesting that they are brain tumor stem cells (BTSCs). After introducing a BTSC-specific knock-out of the Tlx gene in primary mouse tumors, we observed a loss of self-renewal of BTSCs and prolongation of animal survival, accompanied by induction of essential signaling pathways mediating cell-cycle arrest, cell death, and neural differentiation. Our study demonstrates the feasibility of targeting glioblastomas and indicates the suitability of BTSCs as therapeutic targets, thereby supporting the CSC hypothesis.

W4047

DERIVATION AND EXPANSION OF NAïVE HUMAN PLURIPOTENT STEM CELLS IN SUSPENSION BIOREACTORS FOR FUTURE CLINICAL APPLICATION

Rohani, Leili and Rancourt, Derrick, University of Calgary, Calgary, AB, Canada

The recent development of human pluripotent stem cell (hPSC) biobanks indicates that future cell therapy may not be personalized but instead based upon histocompatibility. Hence, the scalable production of clinically relevant hPSC numbers will be required. Suspension bioreactors are potential platform for the scalable generation of hPSCs in a controlled culture environment. Mouse pluripotent stem cells (mPSCs) can be efficiently expanded as aggregates in suspension bioreactors. We have observed that aggregates of mouse PSCs experience "mechano-pluripotency" in stirred bioreactors: fluid shear manifested at adherens junctions (AJs) results in the nuclear translocation of b-catenin and upregulation of pluripotency related genes in the absence of LIF. This phenomenon explains why, in mouse, cellular reprogramming is 1000-fold more efficient in bioreactors. By contrast, conventional (i.e. primed) hPSCs do not undergo mechanopluripotency, presumably because ROCK inhibitor Y-27632 is necessary to stabilize AJs in the bioreactor. However naïve hPSCs, which are functionally equivalent to mPSCs, do not require Y-27632 for passaging, might undergo mechanopluripotency like their mPSC counterpart. In this study we investigated if naïve hPSCs could be derived and expanded in the bioreactor without requiring Y-27632. H9 (i.e. female) hPSCs were inoculated into suspension bioreactors and reverse-toggled to the naïve pluripotent state. Resulting aggregates could be passaged several times without Y-27632 and revealed domed shaped colonies in static culture. Naïve-like aggregates expressed high levels of pluripotency markers, naïve markers, and concomitant disappearance of XIST gene expression. Our research suggests that the conversion of hPSCs to naïve pluripotency in bioreactors for facile expansion may be a viable approach to generate the large numbers of hPSCs required for future clinical application. Given the negative effects that Y-27632 may have on hPSC pluripotency and genetic stability, this bioreactor reverse toggling approach may help to ensure legacy hPSCs can be used in future clinical trials. Currently we are investigating if mechanopluripotency will allow us to eliminate the need for other inhibitors and growth factors, thus improving cell quality and reducing expansion costs.

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W4051

ALPHA2-6SIALYLATION IS A MARKER OF THE DIFFERENTIATION POTENTIAL OF HUMAN MESENCHYMAL STEM CELLS

Tateno, Hiroaki[1], Toyoda, Masashi[2], Onuma, Yasuko[3], Ito, Yuzuru[3], Akutsu, Hidenori[4] and Hirabayashi, Jun[1], [1]National Institute of Industrial Science and Technology, Tsukuba, Japan, [2]Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan, [3]AIST, Tsukuba, Japan, [4]National Center for Child Health & Development, Tokyo, Japan

Human somatic stem cells such as human mesenchymal stem cells (hMSCs) are considered attractive cell sources for stem cell-based therapy. However, quality control issues have been raised concerning their safety and efficacy. Here we used lectin microarray technology to identify cell surface glycans as markers of the differentiation potential of stem cells. We found that α2-6Sia-specific lectins show stronger binding to early passage adipose-derived hMSCs (with differentiation ability) than late passage cells (without the ability to differentiate). Flow cytometry analysis using α2-6Sia-specific lectins supported the results obtained by lectin microarray. Similar results were obtained for bone marrow-derived hMSCs and cartilage tissue-derived chondrocytes. Little or no binding of α2-6Sia-specific lectins was observed for human dermal fibroblasts, which are unable to differentiate, suggesting that the binding of α2-6Sia-specific lectins is associated with the differentiation ability of cells, but not to their capacity to proliferate. Quantitative analysis of the linkage mode of Sia using anion-exchange chromatography showed that the percentage of α2-6Sia linkage type was higher in early passage adipose-derived hMSCs than late passage cells. Integrinα5 was found to be a carrier protein of α2-6Sia. Sialidase treatment significantly reduced the differentiation efficiency of bone marrow-derived hMSCs. Based on these findings, we propose that α2-6sialylation is a marker of differentiation potential in stem cells such as adipose-derived hMSCs, bone marrow-derived hMSCs, and cartilage tissue-derived chondrocytes.

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W4053

SYNERGISTIC TARGETS BETWEEN ASC-DIFFERENTIATED ENDOTHELIAL AND NEURAL LINEAGE CELLS TO PREVENT HYPOXIC-ISCHEMIC BRAIN INJURY

Huang, Chia-Wei[1], Chang, Ya-Ju[1], Shu, Chien[2] and **Wu, Chia-Ching**[1], [1]National Cheng Kung University, Tainan, Taiwan, [2]Director, Institute of Engineering in Medicine, San Diego, CA, U.S.

Neonatal hypoxic-ischemic (HI) brain injury causes disruption of neurovascular integrity and leads to life-long functional deficit in human development. These devastating consequences can be prevented by protecting the architecture of the neurovascular unit. Our lab has demonstrated the possibility to differentiate the adipose-derived stem cells (ASCs) into endothelial lineage cells (ELCs) or neuronal lineage cells (NLCs) via microenvironmental induction and studied the therapeutic effect of the cells by transplanting ASCs, ELCs, NLCs, or combination of ELCs and NLCs (E+N) into HI brain injured neonatal rats. The combined E+N treatment showed a significantly greater decrease of infarction and apoptotic areas, and better preservation of the neurovascular structure. The E+N combinations increased the cell migration under in vitro hypoxia microenvironment. Moreover, the transplanted ELCs and NLCs were able to engraft into host tissue and promote endogenous angiogenesis and neurogenesis. We performed the RNAseq to fish out the potential targets for the differentiated ELCs and NLCs. The ELCs, induced by endothelial growth factors and laminar shear stress, migrated and contributed to the vascular structure by activating the Akt signaling through vascular endothelial growth factor receptor 2 (VEGFR2) and neuropilin 1 (NRP1). NLCs showed better ability in preserving the neural structure. The synergistic benefits in E+N combination were revealed via the cell-cell interactions for NRP1 signaling in ELCs and C-X-C chemokine receptor 4 (CXCR4) and fibroblast growth factor receptor 1 (FGFR1) signaling in NLCs. Blockage of target signals in either ELCs or NLCs diminished the synergistic interaction in cell migration, homing, and protection of neurovasculature in the HI injured brain. The results in current study pointed out the potential molecular targets for the synergistic effect between ELCs and NLCs to improve the therapeutic outcome for preserving brain integrity and function after HI injury.

W4055

INDUCED MESENCHYMAL PROGENITOR CELLS PRODUCE NEUROTROPHIC FACTORS TO AID NERVE REGENERATION

Brick, Rachel and Tuan, Rocky, University of Pittsburgh, Pittsburgh, PA, U.S.

Bone marrow-derived mesenchymal stem cells (MSCs) have well known musculoskeletal regenerative capacities and have trophic and immunomodulatory activities that support regeneration of most tissues. For example, we and others have reported that MSCs have the ability to support nerve regeneration by secreting neurotrophic factors typically produced by Schwann cells. However, MSCs have limited expansion capacity, restricting the time and scale of their use. We have recently described the derivation of induced mesenchymal progenitor cells (iMPCs) from iPSC colonies that retain the differentiation potency of the parent MSCs. Because they may be repeatedly derived from iPSC colonies, iMPCs may provide an unlimited supply of cells for large-scale tissue regeneration. Considering the similarities between iMPCs and MSCs, we hypothesized that iMPCs have the same ability to support neuronal regeneration as the parent MSCs. Immunostaining of iMPCs confirmed expression of mesenchymal and neural cell markers, including CD105, CD44, P74/NGFR and GFAP, demonstrating phenotypic similarities between iMPCs and MSCs. The lack of pluripotency markers (SSEA4, Oct3/4, and Sox-2) indicate a full differentiation from the original iPSCs. After neurotrophic induction, ELISAs confirmed that iMPCs were able to produce 4-fold higher levels of BDNF than that produced by induced MSCs. To assess the biological effects of the induced iMPCs, chick dorsal root ganglia (DRGs) neurite outgrowth was used as a model for peripheral nerve regeneration. Sholl analyses showed that culturing DRGs cultured for 5 days with medium conditioned for 48 hours by neurotrophically-induced iMPCs improved branching complexity by two-fold compared to growth control DRG cultures (supplemented with 10 ng/ml of FGF, EGF, and NGF). A comparable increase in branching complexity was observed in cultures utilizing conditioned medium from non-induced MSCs. These in vitro data strongly suggest the applicability of this approach to generate a renewable source of therapeutic cells to support nerve regeneration. Further studies will use a rat model to examine the efficacy of condensed conditioned medium infused into an aligned nanofiber scaffold to treat peripheral nerve injury.

W4057

PLURIPOTENT STEM CELLS FROM MOUSE ENDOLYMPHATIC SAC

Kim, Hyoung-Mi, CHA University, Seongnam, Korea

Mammalian hair cells have lost their ability of regeneration which is present only in some species of birds by spon-

taneous differentiation induction of stem cells in the inner ear. The aim of the present study is to investigate the pluripotency of epithelial cells of mouse endolymphatic sac. We harvested endolymphatic sacs from neonatal and 7 days old mice. After trypsinization, obtained cells were cultivated in DMEM with F12 nutrient mixtures, B27, N2 supplement, IGF-1 and EGF. We observed that the epithelial cells from endolymphatic sac contain pluripotent stem cells able to form cell clusters (spheres). These inner ear stem cells have the capacity for self-renewal, and form primary and secondary spheres that express marker genes of the developing inner ear and the nervous system. Inner ear stem cells are pluripotent and can give rise to a variety of cell types in vitro, including cells representative of ectodermal and mesodermal lineages. Single spheres harvested and cultivated on collagen produced the differentiation of different cell types including neuron like-cells, positive for myosin VIIA, pax2 and otx2. Our findings suggest that epithelial cells from endolymphatic sac display the characteristic features of stem cells and these stem cells are capable of differentiating into hair cell-like cells, which implies a possible use of such cells for the replacement of lost inner ear sensory cells.

POSTER SESSION I EVEN

PRE-CLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL CELLS

W1002

BONE HEALING EFFECTS OF UNDIFFERENTIATED AND OSTEOGENIC DIFFERENTIATED MESENCHYMAL STROMAL CELL SHEETS IN FRACTURE MODEL DOGS

Yoon, Yongseok[1], KIM, Yongsun[2], Lee, Seung Hoon[2], Kim, Ahyoung[2], Choi, Kyeong Uk[2], Jung, Taeseong[2], **Kweon, Oh-kyeong**[2] and Kim, Wan Hee[2], [1]Seoul national university, Seoul, Korea, South, [2]Department of Veterinary Surgery, College of Veterinary Medicine, Seoul National University, Seoul, Korea, South

Cell sheets technology is being available for fracture healing. This study was performed to evaluate bone healing effects of undifferentiated (UCS) and osteogenic (OCS) differentiated mesenchymal stromal cell (MSC) sheets in fracture model dogs. UCS and OCS were harvested at 10 days of culture. Transverse fracture at radius of six beagle dogs were assigned into three groups (n=4 in each group): UCS, OCS and no treatment. The fractures were fixed with a 2.7mm locking plate and six screws. Three cell sheets were wrapped around fracture site. Radius were harvested 8 weeks after operation, then scanned by micro computed tomography (CT) and histological assessments were performed. The results of micro-CT revealed different aspects of bone regeneration according to the groups. The percentages of external callus volume out of total bone volume in control, UCS, and OCS groups were 42.1%, 13.0% and 4.9% ($P < 0.05$) respectively. However, the percentages of limbs having connectivity of gap were 25%, 12.5%, and 75% respectively. In histopathological assessments, OCS group showed that fracture site was well organized with peripheral cartilage and mature woven bone, whereas control group showed cartilage formation without bone maturation or ossification at fracture site. Meanwhile, fracture site was only filled with fibrous connective tissue without endochondral ossification and bone formation in UCS group. Different healing pathway in relation to the type of cell sheets was observed. It was suggested that the MSC sheets reduced the quantity of external callus, and OCS induced the direct bone healing.

Funding Source: This work was supported by the National Research Foundation of Korea (NRF-2013R1A1A2004506).

W1004

"ALFIE, THE WONDER CAT", OR CORRECTING IN-BORN ERRORS OF LIPID METABOLISM IN LIPOPROTEIN LIPASE DEFICIENT CATS USING ALLOGENEIC MESENCHYMAL STEM CELLS

Vulliet, Richard[1,2], Halloran, Mitch[1], Tallon, Kelli[1] and Rosman, Pamela[2], [1]University of California Davis, Davis, CA, U.S., [2]ReGena-Vet Laboratories, LLC, Davis, CA, U.S.

Veterinary patients with naturally occurring diseases offer excellent opportunities for stem cell trials. Cat mesenchymal stem cells (MSCs) which express LPL mRNA were tested for efficacy in lipoprotein lipase deficient (LPL-/-) feline patients. Feline LPL-/- is essentially identical to human familial hypercholesterolemia (i.e. mutation in the same protein at an equivalent site). Bone marrow was harvested from LPL+/+ cats, MSCs amplified in culture, and the cells administered into LPL-/- cats. In pilot trials, plasma turbidity, triglyceride, cholesterol and lipase levels were monitored prior to and following the injection of allogeneic MSCs. There was a modest decrease in plasma turbidity after the first injection. Following the second injection, a much greater decrease in plasma turbidity and circulating lipids was observed. The enhanced response following the second injection suggests that an accumulation of LPL+/+ cells occurs somewhere in the cat, most likely in the bone marrow. To confirm that the observed decrease in plasma lipids resulted from circulating lipoprotein lipase activity from the injected MSC's, plasma samples were collected and LPL activity measured. Following the first administration of MSCs, a slight increase in LPL activity was observed. After the second administration, a larger increase in LPL activity was noted. Further monitoring revealed that the effect on plasma lipids last-

ed approximately three months. Injection of LPL+/+ MSCs corrects the LPL-/- deficiency and transiently returns the plasma lipid profile to near normal in a dose dependent manner. Multiple injections of allogeneic MSCs demonstrate additivity and increased duration of effect. Behavioral improvement correlated with duration of treatment effects. MSCs are an effective treatment for LPL deficiency in cats, and probably have translational application in human patients with this condition, given the observed decrease in plasma turbidity and improvement in quality of life. The LPL-/- cat is a very powerful and robust animal model for studying cytokinetics and cytotherapeutics. Patients with a naturally occurring disease, such as feline lipoprotein lipase deficiency, provide ideal models for translating stem cell therapies into human patients.

W1006

ERAP1-MEDIATED GENERATION OF STNFR UNDER HYPOXIC CONDITION CONTRIBUTES TO THE CARDIO PROTECTIVE EFFECT OF MSCs

Seo, Hyang-Hee[1], Lee, chang Youn[2], Lee, Jiyun[3], Lim, Kyu Hee[4], Kim, Hye-Min[2], Shin, Sunhye[2], Kim, Sang Woo[5,6], Choi, Eunhyun[5,6], Lim, Soyeon[5,6], Lee, Seahyoung[5,6], Park, Jong-Chul[1,7] and Hwang, Ki-chul[5,6], [1]Brain Korea 21 PLUS project for Medical Science, Yonsei University, Seoul, Korea, [2]Department of Integrated Omics for Biomedical Sciences, Yonsei University, Seoul, Korea, [3]Brain Korea 21 PLUS project for Medical Science, Yonsei University, Seoul, Korea, South, [4]Department of Veterinary Physiology, Chonbuk National University, Jeonju, Korea, [5]Institute for Bio-Medical Convergence, Catholic Kwandong University, Gangneung, Korea, [6]Catholic Kwandong University International St. Mary's Hospital, Incheon, Korea, [7]Department of Medical Engineering, Yonsei University, Seoul, Korea

One of the mechanisms of how MSC exerts beneficial effect is using paracrine pathway. We have previously demonstrated that MSC culture conditioned media prepared by culturing MSCs under hypoxic condition improved the function of damaged heart. Nevertheless, the underlying mechanism, or identity of soluble factors responsible for the beneficial effect of the transplanted MSCs remains to be elucidated. Therefore, identifying the factors mediating in the reported cardio-protective effect of MSC of its conditioned medium will further help us to develop optimized and more effective MSC-based therapeutic strategies. Among many soluble factors increased during cardiac insult such as myocardial infarction, tumor necrosis factor (TNF) is known to be a critical mediator of range of biological processes including inflammation, immune-regulation, cytotoxicity, antiviral actions and transcriptional regulation. In ischemic heart, TNF contributes to cardiac dysfunction by binding to its respective receptor TNFR. Thus, deterring TNF-TNFR binding may effectively inhibits TNF signaling and subsequent cardiac damage induced by it. Regarding negative regulation of TNF signaling, it has been reported that soluble TNF receptor (sTNFR) generated by proteolytic cleavage of TNFR acted as a decoy for TNF suppressing TNF-TNFR signaling. This proteolytic cleavage of TNFR is known to be mediated by endoplasmic reticulum aminopeptidase 1 (ERAP1), and hypoxia-driven increase of its expression has been reported. Therefore, we hypothesized that MSC exposed to hypoxic environment promotes the generation of sTNFR by increasing the expression of ERAP1, and this subsequently protects host cells (i.e cardiomyocytes) by suppressing TNF signaling pathway. As a proof of concept study, we examined the expression of ERAP1 in MSCs under hypoxic condition and measured the amount of sTNFR produced by MSCs exposed to hypoxic condition. Our data show that both are upregulated under hypoxic condition and such increase was abrogated by the treatment of siRNA specific to ERAP1. Our study demonstrates that hypoxic condition induces ERAP1 expression in MSCs and strongly suggest that the MSC-released sTNFR under hypoxic condition is one of the important factors that facilitate the reported cardiac protective effect of MSCs.

W1008

ADIPOSE DERIVED STEM CELLS DISPLAY HIGHER RESISTANCE TO STRESS INDUCED INJURY AND BETTER REGENERATIVE CAPACITIES COMPARED TO BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

El-Badawy, Ahmed, Amer, Marwa and El-Badri, Nagwa, Center of Excellence for Stem Cells and Regenerative Medicine (CESC), Zewail City of Science and Technology, 6th October City, Egypt

Bone marrow mesenchymal stromal cells (BM-MSCs) are the most widely used MSCs, in cell therapies because of their abundance, easy propagation, and lack of ethical concerns in their applications. Recently, adipose tissue has emerged as a viable source for stem cells since it contain approximately a 500-fold higher frequency of adipose stem cells (ASCs) and tissue collection is simple and accessible. In this study we evaluated the regenerative capacities of ASCs compared to BM-MSCs both in vivo and in vitro. Both BM-MSCs and ASCs were evaluated for their abilities to ameliorate stress-induced injury both in vivo and in vitro. This was measured in vivo by improving hind limb ischemia, and in vitro by, vascular tube formation, telomerase activity, and qPCR analysis. Our data demonstrate that ASCs were more effective than BM-MSCs in promoting angiogenesis and neovascularization in an animal model of hind-limb ischemia. When exposed to severe hypoxia, ASCs showed more resistance to hypoxia-induced apoptosis compared to BM-MSCs as

measured by Annexin-V and Propidium Iodine staining. When exposing both cell types to a high dose of 600 μM H_2O_2, ASCs displayed more resistance to oxidative stress-induced senescence than BM-MSCs. ASCs showed more potent proangiogenic activity than BM-MSCs when measured by vascular tube formation assay ($p < 0.005$). Furthermore, ASCs showed a remarkably higher telomerase activity than BM-MSCs ($p < 0.005$). When visualized by confocal microscopy, ASCs displayed a well-organized network of F-actin filaments and a higher colocalization of α-tubulin when compared to BM-MSCs. mRNA expression analysis using quantitative real-time PCR showed that ASCs had a higher expression of Oct4 and VEGF than BM-MSCs ($p < 0.01$), indicating that they have higher pluripotency potentials. However, MMPII expression was higher in BM-MSCs. These data provide strong evidence for superiority of ASCs compared to marrow MSCs as a source of stem cells for regenerative therapies.

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W1010

EFFECT OF MESENCHYMAL STROMAL CELLS (MSC) CARRYING INTERLEUKIN-10 (IL10) OR HEPATOCYTE GROWTH FACTOR (HGF) IN EXPERIMENTAL ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

Islam, Diana[1,2], Grassi, Alice[2], Li, Manshu[2], Khang, Julie[2], Zheng, Junbo[2], Mckillop, Monty[3], Medin, Jeffrey[3], Laffey, John[2], Slutsky, Arthur[2] and Zhang, Haibo[2], [1]University of Toronto, Toronto, ON, Canada, [2]St. Michael's Hospital, Toronto, ON, Canada, [3]Medical College of Wisconsin, Milwaukee, WI, U.S.

Acute respiratory distress syndrome (ARDS) is a complex disorder with high mortality. ARDS is caused by direct or indirect injurious insult to the lung leading to acute inflammation, alveolar barrier disruption, edema and respiratory failure. Most ARDS non-survivors suffer complications of pulmonary fibrosis. MSC delivery in experimental models of endotoxin-induced ARDS demonstrates anti-inflammatory effects. However its role in fibrosis remains unknown. In a mouse model of ARDS induced by intratracheal instillation of hydrochloric acid (HCl), we transduced mouse bone marrow derived MSCs with the gene of the human anti-inflammatory cytokine IL-10 and the anti-fibrotic growth factor HGF using lentivirus (LV). MSC surface markers remained unchanged after LV transduction. Two days after HCl instillation, the mice were divided to receive 1) Vehicle control solution (PBS); 2) MSC; 3) MSC+Luiferase (LV control); 4) MSC+IL-10; 5) MSC+HGF; or 6) MSC+IL-10/MSC+HGF (50% mixture of IL-10 and HGF transduced MSC). The MSCs were delivered via intratracheal and intravenous routes (0.5×10^6 cells each), mice were sacrificed at day 7. Lung injury and inflammation was accessed using histological scoring and differential cell count of bronchioalveolar lavage fluid (BALF). Lung fibrosis was accessed using histological score, fibronectin and fibrinogen levels. Bioluminescence imaging showed persistent MSC localization in the lung up to 7 days after transplantation. Using ELISA hIL-10 and hHGF production was detected in lung tissue and BALF up to 7 days. After HCl-induced injury, MSC alone or MSC+Luciferase did not ameliorate inflammation or fibrosis. However the delivery of MSC+IL10, MSC+HGF or their combination reduced inflammatory responses, the expression of fibrotic markers and fibrotic scores as compared to MSC alone. Our data suggest that MSC overexpressing hIL-10 or hHGF may augment MSC response to lung injury and be protective by supressing both inflammation and pulmonary fibrosis. This novel approach may have therapeutic potential in the management of ARDS.

W1012

HEPARAN SULFATE GLYCOSAMINOGLYCANS: PROMISING BIOMATERIALS FOR STEM CELL SCALE-UP

Ling, Ling[1], Xue, Cao[2], Hassan, Afizah[2], Lee Hui Ching, Michelle[3], Hui, James[2], Raghunath, Michael[3], Nurcombe, Victor[1] and Cool, Simon[1], [1]Institute of Medical Biology, Singapore, Singapore, [2]NUHS, Singapore, Singapore, [3]NUS, Singapore, Singapore

Human mesenchymal stem cells (hMSCs), central to much cell-based therapy, are currently being rigorously assessed in clinical trials. A key challenge to their widespread application is their low abundance in native tissues, which in turn demands strategies to enhance their ex vivo expansion without compromising their stemness. This study explored a novel scale-up strategy that relies on supplementation of a subfraction of the glycosaminoglycan heparan sulfate (HS) into the culture environment. HS, a linear sugar abundant in extracellular matrix, is known to regulate the activity of many growth factors, most notably fibroblast growth factor 2 (FGF2). FGF2 increases hMSC proliferation and is widely used as a stem cell culture adjuvant. Using affinity chromatography, we have isolated an HS variant (HS8) specifically targeted to FGF2. HS8 was made in a cGMP facility and QA/QC tests were performed. ELISA demonstrated that HS8 bound to FGF2 with much greater affinity than to other heparin-binding factors (e.g. PDGF or VEGF). HS8 markedly increased the melting temperature of FGF2, indicating HS8 acts to stabilize FGF2 and prolong its activity. Both FGF2-stimulated ERK signaling and proliferation were amplified by HS8 within hMSCs. Upon meeting these QA release criteria, and passing sterility & toxicity tests, HS8 was used in a GMP-compliant facility as a culture supplement to expand hMSCs freshly isolated from the bone marrow of healthy human donors. Cumulative cell growth

was monitored and the continuing quality of stem cells assessed in vitro for colony-forming efficiency (CFU-F), surface antigen expression, telomere length and multipotency. Our data clearly shows that HS8 enhances hMSC growth over 4 passages compared to control. Flow cytometry analysis revealed increased levels of STRO-1, a key hMSC biomarker, and that the expression of other stem cell markers remained unchanged. CFU-F assays and multilineage differentiation analysis confirmed that hMSCs cultured exposed to HS8 for 4 passages retained a comparable proportion of stem cells compared to the control. Our work suggests that this HS is a promising vehicle for the stem cell scale-up needed to meet the burgeoning clinical need.

W1014

IDENTIFICATION OF A DISTINCT SUBPOPULATION OF BONE MARROW MESENCHYMAL STEM CELLS THAT DIRECTLY CONTRIBUTES TO LIVER REGENERATION

Nishizuka, Satoshi S.[1], Katagiri, Hirokatsu[1], Kushida, Yoshihiro[2], Takahara, Takeshi[1], Nitta, Hiroyuki[1] and Dezawa, Mari[2], [1]Iwate Medical University School of Medicine, Morioka, Japan, [2]Tohoku University Graduate School of Medicine, Sendai, Japan

Although the liver has high regenerative potential, the mechanism of regeneration is not yet fully understood. Living donor liver transplantation (LDLT) is one of the best opportunities to evaluate the contribution of extrahepatic cells to liver regeneration, and has provided information on the involvement of the recipient's extrahepatic cells. LDLT is a surgical procedure in which a living donor undergoes physical partial hepatectomy (PPHx), the transected liver is then transplanted into a recipient body, and the graft subsequently undergoes the process of liver regeneration. By genotyping graft livers after human living-donor liver transplantation, we revealed in this study the presence of chimeric genotypes in hepatocytes (35%), sinusoidal cells (95%), cholangiocytes (70%), and cells in the periportal areas (50%), suggesting the involvement of extrahepatic cells. In the present study, we focused on a distinct type of bone marrow-derived cells, multilineage-differentiating stress-enduring (Muse) cells, which reside in the bone marrow and are a subpopulation of bone marrow-mesenchymal stem cells (BM-MSCs), because of their triploblastic differentiation and tissue repair abilities. Muse cells can be efficiently isolated as cells that are positive for SSEA-3, while also expressing other pluripotency markers, including Oct3/4, Sox2, and Nanog. When human Muse cells were infused into immunodeficient mice with a PPHx using a harmonic scalpel, they were integrated into the regenerating areas of the liver, expressed liver progenitor markers during the early phase, and then differentiated into the major liver component cells, including hepatocytes (~75% of integrated cells), cholangiocytes (~17%), sinusoidal endothelial cells (~2%) and Kupffer cells (~6%). Non-Muse cells in human BM-MSCs were not detected in the liver for up to 4 weeks by either immunohistochemistry or species-specific DNA analysis. Therefore, we suggest that Muse cells are a population of extrahepatic cells that participate in regeneration of the liver tissue. While the pleiotropic actions of BM-MSCs are considered useful for general regenerative medicine applications, our results suggest that efficient use of BM-Muse cells may improve the effectiveness of current BM-MSC transplantation techniques for liver diseases.

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W1016

ANTI-TUMORAL EFFECT OF LOW-DOSE GAMMA-IRRADIATED MOUSE BONE MARROW-DERIVED MESENCHYMAL STROMAL CELLS

Stefani, Francesca Romana, Eberstål, Sofia and Bengzon, Johan, Lund University, Lund, Sweden

Glioblastoma multiforme is the most common and aggressive primary malignant brain tumor in adults and the prognosis remains very poor despite the current standard of care. Bone marrow-derived multipotent mesenchymal stromal cells (MSCs) target glioma metastases efficiently when implanted intra-tumorally and recent findings demonstrate that MSCs can polarize into immune stimulatory cells when exposed to appropriate stimuli. Thus, in the present study we investigated if low-dose γ-irradiation could induce MSCs with an immune stimulatory phenotype and inhibit brain tumor growth in vivo. Gliomas were established by transplanting GL261 mouse glioma cells into the syngeneic mouse strain C57BL/6. Mouse MSCs were isolated from the bone marrow, characterized (plastic adherence, surface markers expression, tri-lineages differentiation) and cultured according to standard techniques. For the survival study, γ-irradiated MSCs (0/2/5/10/15/20 Gy) were injected intra-tumorally in tumor-bearing mice at day 7 and 17 and the animals followed for 100 days after tumor challenge. Flow cytometry was used to analyze the systemic immune response and immunohistochemistry to study the effect at the tumor site. Our in vivoresults show that intra-tumorally transplanted MSCs enhance the cure rate of GL261 tumor-bearing mice. Groups were compared to the tumor-bearing control and the 5 Gy radiation dose showed to highest survival rate (28.6% cure rate). No statistical difference was detected when MSCs were irradiated with 20 Gy or non-irradiated (0% cure rate). The analysis of peripheral blood revealed an increased amount of $CD4^+$ T cells and

a decrease in the myeloid-derived suppressor cell population after treatment with MSCs 5 Gy. The same trend was seen at the tumor site where the increased number of $CD4^+$T cells is further accompanied by a decreased amount of vessels within the tumor in the treated group. The increased survival of tumor-bearing mice suggests that low-dose γ-irradiated MSCs have anti-tumoral activity and their mechanism may involve both immune system and angiogenesis, as shown by the effect on T cells and vessels formation. Low-dose γ-irradiated MSCs may represent a favorable alternative approach in cancer therapy. This strategy is straightforward and can be easily be part of a combination therapy.

W1018

ENHANCED IMMUNOSUPPRESSION OF T-CELLS BY SUSTAINED PRESENTATION OF BIOACTIVE IFN-γ WITHIN 3D MESENCHYMAL STEM CELL CONSTRUCTS

Zimmermann, Josh[1,2], Hettiaratchi, Marian[2] and McDevitt, Todd[1,3], [1]Gladstone Institute of Cardiovascular Disease, San Francisco, CA, U.S., [2]Georgia Tech & Emory University, Atlanta, GA, U.S., [3]University of California - San Francisco, San Francisco, CA, U.S.

Mesenchymal stem/stromal cell (MSC) therapies are currently being explored clinically due to their ability to regulate a myriad of inflammatory responses. However, MSC immunomodulatory activity is dependent upon the local microenvironment where inflammatory cytokines, such as interferon gamma (IFNγ), induce MSC expression of potent immunosuppressive factors. Although MSC therapies modulate acute inflammation in the presence of high concentrations of inflammatory cytokines, MSCs are less effective at suppressing immune responses during chronic inflammation, presumably due to attenuated concentrations of cytokines. To address this limitation, pre-treatment of MSCs with IFNγ prior to delivery has been used to induce indoleamine dioxygenase (IDO) expression, a key factor for suppression of T-cell activation. However, pre-treatment effects are transient and may not induce sustained MSC immunomodulatory activity in weak inflammatory environments. Therefore, the objective of this study was to investigate whether microparticle (MP) mediated presentation of bioactive IFNγ within spheroidal MSC aggregates could induce sustained immunomodulatory activity as an alternative means of inducing MSC immunomodulation irrespective of an ill-defined inflammatory milieu. Delivery of IFNγ via heparin MPs induced sustained IDO expression (~10^4 fold compared to untreated MSC spheroids) over one week, whereas IDO expression of IFNγ pre-treated MSC spheroids rapidly decreased within two days. In MSC co-cultures with CD3/CD28 activated peripheral blood mononuclear cells (PBMCs), delivery of IFNγ loaded MPs also increased and prolonged suppression of T-cell activation and proliferation compared to pre-treated MSC spheroids (~30% proliferating T-cells in cultures with MSC spheroids with MPs after 8 days vs. ~60% proliferating T-cells with pre-treated MSC spheroids). Furthermore, IDO inhibition with 1MT impaired the ability of MSC spheroids with MPs to suppress T-cell activation, confirming that increased T-cell suppression was dependent on induction of IDO. Altogether, biomaterial-based engineering of MSC microenvironments can specifically direct the immunomodulatory activity of transplanted cells and may improve the efficacy of MSC-based therapies for treating inflammatory and immune diseases.

W1020

NETRIN-1 INDUCES SP1-MEDIATED VEGF EXPRESSION VIA LIPID RAFT-MEDIATED INTEGRIN α6β4 SIGNALING IN PROMOTING HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELLS PROLIFERATION

Han, Ho Jae, Lim, Hyeon Su, Lee, Sei-Jung, Kim, Jun Sung, Oh, Ji Young, Kim, Dah Ihm, Song, Eun Ju and Onphachanh, Xaykham, Seoul National University, Seoul, Korea, South

Netrin-1 (Ntn-1), a multifunctional neuronal signaling molecule; however, the functional role of Ntn-1 which improves stem cell therapy has not been characterized. In the present study, we investigate the mechanism of Ntn-1 with regard to cytoskeletal reorganization and cell proliferation of mesenchymal stem cells (MSCs) during the processes of mouse wound healing and vascular regeneration. In a mouse skin excisional wound model, we found that transplantation of human umbilical cord blood derived (hUCB)-MSCs pre-treated with Ntn-1 enhanced wound closure, granulation, and re-epithelialization at mouse skin wound sites, where relatively more angiogenesis with newly formed vessels onto the wound site were detected. Ntn-1 significantly increased the F-actin reorganization and cell proliferation of hUCB-MSCs, which had been inhibited by the blocking antibody for integrin (In) α6β4, but not deleted in colorectal cancer (DCC). We found that Ntn-1 acting through Inα6β4 stimulated the c-Src-dependent activation of Rac1 during actin cytoskeleton remodeling and cell proliferation of hUCB-MSCs. Cytoskeletal reorganization evoked by Ntn-1 is linked to the increased level of Arp2/3, profilin-1, p- cofilin-1, and F-actin in hUCB-MSCs. On the other hand, Ntn-1 induced the recruitment of NADPH oxidase 2 and neutrophil cytosolic factor 1 into the membrane lipid rafts coupled with Rac1 to facilitate the production of reactive oxygen species (ROS) in promoting the cell proliferation rather than the cytoskeletal reorganization process. Interestingly, Ntn-1 has ability to induce cell proliferation by enhancing the region-specific transcriptional occupancy by SP1 in the VEGF promoter, which was significantly down-regulated

by the knockdown of the PKCα. Finally, we verified that transplantation of hUCB-MSCs pre-treated with Ntn-1 enhanced blood perfusion ratio and vascular regeneration in a mouse hindlimb ischemia model. These results demonstrate that Inα6β4 signaling triggered by Ntn-1 regulates c-Src phosphorylation to stimulate Rac1 activation, which is responsible for reorganization of the actin cytoskeleton and cell proliferation of hUCB-MSCs. Ntn-1 distinctively induced the transcriptional regulation of SP1 in VEGF promoter through lipid raft-dependent ROS signaling to promote cell proliferation.

MESENCHYMAL STEM CELL DIFFERENTIATION

W1022

MESENCULT™ OSTEOGENIC DIFFERENTIATION MEDIUM – AN OSTEOGENIC STIMULATORY MEDIUM FOR THE EFFICIENT DIFFERENTIATION OF HUMAN MESENCHYMAL PROGENITOR CELLS

Sampaio, Arthur V[1], Duronio, Chris[1], Wagey, Ravenska[1], Thomas, Terry E.[1], Eaves, Allen C.[1,2], Szilvassy, Stephen J[1] and Louis, Sharon A.[1], [1]STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Mesenchymal Stem and Progenitor Cells (MSCs) can be derived and expanded from bone marrow (BM) using a variety of media; however, the choice of medium and protocol can impact the ability of the resulting MSCs to subsequently differentiate into specific lineages. To overcome this variability, MesenCult™ Osteogenic Differentiation Medium (MODM) was developed to facilitate consistent evaluation of the osteogenic potential of BM MSCs derived and expanded in MesenCult™-FBS (serum), -XF (Xeno Free), and -ACF (Animal Component Free) media, media containing human platelet lysate (hPL), or other commercial MSC expansion media. Human BM mononuclear cells were derived and cultured in these media for up to 5 passages, and the MSC fraction harvested at passages 2 and 5 and reseeded into the same medium at 1 X 10^4 cells/cm^2. When cultures reached ~90% confluence, osteogenic differentiation was induced by replacing the medium with either MODM, or a popular commercial osteogenic medium as a positive control (+ctrl), or maintained in expansion medium as a negative control (-ctrl). Cultures were kept for up to 15 days with media changed every 3 days. Differentiation was assessed by histological analysis at days 5, 10, and 15 (n=3). Alkaline Phosphatase (ALP) activity and bone mineral content were determined by Naphtol AS-MX/Fast Red and von Kossa staining, respectively. In general, MSCs differentiated with MODM exhibited the highest levels of ALP activity, even compared to +ctrl cultures, and this was detected as early as day 5. The only exception was for cultures that were initially expanded in MesenCult™-XF, in which ALP activity was as robust as in MSCs that were differentiated in +ctrl medium. Similarly, bone mineral content was highest in cultures differentiated with MODM and was observed as early as day 10. Indeed, cultures differentiated with the +ctrl medium had little mineral content by day 15, suggesting that this medium was insufficient to induce robust differentiation. Again the only exception was in cultures expanded in MesenCult™-XF, where bone mineral content was as high as in the +ctrl cultures. In summary, MesenCult™ Osteogenic Differentiation Medium is a superior medium for studies of MSC bone lineage commitment as it induces robust differentiation of MSCs previously expanded in various media in as little as 15 days.

W1024

IN VITRO ADIPOGENIC DIFFERENTIATION OF HUMAN ADIPOSE STEM CELLS UNDER SIMULATED MICROGRAVITY

Elcin, Y. Murat[1], Subak, Erdem[1], Seker, Sukran[1] and Elcin, A. Eser[2], [1]Ankara University Faculty of Science, and Ankara University Stem Cell Institute, Tissue Engineering, Biomaterials and Nanobiotechnology Laboratory, Ankara, Turkey, [2]Ankara University Stem Cell Institute, Ankara, Turkey

The concept of culturing cells under microgravity conditions has come into question during space research. It is known that microgravity conditions lead to a sharp decrease in mechanotransductive forces, while cell-medium interactions increase and 3D tissue organoids resembling living tissues can be formed. MSC spheroid cultures have greater differentiation ability, and enhanced anti-inflammatory and immunomodulatory properties compared with their 2D counterparts. Besides they can be used on clinically relevant scale-up productions. There is evidence on depression of osteogenesis under osteogenic microgravity conditions while adipogenesis is stimulated. However the effects of adipogenic microgravity conditions on adipose stem cells (ASCs) have not been comprehensively investigated. The aim of this study was to comparatively evaluate adipogenic differentiation of human ASCs in a rotating wall vessel bioreactor simulating microgravity conditions, and in standard 2D culture. Plastic-adherent cells were isolated from the adipose tissue of adult patients collected during elective operations under ethical approval. ASCs were expanded in DMEM-LG containing 10% FBS, Pen-strep, 1% L-glutamine at 37°C, 5% CO_2-95% air. Spheroids were formed inside STLV vessels. Flow cytometric analysis was performed to determine ASC immunophenotype. Adipogenic induction was carried out by using a two-step differentiation protocol: (i) 3 days of culture in DMEM-HG supplemented with 10% FBS, Pen-strep, 1% L-Glu, 1 μM dexamethasone, 0.5 μM IBMX, 10 μM

insulin; (ii) then, another 3 days of culture in DMEM-HG supplemented with 10% FBS, Penstrep, 1% L-Glu, 1 μM dexamethasone, 0.5 μM IBMX, 20 μM insulin, either with or without 0.5 mM linoleic acid (maturation medium). Adipogenesis was investigated using histochemistry, immunohistochemistry and PCR methods. Differentiation into preadipocytes and adipocytes was assessed by evaluating changes in marker expression levels. Thus, useful information was obtained to elucidate fate decisions of human ASCs under adipogenic 3D simulated microgravity and 2D culture conditions.

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W1026

ISOLATION OF MESENCHYMAL STEM CELLS DERIVED FROM HUMAN CRANIAL BONE MARROW

Imura, Takeshi[1], Nakagawa, Kei[1], Otsuru, Naofumi[1], Tomiyasu, Mayumi[1], Otsuka, Takashi[1], Baba, Tatsuya[1], Kawahara, Yumi[2], Shinagawa, Katsuhiro[3], Takahashi, Shinya[4], Sueda, Taijiro[4], Kurisu, Kaoru[3] and Yuge, Louis[1,2], [1]Bio-Environmental Adaptation Sciences, Graduate School of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan, [2]Space Bio-Laboratories Co., Ltd., Hiroshima, Japan, [3]Department of Neurosurgery, Graduate School of Biomedical & Health Science, Hiroshima University, Hiroshima, Japan, [4]Department of Cardiovascular Surgery, Graduate School of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan

Cell based therapy using mesenchymal stem cells (MSCs) has been attracting attention as a novel treatment for central nervous disorders such as stroke and spinal cord injury. Recently, we reported that MSCs derived from cranial bone marrow (cMSCs) have high neurogenic potential compared with MSCs derived from iliac bone marrow. However, cranial bone has less bone marrow relative to other tissue such as iliac or femoral bone. Therefore, original efficient isolation method of cMSCs was required. Here, we consider several methods to isolate cMSCs. Cranial bone marrow samples were obtained from front-temporal cranial bone waste following neurosurgical procedures after informed consent, according to the Hiroshima University hospital's guidelines. We tried to isolate cMSCs by three different methods at the same time. The characteristics of each isolated cMSCs were analyzed using proliferation curve and multi-lineage cell differentiation including neural differentiation, osteogenic differentiation, and adipogenic differentiation. For differentiation analysis, immunostaining (neurofilament) and histological staining (Alizarin red S staining and Oil red O staining) were examined. This study was approved by the Hiroshima University ethical comittee. In results, cMSCs could be isolated by all three isolation methods. There are no any differences among cMSCs isolated by three different isolation methods. These results provide efficient isolation from limited cranial bone tissue, which will be useful for preparation of graft cells in clinical application.

W1028

MODULATION OF OSTEOBLASTIC DIFFERENTIATION OF BONE STEM CELLS IN RAT BY A NOVEL OSTEOPROTECTIVE ACTIVITY IN ORMOCARPUM COCHINCHINENSE LEAF EXTRACT

Kumaran, R. Ileng[1], Daniel, Winnie[2], Satyanarayana, Ramya[2], Krishnamoorthy, Senthilkumar[2], Sadhasivam, Balaji[2], Ramadoss, Sivakumar[3], Bhaskaran, Ravi Sankar[2], Queimado, Lurdes[4], Aruldhas, Mariajoseph Michael[2], Narasimhan, Srinivasan[5] and Ramachandran, Ilangovan[2], [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, U.S., [2]Department of Endocrinology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai, India, [3]Department of Obstetrics and Gynecology, David Geffen School of Medicine at UCLA, Los Angeles, CA, U.S., [4]Departments of Otorhinolaryngology, Cell Biology and Pediatrics, Oklahoma Tobacco Research Center and The Peggy and Charles Stephenson Cancer Center, The University of Oklahoma Health Sciences Center, Oklahoma City, OK, U.S., [5]Tissue Engineering & Regenerative Medicine, Faculty of Allied Health Sciences, Chettinad Academy of Research and Education, Kelambakkam, Chennai, India

Osteoporosis is a metabolic disorder associated with a high risk of bone fractures, increased morbidity and mortality in women. Currently available therapies for osteoporosis have various side effects. In this context, the leaf extract of the plant Ormocarpum cochinchinense (OC) has been reported to heal bone fractures in traditional medicine. However, the molecular mechanisms of OC-mediated effects on osteoblast differentiation from bone stem cells, and bone remodeling during fracture healing are largely unexplored. Therefore, in our current study, we have assessed the effects OC extract on bone stem cell differentiation and bone turnover. For this, we induced unilateral fractures in the femur of adult female Wistar rats using standard ethical procedures. Then, OC (200 mg/kg body weight) was administered orally, every day, for a period of 21 days. After that, the serum levels of bone formation marker [alkaline phosphatase (ALP)], resorption marker [tartrate-resistant acid phosphatase (TRAP)], and calcium and phosphorus were quantified using standard methods. Moreover, computed tomography (CT) scan of the femur was performed to examine fracture healing. In

addition, the expression of critical genes, such as Runx2/Cbfa1, a master regulator of osteoblastic differentiation, and bone morphogenetic proteins (BMPs) were assessed by real-time RT-PCR and Western blot analysis using the callus collected from the fracture site. Our results show that OC significantly increased the serum ALP activity (bone formation marker) and decreased the TRAP activity (bone resorption marker) during fracture healing. Most importantly, the extract of OC modulated the expression of master genes (Runx2/Cbfa1 and BMPs) that control osteoblastic differentiation of bone stem cells. Furthermore, the effects of OC extract on mesenchymal stem cells are also being examined. Our findings demonstrate for the first time that the extract of OC exerts its osteoprotective effect and facilitates bone fracture healing by regulating the osteoblastic differentiation of bone stem cells.

W1030

EFFECTS OF GRAPHENE 2D CELL CULTURE SURFACES ON THE DIFFERENTIATION POTENTIAL OF HUMAN OR RAT ADIPOSE STEM CELLS (ASC)

Siennicka, Katarzyna[1], Aleksandra, Zolocinska[1], Alicja, Bachmatiuk[2], Juliusz, Wysocki[1], Trzebicka, Barbara[2], Janik-Kosacka, Karolina[1] and **Pojda, Zygmunt**[1], [1]Oncology Center-Institute, Warsaw, Poland, [2]Centre of Polymer and Carbon Materials PAS, Zabrze, Poland

Graphene consists of carbon molecules organized into single atomic sheet. Mechanical properties make graphene one of the thinnest, lightest and durable materials available for medical purposes. The additional possible advantage for regenerative medicine applications , especially concerning nerve system or heart may be the electrical conductivity of graphene sheets. The aim of the study was to test the graphene biocompatibility of adipose stem cells (ASCs) cultured and differentiated on graphene-coated glass. Rat or human ASCs were enzymatically extracted from adipose tissue, in vitro expanded until 3rd passage and seeded on the graphene coated glass slides. Subsequently cells were cultured in DMEM + 15%FCS until reaching the 75% confluence phase, at this stage the medium was replaced by osteogenic differentiation medium (LONZA, Cat. No PT-3002) and cultured for 3-4 wks. Finally, slides were stained with Alizarin Red and quantitative analysis of stain deposits was performed. The proliferative potential of human and rat ASCs cultured on glass surface (controls) was comparable to those cultured on graphene, similarly there were no statistic significant differences in CFU-F frequencies. The osteogenic differentiation potential on graphene layers was similar or higher when compared to control cultures. It may be concluded, that graphene biocompatibility with ASCs allows for the construction of scaffolds consisted partially from this material. Cells growing on graphene surfaces have normal or enhanced osteogenic potential, allowing for the conclusion of the specific usefulness of graphene as a composit for scaffold constructions for bone regeneration.

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W1032

DIFFERENTIATION OF C-KIT+ MESENCHYMAL STEM CELLS TOWARD ENDOTHELIAL CELLS AFTER TRANSPLANTATION INTO THE ISCHEMIC LIMB

Wang, Hai-jie, Shanghai Medical School of Fudan University, Shanghai, China

Resent preclinical studies and clinical trails prove that transplantation of mesenchymal stem cells (MSCs) is a promised therapy for ischemic diseases such as critical limb ischemia resulted from diabetes. However, the differential potential of c-kit$^+$ cells in MSCs remains unclear. This investigation was designed to examine differentiation of c-kit cells after transplantation into the ischemic limb. c-kit$^+$ cells were isolated from rat marrow-derived MSCs and expanded with SCF. After induction with VEGF, MyoD, TGF-b or BMP-2, differentiation of c-kit$^+$ cells toward endothelial cells and myogenesis of the cells was determined with CD31, cTnT and α-SMA immunostaining respectively. The model of rat hind-limb ischemia was established by ligation of the femoral artery. Rats were divided into PBS, c-kit$^+$ cell group and c-kit$^+$ cell + EPC (endothelial progenitor cells) group. EPCs were isolated from rat marrow. GFP-labelled c-kit$^+$ cells were transplanted by intramuscular injection in the ischemia limb. At three weeks after cell transplantation, blood flow of the ischemia limb was evaluated with a laser Doppler perfusion image analyzer. Formation of the collateral vessels was analyzed by angiography. To determine angiogenesis and myogenesis, expression of CD31, α-SMA or dystrophin in the engrafted cells was examined with immunostaining. After induction with VEGF, MyoD, TGF-b or BMP-2, c-kit$^+$ cells differentiated toward endothelial cells and muscle cells. After transplantation, blood perfusion, formation of collateral vessels and density of the microvessels in c-kit$^+$ cell group increased significantly. Combination of c-kit$^+$ cell and EPC transplantation enhanced blood perfusion and angiogenesis in the ischemic limbs. Some GFP-labelled c-kit$^+$ cells expressed CD31 or α-SMA. GFP$^+$CD31$^+$ cells and GFP$^+$α-SMA$^+$ cells incorporated into the new-formed microvessels. These results demonstrate that MSC-derived c-kit$^+$ cells have potential to differentiate toward endothelial cells and vascular smooth muscle cells. After transplantation, c-kit$^+$ cells promote angiogenesis and improve blood perfusion of the ischemic limb effectively.

MESENCHYMAL CELL LINEAGE ANALYSIS

W1036

ROLE OF NOTCH SIGNALING IN THE MAINTENANCE OF HUMAN MESENCHYMAL STEM CELLS UNDER HYPOXIC CONDITIONS.

Moriyama, Hiroyuki[1], Moriyama, Mariko[2] and Hayakawa, Takao[2], [1]Kindaii University, Higasi-Osaka, Japan, [2]Kindai University, Higashi-Osaka, Japan

Human adipose-derived mesenchymal stem cells (hADSCs) are an attractive material for cell therapy and tissue engineering because of their multipotency and ease of availability without serial ethical issues. However, their limited lifespan in in vitro culture system hinders the therapeutic applications of hADSCs. Some somatic stem cells including hADSCs are are known to be localized in hypoxia regions. These stem cells exhibit a high level of glycolytic metabolism despite the presence of high oxygen, and further increase their glycolysis rate under hypoxia. However, physiological role of glycolytic activation and its regulatory mechanisms is still incompletely understood. Here we show that Notch signaling is required for glycolysis regulation under hypoxic condition. Our results demonstrate that hypoxia dramatically increased the glycolysis rate, improved the proliferation efficiency, prevented the senescence, and maintained the multipotency of hADSCs. These effects were mediated by Notch signaling pathway. Hypoxia significantly increased the level of activated Notch1 and expression of its downstream gene, HES1, in a Hypoxic Inducible Factor-independent manner. Furthermore, hypoxia markedly increased glucose consumption and lactate production of hADSCs, which decreased back to normoxic levels upon treatment with a γ-secretase inhibitor and knockdown of HES1 expression. We also found that Notch signaling was involved in induction of SLC2A1, SLC2A3, TPI, PFKFB3, and PGK1 in addition to reduction of TIGAR and SCO2 expression. In addition, Notch signaling suppressed p53 activation, which in turn activated glycolysis. Furthermore, we also found that Notch signaling was involved in the induction of GLUT3 and TPI expression through activation of NF-κB signaling. Finally, a glycolysis inhibitor attenuates the proliferation rate of hADSCs, 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 hADSCs. These results clearly suggest that Notch signaling regulates glycolysis under hypoxic conditions and thus likely affects the cell lifespan via glycolysis.

W1038

COMPREHENSIVE MODELING OF CELL FATE IN MOUSE PANCREATIC EMBRYONIC MESENCHYME USING A SYSTEMS DEVELOPMENTAL BIOLOGY APPROACH

Buzzard, Joshua William[1], Swegal, Warren[2], Sears, Katie[1], Bukys, Michael[3], Leach, Steven[4] and Jensen, Jan[1], [1]Cleveland Clinic Lerner Research Institute, Cleveland, OH, U.S., [2]Henry Ford Hospital, Detroit, MI, U.S., [3]Cleveland Clinic, Cleveland, OH, U.S., [4]Memorial Sloan Kettering Cancer Center, New York, NY, U.S.

During embryogenesis, pancreatic mesenchyme is critical for pancreatic fate induction in the endoderm, and later serves important roles in organ homeostasis and regeneration. Investigating primary cultures of E13.5 pancreatic mouse embryonic fibroblasts (MEF^{panc}) and comparing such to similar cells obtained from liver, lung, stomach and gut using a combination of genomics and proteomics approaches, we concluded that MEF^{panc} cells represent unique, organ-specific mesodermal descendants. This is reflected by a unique secretome, which includes expression of morphogens such as FGF10 and Inhibin-bA (Activin). Furthermore, short term incubation of pluripotent cells with MEF^{panc}-conditioned media resulted in decreased pluripotency and an increase in endodermal gene expression and pancreatic marker induction. Because single cell analysis using flow cytometry indicated that primary MEF^{panc} cultures consisted of a heterogenous cell mixture, we explored the 'fate universe' of these cells using a novel, comprehensive developmental biology systems approach. Specifically, to map phenotypic states in MEF^{panc} cultures we utilized Design of Experiments Theory (DoE) and multivariate data analysis (MVDA). MEF^{panc} cultures were exposed to a perturbation matrix of thirteen different morphogens for 48 hours, and gene expression was measured over a large set of 53 phenotype-specific genes. Computational modeling and extraction of input logics for relevant genes allowed us to perform a cluster analysis which revealed a phenotypic landscape represented by the culture. The co-regulated gene clusters were shown to represent various pancreatic stromal states including pericytes, stellate cells, and endothelial cells. In addition, evidence suggested the presence of telocytes, a cell type sharing similarities to interstitial cells of Cajal in the intestine. Importantly these analyses highlighted the presence of a Wnt5a+/Inhibin A+ signaling state, and also a distinct, cMET+ population expressing Fgf7 and Fgf10 acting as a potential organ-specific niche supportive cell.

HEMATOPOIETIC CELLS

W1042

REGULATION OF HUMAN HSC SELF-RENEWAL BY THE TRANSCRIPTIONAL ELONGATION FACTOR MLLT3

Calvanese, Vincenzo[1], Nguyen, Andrew[1], Dou, Diana[2], Galic, Zoran[1] and Mikkola, Hanna K A[1], [1]UCLA, Los Angeles, CA, U.S., [2]University of California, Los Angeles, Los Angeles, CA, U.S.

Hematopoietic stem cells (HSC) have been used successfully to treat hematopoietic malignancies; however, the availability of HSCs and the spectrum of treatable diseases would increase greatly if they could be expanded in culture or derived from pluripotent stem cells (PSC). To improve HSC maintenance in culture, we sought for genes whose expression is enriched in the self-renewing human hematopoietic stem and progenitor cell (HSPC) population at all developmental stages. MLLT3, which has been identified as a component of the super elongation complex, enhancing RNA polymerase II elongation, was consistently enriched in the HSPC subset. shRNA-mediated MLLT3 depletion in human fetal liver HSPC caused loss of proliferative capacity in OP9 stroma co-culture, as well as impaired engraftment in NSG mice, indicating an essential role for MLLT3 in HSPC function. Conversely, MLLT3 overexpression in human HSC during prolonged stroma co-culture, which otherwise leads to decline in MLLT3 expression, enhanced the expansion of immunophenotypic HSPC. In addition, MLLT3 overexpression in human PSC-derived HSPC was sufficient to extend the maintenance of immunophenotypic HSPC in culture, and induce the expression of known self-renewal regulators such as HLF. Notably, MLLT3 overexpression in fetal liver HSPC did not sustain HSPC expansion in the absence of OP9 stroma, implying that MLLT3 overexpressing HSPC retain niche-dependence and do not acquire uncontrolled proliferative potential. Likewise, MLLT3 overexpression in non-self-renewing fetal CD34+CD38-CD90+GPI80- progenitors did not confer self-renewal properties, nor did it prevent differentiation, demonstrating a specific action in undifferentiated HSPC. ChIP-seq analysis of MLLT3 in human HSPC revealed binding to the transcriptional start site and proximal gene body region of several highly expressed genes, including early response genes JUNB, FOS and MYC, and known HSC regulators MEIS1, HOXA9 and BCL11A. This study identifies MLLT3 as a novel human HSC self-renewal regulator, whose impaired expression in culture contributes to the defective HSC maintenance in vitro. Future studies will define if enforced MLLT3 expression can help generate HSC for therapeutic use, and determine mechanistically how MLLT3 conducts the HSC self-renewal network.

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W1044

PERIVASCULAR STROMAL CELLS ARE THE ORIGIN OF BONE MARROW FIBROSIS AND PLAY CRITICAL ROLES IN THE PATHOGENESIS OF MYELOFIBROSIS

Ding, Lei, Decker, Matthew, Martinez-Morentin, Leticia, Wang, Guannan, Liu, Qingxue and Leslie, Juliana, Columbia University, New York, NY, U.S.

The bone marrow niche maintains hematopoietic stem cells (HSCs) and contributes to hematological disease development. Primary myelofibrosis is a clonal disease thought to originate from mutant HSCs. Its clinic features include myeloid proliferation, extramedullary hematopoiesis and bone marrow fibrosis (deposits of reticulin fiber). Most myelofibrosis patients are the elderly. Although HSC transplantation is the only curative treatment, the procedure-associated toxicity precludes most patients from this option. Thus, novel therapeutics is in great need. Recent advances have identified the critical role of hematopoietic activation of the MPL-JAK pathway for the pathogenesis of this disease. However, how activation of this pathway leads to bone marrow fibrosis and extramedullary hematopoiesis is not clear. Furthermore, the cells that generate reticulin fiber have not been identified. We have recently identified bone marrow leptin receptor+ (Lepr+) perivascular stromal cells as an important component of the HSC niche. Here we have characterized the niche in myelofibrosis and examined its contribution to disease progression. In myelofibrosis, HSCs over-proliferate and mobilize to extramedullary sites. We performed in vivo genetic fate mapping to investigate the cells that are responsible for generating reticulin fiber. We found that Lepr+ perivascular stromal cells undergo extensive proliferation and generate cells depositing reticulin fiber in the bone marrow. These perivascular stromal cells down-regulate critical niche factors and up-regulate genes involved in fibrogenesis, suggesting that perivascular stromal cells lose HSC supporting activity and gain fibrogenic potential. To identify pathways leading to fibrogenic cells, we conditionally deleted platelet-derived growth factor receptor a (Pdgfra) from these cells. This leads to blockage of bone marrow fibrosis. Our results demonstrate that perivascular stromal cells are the cells responsible for generating reticulin fiber. Their fibrogenic conversion and losing HSC niche function underlies a critical step in the pathogenesis of myelofibrosis. The identification of Pdgfra signaling as a critical pathway in bone marrow fibrosis suggests the possibility to target this pathway in the niche to better treat myelofibrosis.

W1046

MICRORNA ARE ESSENTIAL TO MURINE HEMATOPOIETIC STEM CELL FUNCTION

Heiser, Diane[1], Haro-Acosta, Veronica[1], Tan, Yee Sun[2], Civin, Curt I.[2] and Clarke, Michael[1], [1]Stanford University School of Medicine, Institute for Stem Cell Biology/ Regenerative Medicine, Stanford, CA, U.S., [2]University of Maryland School of Medicine, Baltimore, MD, U.S.

While microRNA have been shown to be important players in both development and disease, their precise role as a species in hematopoietic stem cells (HSCs) remains undefined. Using two different floxed mouse models, we ablated microRNA expression in HSCs with lentiviral CRE, and assessed HSC engraftment capacity by transplant into congenic recipients. Deleting either Dicer or DGCR8 – each key players at different steps in the microRNA biogenesis pathway – resulted in dramatic HSC engraftment defects. As the DGCR8 protein is more specific to microRNA processing than Dicer (which performs many other functions in the cell), we performed a genetic knockout by crossing DGCR8 floxed mice to mice expressing CRE under the hematopoietic-specific promoter Vav. A DGCR8$^{FL/-}$; vav-iCRE genotype resulted in embryonic lethality, while heterozygous DGCR8$^{FL/+}$; vav-iCRE mice were born at normal Mendelian ratios. Fetal livers from DGCR8$^{FL/-}$; vav-iCRE embryos contained phenotypic HSCs at E14.5. However, when these fetal liver HSCs were transplanted, no donor-derived engraftment was observed. This indicated that these HSCs cannot generate definitive adult hematopoiesis and suggests that HSC exhaustion may occur before birth in these mice. To confirm that DGCR8 is essential for HSC homeostasis, rather than causing a defect intrinsic to the engraftment process (ie. homing to the bone marrow niche), we infected DGCR8$^{FL/-}$ bone marrow with a Tamoxifen-responsive GFP-CRE-ERT2-expressing lentivirus and performed transplants. After 10-12 weeks engraftment, recipient mice were fed a Tamoxifen diet for 16 additional weeks. DGCR8 knockout donor-derived engraftment levels decreased over time in contrast to controls, and endpoint bone marrow analysis revealed an exhaustion of DGCR8 knockout HSCs. A small number of DGCR8$^{FL/-}$ GFP+ cells remaining were FACS sorted and genotyped, revealing retention of a functional copy of DGCR8 despite lentiviral infection. DGCR8$^{FL/-}$ GFP+ bone marrow cells also failed to engraft in a competitive secondary transplant. Here we demonstrate genetically that microRNA are essential for HSC engraftment and homeostasis. Identifying the microRNAs responsible for this phenotype through rescue experiments may provide new targets for HSC-related diseases, or avenues to enhance ex vivo expansion of HSCs.

W1048

CD11A AND EPCR AS HIGH-RESOLUTION MARKERS TO IDENTIFY MURINE LONG-TERM HEMATOPOIETIC STEM CELLS

Karimzadeh, Alborz, University of California, Irvine, Irvine, CA, U.S.

Hematopoietic stem cells (HSCs) are multipotent progenitors with self-renewal capacity that give rise to all downstream progenitor and effector blood cells. Molecular characterization of HSCs would elucidate governing pathways for generation of patient-specific HSCs. However, current markers used for identification of long-term HSCs are limited to purification of a functionally heterogeneous population. Furthermore, some HSC markers show inconsistent expression in different mouse models and in different conditions. Our preliminary data identified CD11a as a novel marker for purification of long-term HSCs. CD11a is highly expressed on downstream progenitor and effector blood cells, however it is absent on a subset of HSCs. In vitro and in vivo, CD11a- HSCs show higher multipotency potential, and higher engraftment and self-renewal capacity compared to their CD11a+ counterpart. EPCR expression is also correlated with higher HSC activity. Our data suggests CD11a and EPCR are consistently expressed in a number of mouse models. Therefore, we hypothesize that CD11a and EPCR together can be used to highly enrich for HSCs without the need for any other aforementioned inconsistent markers, potentially simplifying the purification procedure and making HSC sorting more accessible in different contexts. Indeed, we show that CD11a and EPCR alone are sufficient for purification of a rare population which contains *all* functional HSCs. Therefore, in combination with classical markers and also in a two-color sorting method, CD11a and EPCR are promising markers for purification of adult HSCs. Inconsistent expression of markers is also true in the developing embryo. However, we have determined that CD11a and EPCR can be used to identify embryonic populations equivalent to HSCs (embryonic precursors to HSCs) across multiple timepoints and tissues in vitro.

W1050

ENHANCED ANTI-TUMOR ACTIVITY OF HUMAN PLURIPOTENT STEM CELL-DERIVED NATURAL KILLER CELLS BY EXPRESSION OF CHIMERIC ANTIGEN RECEPTORS

Li, Ethan[1], Blum, Robert[1], Hermanson, David[2], Moriarty, Branden[2] and Kaufman, Dan S.[3], [1]University of California, San Diego, La Jolla, CA, U.S., [2]University of Minnesota, Minneapolis, MN, U.S., [3]University of California- San Diego, La Jolla, CA, U.S.

Human pluripotent stem cells can efficiently differentiate into diverse hematopoietic cell lineages including my-

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eloid, erythroid and lymphoid cells. Human natural killer (NK) cells are a promising source of lymphocytes for anti-cancer immunotherapy. NK cells are part of the innate immune system and exhibit potent anti-tumor and anti-viral activity without need for HLA matching and without prior antigen exposure. Clinical trials using allogeneic NK cells isolated from peripheral blood demonstrate efficacy against chemotherapy-refractory tumors, mainly acute myelogenous leukemia. Derivation of NK cells from pluripotent stem cells can provide an unlimited source of lymphocytes for "off-the-shelf" therapy, rather than requiring these cells to be isolated from individual donors. We have developed a two-stage culture system to efficiently produce NK cells from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) that requires neither cell sorting nor xenogeneic stromal cell co-culture. These hESC and iPSC-derived NK cells have phenotype and function similar to NK cells isolated from peripheral blood. Here, we demonstrate the ability to enhance the activity of the hESC and iPSC-derived NK cells via expression of chimeric antigen receptors (CARs) that recognize antigens on NK cell-refractory tumors. Specifically, we have stably expressed both anti-CD19 and anti-mesothelin (meso) CARs in hESCs and iPSCs using the Sleeping Beauty transposon system. CAR-expressing hESCs and iPSCs can differentiate into NK cells with CAR surface expression. The anti-CD19 CAR-expressing hESC/iPSC-derived NK cells are able to effectively kill B cell leukemia cells and the anti-meso CAR-expressing hESC/iPSC-derived NK cells kill ovarian cancer cells that are refractory to CAR-negative NK cells. For these studies, 3rd generation T cell-based CAR were used that contain CD28, CD137, and CD3-zeta signaling domains. Additionally, use of hESCs/iPSCs provides an effective platform to rapidly screen novel CAR constructs. Currently, we are testing novel NK cell-specific CAR constructs to further enhance stable expression and function in hESC/iPSC-derived NK cells. Additional cellular engineering is also in progress to augment anti-tumor and anti-viral activity of hESC and iPSC-derived NK cells.

W1052

A SPATIO-TEMPORAL MOLECULAR FRAMEWORK OF THE DEVELOPING HAEMATOPOIETIC STEM CELL NICHE

McGarvey, Alison, Rybtsov, Stanislav, Souilhol, Celine, Godwin, Duncan, Tomlinson, Simon and Medvinsky, Alexander, University of Edinburgh, Edinburgh, U.K.

In the developing embryo the emergence of haematopoietic stem cells (HSCs) is driven by signalling between precursor cells and their niche. Whilst the timing and location of HSC ontogenesis is well described in the mouse as being within the aorta-gonad-mesonephros (AGM) region between embryonic day 10.5 and 11.5, the underlying molecular processes driving this are poorly characterised. By generating and analysing a transcriptome-wide RNA-sequencing dataset from subdomains of the developing mouse AGM region we have elucidated key in vivo expression signatures of the early embryonic HSC niche. Within these profiles we identified known HSC regulators such as Runx1, SCF, and other haematopoietic cytokines, suggesting that these signatures do indeed represent a supportive profile for HSC development. Through a functional screen in ex-vivo culture we identified a novel candidate which improves the outcome of maturation from early precursors in terms of long-term multilineage reconstitution. We validated, by immunostaining, its in vivo proximity to emerging HSCs and propose that through its action as a modulator of BMP signalling it facilitates maturation of precursors into HSCs. Moreover we present the transcriptome data as a useful resource for future studies of the developing HSC niche which, as we have demonstrated here, can yield novel molecular insight into this highly dynamic process.

W1054

DPP4 TRUNCATED COLONY STIMULATING FACTORS MANIFEST DISTINCT REGULATORY FUNCTIONS COMPARED TO THEIR FULL LENGTH FORMS AND DPP4 IS ALTERED BY, AND MODULATES SENSITIVITY TO, EPHOSS.

O'Leary, Heather Ann[1], Jiang, Guanglong[1], Lai, Xianyin[1], Li, Sujun[1], Cooper, Scott[1], Lee, ManRyul[2], Boswell, H. Scott[1], Witzmann, Frank[1] and **Broxmeyer, Hal E.**[1], [1]Indiana University School of Medicine, Indianapolis, IN, U.S., [2]Soon Chun Hyuang University, Asan-Si, Korea

Hematopoietic stem cells (HSC) reside in hypoxic niches (~1-4% O_2), yet, HSC studies are typically performed using cells isolated in ambient air (~20% O_2). Inhibiting ambient air exposure/harm of cells, which we termed Extra Physiologic Oxygen Shock/Stress (EPHOSS), by collecting/processing human cord blood (hCB) or mouse bone marrow (mBM) stem cells in hypoxia (3% O_2), enhances recovery of phenotypic/functional long-term repopulating HSC (LT-HSC) and is mechanistically linked, in part, to the mitochondrial permeability transition pore, Reactive Oxygen Species (ROS) and cyclophilin D. We hypothesized that Dipeptidyl Peptidase 4 (DPP4), a peptidase that N terminally cleaves select proteins altering homing/engraftment of HSC, may be altered by EPHOSS and involved in EPHOSS effects on HSC. Proteomic and bioinformatic analysis identified many unexpected intracellular/secreted proteins with DPP4 truncation (T) sites. Intracellular signaling of T-cytokines compared to their full length (FL) forms showed specific, as well as overlapping, modifications in phosphorylated and global proteins, leading to differential regulation of signaling/function in normal and leukemic cells. T-GM-CSF and T-IL-3 had enhanced receptor binding compared to their FL

forms with significant, and reciprocal, blunting of functional activity of both factors in vitro and in vivo. To investigate effects of DPP4 on EPHOSS, and vice versa, mBM was harvested (air/ hypoxia) with a DPP4 inhibitor (DPA), or from DPP4 K/O mice. This resulted in significant increases in the number of phenotypic LT-HSC (p=.017) in air, suggesting that DPP4 inhibition blunts EPHOSS mediated loss of phenotypic LT-HSC. Also, the percentage of DPP4+ cells is increased in primitive fractions of mBM or hCB, (LSK ~15%, LSKCD150 40%, CD34+CD38- ~10%, CD34+CD38-CD45RA-CD90+CD49F+ ~40% p=.007) and further enhanced 15- 20% when cells are isolated in hypoxia (p=.005). Unexpectedly, LT-HSC ROS levels (mitochondrial/total) were not diminished in DPA or DPP4 K/O groups harvested in air despite the increase in phenotypic LT-HSC over air harvest alone. These data suggest additional pathways, such as DPP4 expression/activity, may be influencing LT-HSC response to EPHOSS as well as being modulated by EPHOSS and DPP4 T-molecules may serve regulatory roles in hematopoiesis.

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W1056

THE Gadd45 FAMILY AS MEDIATORS OF HEMATOPOIETIC STEM CELL FATE CONTROL – FROM GENOTOXIC STRESS TO CYTOKINE-INDUCED DIFFERENTIATION

Wingert, Susanne[1], Thalheimer, Frederic B.[1], Haetscher, Nadine[1], Rehage, Maike[1], Schroeder, Timm T.[2] and **Rieger, Michael A**[1,3], [1]Goethe University Frankfurt, Frankfurt am Main, Germany, [2]Swiss Federal Institute of Technology (ETH) Zurich, Basel, Switzerland, [3]German Cancer Consortium, Heidelberg, Germany

The growth arrest and DNA-damage-inducible 45 (Gadd45) protein family is encoded by three genes, Gadd45a, b and g. They are early responders of cellular stress with tumor-suppressive activity, besides many essential functions for cell integrity such as survival/death, cell cycle control, DNA damage repair and epigenetic regulation. Thereby they are pivotal for development, regeneration, ageing and cancer. However, their function in hematopoietic stem cell (HSC) fate decision control remained elusive. Here we investigated the regulation and function of all three family members in murine HSCs using genetic gain- and loss-of-function studies at single cell resolution. In contrast to other cellular systems, the DNA-damage-induced p53-dependent GADD45A expression does not cause a cell cycle arrest or an alteration in the decision between cell survival and apoptosis in HSCs. However, GADD45A rapidly induces and accelerates the differentiation program. Therefore, GADD45A expression in HSCs prevents their fatal transformation by directing them into differentiation to clear them from the system, proposing a new DNA-damage response mechanism. Furthermore, we identified the long envisioned molecular link between differentiation-promoting cytokines and the intracellular transmission into a genetic differentiation program in HSCs: the cytokine signaling via STAT5 selectively upregulates GADD45G expression causing an immediate switch from self-renewal into differentiation. Continuous tracking of individual HSCs and their progeny via time-lapse microscopy elucidated an extraordinary fast differentiation of HSCs into committed progenitors within about 30h, once GADD45A or G were expressed. The ectopic expression of the inflammation-induced GADD45B also promotes the differentiation program, though with a different kinetics.GADD45A and G expression initiate a MAP3K4-driven cascade of mitogen-activated protein kinases leading to selective p38 activation that locks-in the differentiation program in HSCs. Interestingly, GADD45B promotes its function independent of p38 activation. The molecular decipherment of GADD45-mediated differentiation will allow rationally modulating the delicate balance between self-renewal and differentiation for regenerative medicine and leukemia therapy.

W1058

ROBUST HEMATOPOIETICS DIFFERENTIATION FROM PLURIPOTENT STEM CELL

Xie, Fei[1], Bayer, Ashley I.[2], Zhang, Mingliang[3], Li, Ke[4], Muench, Marcus O.[1,2] and Kan, Yuet Wai[1], [1]UCSF, San Francisco, CA, U.S., [2]Blood Systems Research Institute, San Francisco, CA, U.S., [3]Gladstone Institutes, San Francisco, CA, U.S., [4]Gladstone Institutes of Cardiovascular Disease, San Francisco, CA, U.S.

Treatment of sickle cell disease and β-thalassemia, two most important genetic blood disorders, is often directed at their complications. Hematopoietic stem and progenitor cells (HSPC) transplantation is potentially curative for the diseases, but histocompatible donors are not available for most patients. The discovery of induced pluripotent stem cells (iPSCs) raises the possibility of generating iPSCs from the patients, correcting the mutations by genome editing, differentiating the corrected iPSCs into HSC and auto-transplanting them back to the patients. However, most protocols for HSC differentiation from pluripotent stem cells have been low in efficiency and poor in reproducibility. Here, we have developed an efficient and controlled strategy to increase hematopoietic differentiation from iPSCs in culture. CD34, CD43 and CD45 were used to analyze the efficiency of HSC differentiation. After 11-days' differentiation, iPSCs could generate up to 96% CD34+, 94% CD43+ and 94% CD45+ hematopoietic stem and progenitor cells (HSPC), most of which were

doubly positive for 2 of the 3 markers. Importantly, representative colonies from these hematopoietic progenitors showed typical morphology of various CFC subtypes, including macrophages, granulocytes, and erythrocytes as well as mixed colonies comprising different types of these cells. Taken together, our system presented here provides a useful tool not only for the study of hematopiesis, but also for practical application of iPSCs in the treatment of hematologic and immunologic diseases.

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W1060

ICAM-1 EXPRESSING MESENCHYMAL STEM CELLS POTENTLY INHIBIT GRAFT-VERSUS-HOST DISEASE BY REGULATING Notch1 SIGNALING

Zhang, Yi[1], Tang, Bo[1], Li, Xue[1], Zhu, Heng[1], Li, Ximei[2], Chu, Yanan[2], Chen, Xiuhui[1], Liu, Yuanlin[1] and Zheng, Rongxiu[2], [1]Institute of Basic Medical Sciences, Beijing, China, [2]Tianjin Medical University General Hospital, Tianjin, China

Inefficient homing of systemically infused mesenchymal stem cells (MSCs) limits the efficacy of existing MSC-based clinical graft-versus-host disease (GvHD) therapies. Intercellular adhesion molecule-1(ICAM-1) plays an important role in the maintenance of MSCs homing and immunomodulation, but is not expressed on the surface of MSCs. Thus we hypothesized that targeted migration ability of MSCs may significantly improve their immunomodulatory effect. Here, ICAM-1 gene was engineered into a murine MSC line C3H10T1/2 by retrovirus transfection system (MSCs/ICAM-1). We found that ICAM-1 overexpressed MSCs inhibited dendritic cells (DCs) dendrite formation, as well as differentiation and maturation. Functionally, ICAM-1 overexpressed MSC-educated DCs significantly reduced antigen-specific T-cell activation. Moreover, infusion of MSCs/ICAM-1 displayed enhanced effect in prolonging the survival and alleviating the clinical scores of the GvHD mice than normal MSCs. ICAM-1 overexpressed MSCs increased regulatory T and Th17 cells but decreased the Th1 cell numbers in the host spleens. Immunofluorescent staining showed that MSCs/ICAM-1 can migrate to and relocate in target organ in vivo. Mechanistically, we determined that ICAM-1 regulated MSC immune function via Notch1 signal pathway. In summary, our finding demonstrates that ICAM-1 guides migration of MSCs to homing and thus highly intensify their in vivo immunomodulatory effect. This exciting therapeutic strategy may improve the clinical efficacy of MSC based therapy for immune diseases.

CARDIAC CELLS

W1064

LATROPHILIN-2 IS A SPECIFIC CELL-SURFACE MARKER FOR CARDIAC PROGENITOR CELLS AND SPECIFIES CARDIAC LINEAGE COMMITMENT AND DEVELOPMENT

Cho, Hyun-Jai, Lee, Choon-Soo, Lee, Jin-Woo, Yang, Han-Mo, Kwon, Yoo-Wook and Kim, Hyo-Soo, Seoul National University Hospital, Seoul, Korea, South

The identification of a lineage-specific marker plays a pivotal role in understanding developmental process and is utilized to isolate a certain cell type with high purity for the therapeutic purpose. We here report a new cardiac-specific marker, and demonstrate its functional significance in the cardiac development. When mouse pluripotent stem cells (ES and iPS cells) were stimulated with BMP4, Activin A, bFGF and VEGF, they differentiated into cardiac cells. To screen cell-surface expressing molecules on cardiac progenitor cells compared to undifferentiated mouse iPS and ES cells, we isolated Flk1+/PDGFRa+ cells at differentiation day 4 and performed microarray analysis. Among candidates, we identified a new G protein-coupled receptor, latrophilin-2 (LPHN2). In sorting experiments under cardiac differentiation condition, LPHN2+ cells derived from pluripotent stem cells strongly expressed cardiac-related genes (Mesp1, Nkx2.5, aMHC and cTnT) and exclusively gave rise to beating cardiomyocytes, as compared with LPHN2- cells. LPHN2-/- mice revealed embryonically lethal and huge defects in cardiac development. Interestingly, LPHN2+/- heterozygotes were alive and fertile. For the purpose of cardiac regeneration, we transplanted iPS-derived LPHN2+ cells into the infarcted heart of adult mice. LPHN2+ cells differentiated into cardiomyocytes, and systolic function of left ventricle was improved and infarct size was reduced. We confirmed LPHN2 expression on human iPS and ES cell-derived cardiac progenitor cells and human heart. We demonstrate that LPHN2 is a functionally significant and cell-surface expressing marker for cardiac progenitor and cardiomyocytes. Our findings provide a valuable tool for isolating cardiac lineage cells from pluripotent stem cells and an insight into cardiac development and regeneration.

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W1066

WNT/BETA-CATENIN SIGNALING ENABLES DEVELOPMENTAL TRANSITIONS DURING VALVULOGENESIS

Bosada, Fernanda Maria, Devasthali, Vidusha, Jones, Kimberly and Stankunas, Kryn, University of Oregon, Eugene, OR, U.S.

Heart valve development proceeds through coordinated steps by which endocardial cushions (ECs) form thin, elongated, and stratified valves. Wnt signaling and its canonical effector β-catenin are proposed to contribute to endocardial-to-mesenchymal transformation (EMT) through postnatal steps of valvulogenesis. However, genetic redundancy and lethality have made it challenging to define specific roles of the canonical Wnt pathway at different stages of valve formation. We developed a transgenic mouse system that provides spatiotemporal inhibition of Wnt/β-catenin signaling by chemically-inducible overexpression of Dkk1. Unexpectedly, this approach indicates canonical Wnt signaling is required for EMT in the proximal outflow tract (pOFT) but not atrioventricular canal (AVC) cushions. Further, Wnt indirectly promotes pOFT EMT through its earlier activity in neighboring myocardial cells or their progenitors. Subsequently, Wnt/β-catenin signaling is activated in cushion mesenchymal cells where it supports FGF-driven expansion of ECs and then AVC valve extracellular matrix patterning. Mice lacking Axin2, a negative Wnt regulator, have larger valves, suggesting that accumulating Axin2 in maturing valves represents negative feedback that restrains tissue overgrowth rather than simply reporting Wnt activity. Disruption of these Wnt/β-catenin signaling roles that enable developmental transitions during valvulogenesis could account for common congenital valve defects.

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W1068

IMMUNOCOMPATIBILITY OF PARTHENOGENETIC STEM CELL DERIVED CARDIOMYOCYTES

Galla, Satish[1,2], Didié, Michael[2,3], Muppala, Vijayakumar[2,3], Dressel, Ralf[2,4] and Zimmermann, Wolfram Hubertus[2,3], [1]University Medicine Goettingen, Göttingen, Germany, [2]DZHK (German Center for Cardiovascular Research), partner site Göttingen, Goettingen, Germany, [3]Institute of Pharmacology and Toxicology, University Medical Center Göttingen, Georg-August-University, Goettingen, Germany, [4]Institute for Cellular and Molecular Immunology, University Medical Center Göttingen, Georg-August-University, Goettingen, Germany

Pluripotent parthenogenetic stem cells (PSC) derived from chemically activated murine oocytes can be differentiated into cardiomyocytes (CM) and utilized in tissue engineered based heart repair approaches. However, tissue engineered allografts are often rejected after transplantation by a mismatch of major histocompatibility complex protein haplotypes (H2) between donor and acceptor. In this study we have investigated the immunological properties of PSC-derived cardiomyocytes (PSC-CM). Haploidentical Mouse PSCs ($H2^{d/d}$) expressing a CM-specific neomycin-resistance were differentiated and CM were purified. We generated engineered heart muscle (EHM) construct from 70% PSC-CM, 30% growth-inhibited murine embryonic fibroblasts (MEF), and collagen type I. The expression of immunemodulatory molecules (MHC-I, MHC-II, CD80, CD86, CD40, PD-L1) was assessed on PSC-CM and PSC derived non myocytes (PSC-NCM) in monolayer culture and from EHM derived cells by FACS. Interferon gamma (IFNg) was added for 48 h to mimic inflammatory conditions. Immune cell proliferation was assessed by coculturing eFluor 670 labeled splenocytes with PSC derivatives for 4 days. PSC-CM and PSC-NCM showed low expression of costimulatory molecules regardless of IFNg stimulation. PSC-CM, in contrast to PSC-NCM, showed low expression of MHC-I (3±1% vs. 35±7%; n=3) in 2D and after EHM-culture. After IFNg-treatment MHC-I (40±6%) and MHC II (30±5) was upregulated in PSC-CM in 2D, in EHM only MHC I (30±8%) was upregulated. Of all immunomodulatory molecules IFNg increased only PDL-1 expression in 2D (90±5%). We cocultred PSC-CM, PSC-NCM and EHMs with splenocytes from DBA-mice ($H2^{d/d}$, H2-match) and BL6-mice ($H2^{b/b}$, H2-mismatch). Compared to a matched cocultivation, mismatched NCM but not CM induced a higher splenocyte proliferation after IFNg stimulation. EHMs showed no difference in splenocyte proliferation regardless of match or mismatch between CM or MEFs and splenocytes. In conlusion PSC-CM show a low expression of MHC-I and respond with MHC-I and MHC-II upregulation to IFNg stimulation. This, together with the high upregulation of PD-L1 as an immunosuppressive

molecule after IFNg stimulation, and no activation of immune cells after co culture could play an important role in tissue engineered cardiac repair applications.

W1070

INTEGRATED ANALYSIS OF –OMICS DATA FROM HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES EXPOSED TO DOXORUBICIN

Holmgren, Gustav[1,2], Synnergren, Jane Marie[3], Andersson, Christian X[4], Lindahl, Anders[2] and Sartipy, Peter[5], [1]University of Skövde, Skövde, Sweden, [2]Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden, [3]TakaraBio / University of Skovde, Lidkoping, Sweden, [4]Takara Bio Europe AB, Gothenburg, Sweden, [5]University of Skovde, Skovde, Sweden

Doxorubicin, a member of the anthracycline class, is a highly efficient, well established chemotherapeutic agent used for treatment of various cancer forms including breast cancer, solid tumors, leukemia, and lymphomas. The clinical use of doxorubicin is, however, limited by the severe cardiotoxic side effects associated with the treatment. The doxorubicin-induced cardiotoxicity show a dose-dependency, as well as age and gender differences in the sensitivity to the treatment, and the time-to-onset of the toxicity can be several years after termination. Despite intense research the exact mechanisms of the cardiotoxicity is not fully understood, though the formation of reactive oxygen species and cellular iron accumulation has been suggested to be involved. In the present study we used cardiomyocytes derived from human pluripotent stem cells to investigate mechanisms of the toxicity caused by doxorubicin exposure. The cells were incubated with various concentrations of doxorubicin for up to 48h, followed by a 12 days wash-out period. Total RNA and protein was isolated at multiple time points throughout the experiment and an integrated analysis approach was applied to the data. The cells showed an altered morphology and dysfunctional contractility upon doxorubicin exposure and evident effects of the treatment were observed both at the mRNA- and protein level. Approximately 2400 mRNAs and 200 proteins were differentially expressed between treatment and control in at least one time point. Several interesting patterns that showed a correlation between mRNA and protein expression, were identified. Pathway enrichment analysis of the differentially expressed mRNAs and proteins revealed several overrepresented pathways with connection to cardiomyocyte function and cardiotoxicity, such as regulation of actin cytoskeleton, hypertrophic cardiomyopathy, and p53 signaling pathway. The present study demonstrates the utility of human pluripotent stem cell-derived cardiomyocytes for mechanistic studies of doxorubicin-induced cardiotoxicity and provides further molecular insights into the cellular responses towards the compound.

W1072

PHENOTYPE VARIABILITY OF iPSC-DERIVED CARDIOMYOCYTES FROM PATIENTS WITH DISTINCT MUTATIONS FOR FAMILIAL HYPERTROPHIC CARDIOMYOPATHY CAN INFORM DRUG DISCOVERY

Lam, Jason, Sanchez-Freire, Veronica, Park, Eunhye, Pike, Nirupama and Armstong, Chris, Stem Cell Theranostics, Redwood City, CA, U.S.

Familial hypertrophic cardiomyopathy (HCM) is the leading cause of sudden cardiac death in the young people, and is the most common inherited heart defect affecting 1 in 500 individuals worldwide. To date, over 1000 unique HCM mutations have been identified in over 13 different genes encoding for the cardiac sarcomere. Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) have been known to model certain aspects of HCM. We have successfully generated cardiomyocytes from iPSCs of patients with HCM. We compared disease phenotypes across a library of patient-specific HCM iPSC-CMs carrying distinct mutations to assess the range of phenotypes that may present in iPSC-CMs derived from different patient cohorts. iPSCs were generated from three patient cohorts carrying known hereditary mutations for HCM in MYBPC3 and MYH7, as well as family-matched controls. Disease phenotypes in patient-specific iPSC-CMs were evaluated using immunofluorescence, Ca^{2+} transient analysis, multi-electrode array, and video analysis of contractile motion. HCM iPSC-CMs displayed a range of disease phenotypes as assessed by cell size, Ca^{2+} homeostasis, electrophysiology, and contractile arrhythmia. These disease models were then used to screen for compounds that may attenuate the HCM disease phenotypes. Interestingly, different HCM mutations resulted in distinct disease phenotype presentation and response to compound treatments. These findings indicate that disease-specific iPSC-CMs present with a range of phenotypes for HCM that vary by specific mutation. iPSC-CMs libraries are therefore critical for cellular characterization of diseases such as HCM and will serve as a valuable tool in the process of drug discovery.

W1074

IDENTIFICATION OF THE MOLECULAR SIGNATURE OF iPSC-DERIVED AHF CARDIOVASCULAR PROGENITORS

Linares, Javier[1,2], Arellano-Viera, Estibaliz[1,2], Ruiz-Villalba, Adrian[1,2], Guruceaga, Elizabeth[1,2], Iglesias, Elena[1,2], Abizanda, Gloria[1,2], Prosper, Felipe[1,3] and Carvajal-Vergara, Xonia[2,4], [1]Fundación Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain, [2]Foundation for Applied Medical Research (FIMA), University of Navarra, Pamplona, Spain, [3]Clínica Universidad de Navarra, University of Navarra, Pamplona, Spain, [4]FIMA, Pamplona, Spain

Different stem cells have been explored in cardiac cell therapy: bone marrow-, adipose tissue- and cardiac-derived stem cells. However, any improvement in cardiac function is likely to be the result of a paracrine action. The optimal cell type for regeneration of the failing heart should ensure safety, be expandable, immunocompatible and integrate within the host myocardium. Cardiovascular progenitors (CVP) could fulfill these prerequisites. Mef2c-AHF (Myocyte Enhancer Binding Factor 2C - Anterior Heart Field) enhancer is active in multipotent CVP identified in the pharyngeal mesoderm of the developing embryo, termed the second heart field (SHF). We crossed Mef2c-AHF-Cre mice with Ai6(RCL-ZsGreen) mice to obtain Mef2c-AHF-CVP reporter mice. Tail-tip fibroblasts (TTF) and cardiac fibroblasts (CF) derived from these mice were transduced with Oct4, Sox2, Klf4 and c-Myc (OSKM) with the pMXs retrovirus vectors. Established AHF-iPSC derived from TTF and CF encoded the expected genomic insertions, showed normal karyotypes and the transgenes were silenced. AHF-iPSC expressed the endogenous pluripotency-associated markers such as Oct4, Sox2, Nanog and Zfp42 and demonstrated alkaline phosphatase activity. The AHF-iPSC lines were capable to differentiate into the three germ layers using in vivo teratoma and in vitro embryoid body (EB) differentiation assays. ZsGreen was not detected in undifferentiated AHF-iPSC. However, the expression of ZsGreen was confirmed by qRT-PCR and fluorescence microscopy from EB day 6 onwards. The differentiation potential of AHF-CVP towards cardiomyocyte (CM) and endothelial cell (EC) lineages was demonstrated. To further characterize these iPSC-derived AHF-CVP we analyzed their whole genome expression profile using microarrays. To this end, we collected RNA from undifferentiated AHF-iPSC and ZsGreen+ cells isolated at early stage of differentiation (EB day 6, enriched in CVP) and late stage of differentiation (EB day 14, enriched in CVP-derived differentiated cell progeny). Comparative functional analysis will be presented. Finally we will show preliminary in vivo results of the engraftment capacity and differentiation potential of the AHF-CVP in a murine model of myocardial infarction.

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W1076

PATIENT-SPECIFIC HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES FOR RATIONAL THERAPEUTIC DESIGN IN DANON DISEASE

Hashem, Sherin I, **Nelson, Bradley**, Gault, Emily and Adler, Eric D, University of California, San Diego, San Diego, CA, U.S.

Danon disease is a lethal X-linked disorder caused by mutations in the gene encoding lysosomal associated membrane protein type-2 (LAMP-2). Loss of LAMP-2 expression impairs autophagosome-lysosome fusion and severely disrupts autophagic flux. Clinically, Danon patients develop mild cognitive impairment and severe skeletal and cardiac myopathies. There are no FDA-approved therapies for the treatment of this rare disorder. We previously demonstrated that human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) from Danon patients recapitulated key features of the disease including the accumulation of immature autophagic vacuoles, fragmented mitochondria, and increased production of naturetic peptides and that increased oxidative stress mediated cell apoptosis. We have further shown that Danon hiPSC-CMs have low mitochondrial membrane potentials, significant translocation of PARKIN and p62 to mitochondria, and severely reduced mitochondrial respiratory capacity, indicating significant mitochondrial damage. Overexpression of LAMP-2 using a lentiviral delivery system, however, reversed these abnormalities. We have further characterized an established line of LAMP2 knockout mice crossed with a CAG-RFP-GFP-LC3B autophagy reporter line. Hearts from these mice also demonstrate features consistent with Danon disease including impaired autophagic flux, disrupted myofibrils, and mitochondrial damage/dysfunction. The ability of LAMP2 gene replacement to rescue the Danon phenotype in vitro supports the feasibility of gene therapy for this disease. We have now designed an adeno-associated virus vector carrying the LAMP2 gene for a preclinical gene therapy study in this Danon mouse model. These experiments outline a methodology for using patient-specific hiPSCs to expedite rational therapeutic design for rare diseases in the future: Proof-of-concept testing in patient-specific hiPSC-derived cells –> Preclinical studies in a validated

mouse model of the disease –> Therapeutic trials in human patients.

Funding Source: California Institute for Regenerative Medicine TR3-05687, Identification of Novel Therapeutics for Danon Disease Using an iPS Model of the DiseaseCalifornia Institute for Regenerative Medicine Stem Cell Training Grant 2

W1078

MICRORNA EXPRESSION PROFILING OF DOXORUBICIN-TREATED HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Holmgren, Gustav[1,2], Synnergren, Jane Marie[3], Andersson, Christian X[4], Lindahl, Anders[2] and **Sartipy, Peter**[5], [1]University of Skövde, Skövde, Sweden, [2]Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden, [3]TakaraBio / University of Skovde, Lidkoping, Sweden, [4]Takara Bio Europe AB, Gothenburg, Sweden, [5]University of Skovde, Skovde, Sweden

Anthracyclines, such as doxorubicin, are well-established, highly efficient anti-neoplastic drugs used for treatment of a variety of cancers, including solid tumors, leukemia, lymphomas, and breast cancer. The successful use of doxorubicin has, however, been hampered by severe cardiotoxic side-effects, often resulting in early discontinuation of the treatment. In order to prevent or reverse negative side-effects of doxorubicin, it is of great importance to find useful early biomarker of heart injury and drug-induced cardiotoxicity. The high stability under extreme conditions, presence in various body fluids, and tissue-specificity, of microRNAs make them very suitable as clinical biomarkers. The present study aimed towards evaluating the early and late effects of doxorubicin on the microRNA expression in cardiomyocytes derived from human pluripotent stem cells. The cardiomyocytes were exposed to different doses of doxorubicin for 48 hours, followed by a 12 days wash-out period. The doxorubicin exposure displayed a clear effect on the cardiomyocytes, manifested by a changed morphology and dysfunctional contractility. An evident influence on the global microRNA expression profile could also be observed. We report on several microRNAs, including miR-34a, miR-34b, miR-187, miR-199a, miR-199b, miR-146a, miR-15b, miR-130a, miR-214, and miR-424, that are differentially expressed upon, and after, treatment with doxorubicin. Investigation of the biological relevance of the identified microRNAs revealed connections to cardiomyocyte function and cardiotoxicity, strengthening the findings of these microRNAs as potential biomarkers for drug-induced cardiotoxicity.

W1080

VINCULIN AND TROPOMYOSIN VARIANTS ALTER CARDIOMYOCYTE FUNCTION AND CONTRIBUTE TO FAMILIAL DILATED CARDIOMYOPATHY

Happe, Cassandra[1], **Tenerelli, Kevin**[1], Deacon, Dekker[1,2], Erbe, Rossin[1], Tran, Vivien[1], Taylor-Weiner, Hermes A.[1], Mali, Prashant[1], Adler, Eric[1,3], Chi, Neil C.[3,4] and Engler, Adam[1,4], [1]University of California, San Diego, La Jolla, CA, U.S., [2]Biomedical Sciences Program, University of California, San Diego, La Jolla, CA, U.S., [3]Division of Cardiovascular Medicine, University of California, San Diego, La Jolla, CA, U.S., [4]Sanford Consortium for Regenerative Medicine, La Jolla, CA, U.S.

Dilated cardiomyopathy (DCM) is a multivariate disease with poorly understood mechanisms, but recently 30+ different mutations have been suggested to contribute to disease pathology. We have identified a family with high DCM incidence where heterozygous mutations in two dissimilar cytoskeletal proteins, i.e. thin filament-associated a-tropomyosin (TPM1; +/c.G97A) and intercalated disc-associated vinculin (VCL; +/c.659dupA), co-segregate with DCM (Fischer Exact p-value $< 10^{-3}$; LOD score = 3.21). To better understand disease mechanism, we generated human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) from the family cohort carrying the variants (proband and affected cousin vs. non-carrier mother and aunt) and modeled these variants in human embryonic stem cell-derived CMs (hESC-CMs) via RNAi and CRISPR to discern the functional consequences that could induce DCM. Proband and affected cousin CMs had half of the normal protein expressed for both variants with morphological and cytoskeletal abnormalities and a lower aspect ratio relative to non-carriers. They also contracted with decreased energy and more irregular timing compared to non-carrier control CMs. Decrease sodium and potassium channel expression accompanied slowed action potential kinetics in affected vs. non-carrier patient-derived CMs, which together suggests that VCL and/or TPM1 mutations may cause unique downstream transcriptome regulation that leads to the dysfunction we observed in vitro. To assess the combinatorial regulation by mutations in these dissimilar genes, hESC-CMs were altered to mirror decreased protein expression, and we found that hESC-CMs lost expression of gap junction protein connexin43 after 50% loss of VCL; these cells also exhibited prolonged calcium transients and contractions with decreased energy and irregular timing, suggesting that reduced VCL creates CMs with dysfunctional mechanical properties, resulting in part from prolonged Ca^{2+} handling, which would likely adversely affect TPM1 mutant phenotypes leading to more severe disease and DCM observed when the variants co-segregated. Given the lack of disease in single variant carriers, these data pro-

vide a unique set of analyses that result in identification of how dissimilar but co-segregating variants can result in disease.

Funding Source: National Institutes of Health and the American Heart Association

W1082

INDUCED EXPANDABLE CARDIOVASCULAR PROGENITOR CELLS REPROGRAMMED FROM FIBROBLASTS

Zhang, Yu[1,2], Cao, Nan[1,2], Huang, Yu[1], Spencer, Ian[1], Fu, Ji-Dong[3], Yu, Chen[1,2], Liu, Kai[1,2], Nie, Baoming[1,2], Xu, Tao[1,2], Li, Ke[1,2], Xu, Shaohua[1,2], Bruneau, Benoit[1,4], Srivastava, Deepak[1,4] and Ding, Sheng[1,2], [1]Gladstone Institutes of Cardiovascular Disease, San Francisco, CA, U.S., [2]University of California, San Francisco, CA, U.S., [3]Heart and Vascular Research Center, MetroHealth Campus, Case Western Reserve University, Cleveland, OH, U.S., [4]Roddenberry Center for Stem Cell Biology and Medicine, Gladstone Institutes, San Francisco, CA, U.S.

Advances in stem-cell biology make cell-based cardiac regeneration a more feasible therapeutic approach to heart failure. Nonetheless, significant challenges remain in generating autologous cardiac cells for transplantation that are abundant and functional. In this work, we captured cells with extensive proliferative ability and restricted cardiovascular differentiation potentials during cardiac transdifferentiation from mouse fibroblasts. These induced, expandable cardiovascular progenitor cells (ieCPCs) propagated robustly in optimized chemically defined conditions for more than 18 passages, producing >10^{16} ieCPCs from 10^5 starting fibroblasts. When expanded long-term, they broadly expressed cardiac-signature genes and retained their potential for single-step, direct differentiation into functional cardiomyocytes (CMs), endothelial cells (ECs), and smooth muscle cells (SMCs) in vitro. When transplanted into infarcted mouse hearts, ieCPCs spontaneously generated CMs, ECs, and SMCs and improved heart performance up to 12 weeks post-infarction. Thus, ieCPCs represent a powerful platform to study cardiovascular specification and provide promising strategies for large-scale drug screening, disease modeling, and cardiac-regenerative medicine.

MUSCLE CELLS

W1084

SATELLITE CELLS IN MDX DUCHENNE MUSCULAR DYSTROPHY MICE ARE AFFECTED BY HEME OXYGENASE-1

Pietraszek-Gremplewicz, Katarzyna, Kozakowska, Magdalena, Ciesla, Maciej, Bronisz, Iwona, Seczynska, Marta, Bukowska-Strakova, Karolina, Jozkowicz, Alicja and **Dulak, Jozef**, Jagiellonian University, Krakow, Poland

Differentiation of myoblasts is dependent on myomiRs, such as miR-206,-1 and -133a/b. Recently, we showed that expression of myomiRs and consequently, differentiation of C2C12 myoblasts was inhibited by heme oxygenase-1 (Hmox1), an enzyme degrading heme to CO, iron ions and biliverdin. Inversely, differentiation of Hmox1-deficient murine primary myoblasts with enhanced expression of myomiRs was accelerated (Kozakowska et al, Antioxid Redox Signal 16:113-27; 2012). Since Hmox1 is crucial anti-inflammatory mediator we wondered whether it can influence the stem cells properties and disease progression in Duchenne muscular dystrophy. In skeletal muscles of mdx (dystrophin-deficient) mice the expression of Hmox1 is consistently increased from week 8 up to 20 months. Elevated expression of Hmox1 is observed in myeloid cells, which number increased in inflamed dystrophin-deficient muscles. Accordingly, the expression of mature fibers-specific miR-1,-133a and -133b were decreased in mdx mice. Surprisingly, expression of satellite cells (SCs)-specific miR-206 was upregulated in gastrocnemius and diaphragm of mdx mice starting from 2nd week of age till at least one year. Interestingly, in activated SCs (α7integrin+ CD34-), which were more abundant in mdx mice, the expression of Hmox1 was decreased while miR-206 was increased, indicating for SCs-specific regulation of Hmox1and miR-206. Double knockout mice (dKO), lacking Hmox1 and dystrophin, were generated. The dKO mice showed significant impairment of exercise capacity on treadmill in comparison to mdx mice, aggravated muscle injury as evidenced by higher level of creatinine kinase and lactate dehydrogenase, increased infiltration with inflammatory cells and increased expression of miR-146a. Similar effects were observed in mdxmice treated with SnPPIX (tin protoporphyrin), a pharmacological inhibitor of Hmox1 activity.

The results indicate for the relationship between Hmox1 and miR-206 in SCs of mdx mice. Diminished Hmox1 and increased miR-206 are linked to accelerated differentiation of SCs. This effect is independent on increased Hmox1 in inflamed dystrophin-deficient muscles. The data indicate both for the stem cells-specific effect of Hmox1

and its important role in modulation of inflammation in dystrophic muscles.

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W1086

GENERATION OF A SYNTHETIC RNA OVEREXPRESSION SYSTEM TO GENERATE SKELETAL MUSCLE PROGENITOR CELLS FROM HPSCS

Jan, M.S., Majib[1,2], Younesi, Shahab[3], Young, M.S., Courtney[1,2], XI, Haibin[2], Hicks, Michael R.[4] and Pyle, April D[2], [1]Center for Duchenne Muscular Dystrophy, Westwood, CA, U.S., [2]University of California Los Angeles, Los Angeles, CA, U.S., [3]University of California, Los Angeles, Tarzana, CA, U.S., [4]University of California, Los Angeles, Santa Monica, CA, U.S.

Duchenne Muscular Dystrophy (DMD) results in progressive muscle deterioration and ultimately death due to mutations in the *DMD* gene encoding dystrophin. Lack of dystrophin leads to membrane tears and activation of the endogenous muscle stem cell called the satellite cell. Continuous rounds of degeneration and regeneration in DMD ultimately lead to satellite cell exhaustion. No effective treatment for DMD is currently available, but one potential therapy involves deriving skeletal muscle progenitor cells (SMPC) from human induced pluripotent stem cells (hiPSCs) from DMD patients. Once expanded, these SMPCs could be corrected to restore dystrophin expression and then engrafted 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 lentiviral-mediated overexpression of key muscle transcription factors including PAX7. However, a method that does not require viral integration would be required for clinical applications. To obtain a robust SMPC population, we are overexpressing PAX7, using a Venezuelan Equine Encephalitis (VEE) non-integrating synthetic RNA platform. After cloning the PAX7 gene into the VEE backbone, we transfected pre-differentiated hiPSCs with synthetic RNA. Where we have seen a significant increase in PAX7 expression 10 days after transfection. After an additional 10 days in differentiation medium, cells expressed further myogenic differentiation markers such as MYOD and MyHC. Thus, synthetic RNA overexpression of PAX7 has potential to generate myogenic precursor cells. We next plan to study the dynamics of PAX7 expression over time, assess additional myogenic markers and in vitro function, as well as in vivo engraftment of synthetic RNA generated SMPCs. The refinement of this differentiation protocol for producing and maintaining PAX7^{+} progenitor populations will aid in the development of regenerative therapies for muscle disorders including DMD.

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W1088

SMAD4 IS REQUIRED FOR PROPER FUNCTION OF SKELETAL MUSCLE STEM CELLS DURING REGENERATION IN AGED AND YOUNG MICE

Paris, Nicole, Soroka, Andrew, Liu, Wenxuan and Chakkalakal, Joe, University of Rochester, Rochester, NY, U.S.

The TGFβ/SMAD pathway members are conserved regulators of stem cell homeostasis and strongly inhibit myogenic differentiation. It has been proposed that accumulating TGFβ ligand in aging muscle tissue is responsible for regenerative deficits and skeletal muscle loss, making it clinically relevant to investigate inhibition of these signals. Here we utilize a tamoxifen-inducible Cre recombinase, regulated by the satellite cell-specific gene Pax7, to disrupt all canonical TGFβ signaling via SMAD4 deletion in muscle stem cells. In uninjured muscle tissue, SMAD4 protein is virtually absent from satellite cells, whereas it can be readily detected in regenerating tissue. Although at homeostasis there is no noticeable effect on satellite cell maintenance following SMAD4 loss, satellite cell function during regeneration is severely impaired. When isolated by flow cytometry, both young and aged cultured SMAD4-deficient satellite cells fail to expand clonally or incorporate EdU to the extent of control cells. Examination of regenerated skeletal muscle revealed that SMAD4 disruption in satellite cells leads to significantly reduced numbers of Pax7-expressing stem cells and MyoD-expressing myogenic progenitors as well as a deficit in their proliferative potential, assayed by BrdU incorporation. Furthermore, fusion competent myogenic progenitors, labeled by Myogenin, are reduced in number. This failure of myogenic progenitor expansion and proliferation was accompanied by critically impaired myofiber regeneration. These findings identify Smad4 as a critical regulator of satellite cell-derived myogenic progenitor expansion during skeletal muscle regeneration, and raise new questions as to the cellular targets and mechanisms whereby TGFβ inhibition influences aged skeletal muscle regeneration.

W1090

THE EFFECT OF MOTOR DENERVATION ON MUSCLE SATELLITE CELLS

Wong, Alvin, Dreux, Joanna, Garcia, Steven, Tamaki, Stanley and Pomerantz, Jason, University of California San Francisco, San Francisco, CA, U.S.

Satellite cells (SCs) reside between the basal lamina and sarcolemma, express transcription factor paired box protein 7 (Pax7), and repair muscle following injury. After injury, SCs activate, divide, migrate, and fuse with existing myofibers. Previous studies reported SC depletion after long-term denervation, possibly explaining the irreversibility of denervation atrophy, but did not quantify Pax-7^{+}cells nor perform functional studies. It is unknown to what extent SC depletion occurs or limits recovery after delayed reinnervation. Determining whether SCs in denervated muscle survive and retain regenerative ability would direct future approaches to muscle regeneration after denervation injuries. A 4mm segment of the left sciatic nerve in C57Bl6 mice was excised. After 3 months, tibialis anterior muscles (TAs) were weighed and processed for immunohistochemical analysis, and flow cytometry used to deplete Sca-1/CD31/CD45 and select for calcein/VCAM/ITGA7 cells from lower leg muscles. Sorted cells were stained for Pax7 and transcription factor MyoD. TAs weighed 11.9±0.6mg after 3-month denervation vs 49.5±2.5mg ($p<0.0001$). Staining demonstrated no difference in the number of sublaminar Pax7^{+} SCs in denervated TAs compared to contralateral TAs (2.5±0.8 Pax7^{+} cells/100 muscle fibers vs 2.0±0.5, $p=0.4$). 31.2±2.8% of total Pax7^{+} cells in denervated TAs were extralaminar compared to 20.3%±2.8 of Pax7^{+} cells in contralateral TAs ($p<0.01$). FACS analysis revealed 2 distinct populations of cells isolated from denervated and contralateral legs, expressing either relatively high levels of VCAM (VCAM^{hi}) or ITGA7 (ITGA7^{hi}). >90% of SCs isolated from the VCAM^{hi} population expressed Pax7, while cells isolated from the ITGA7^{hi} population expressed MyoD but not Pax7. VCAM^{hi} cells were larger in denervated legs than in the contralateral. The proportion of VCAM^{hi} cells decreases following denervation (62.8%±6.5 vs 78.8%±5.7, $p=0.05$) while ITGA7^{hi}cells increase (37.2%±6.5 vs 21.2%±5.7, $p=0.05$). Muscle denervation alters SC phenotype from quiescence towards activation. While sublaminar SC number is unchanged 3 months after denervation, the percentage of extralaminar Pax7^{+} cells increases, and the proportion of VCAM^{hi} cells decreases. SCs may retain intrinsic regenerative capacity following denervation.

Funding Source: Eli and Edythe Broad Regeneration Medicine and Stem Cell Fellowship California Institute for Regenerative Medicine (CIRM) Grant

PANCREATIC, LIVER, LUNG, OR INTESTINAL/GUT CELLS

W1092

A LOSS-OF-FUNCTION MUTATION IN MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN UNDERLIES THE PATHOPHYSIOLOGY OF ABETALIPOPROTEINEMIA

Liu, YIng[1,2], Bi, Xin[1,3], Slovik, Kate[1,3], Shi, Jianting[1,3], Javaheri, Ali[1,3], Cuchel, Marina[1,3], Yang, Wenli[3], Duncan, Stephen A.[4], Rader, Daneil[1,3] and Morrisey, Edward[1,3], [1]Penn Cardiovascular Institute, Philadelphia, PA, U.S., [2]Cardiovascular Institute, University of Pennsylvania, Philadelphia, PA, U.S., [3]University of Pennsylvania, Philadelphia, PA, U.S., [4]Medical University of South Carolina, Charleston, SC, U.S.

Abetalipoproteinemia (ABL) is an autosomal recessive disease caused by mutations in the gene encoding Microsomal Triglyceride Transfer Protein (MTTP), which transfers lipids onto apolipoprotein B (APOB), thereby promoting secretion of very low density lipoprotein (VLDL) from hepatocytes. Although hepatic MTTP has been studied extensively in mouse models of ABL, little is known about its role in human liver and heart due to the lack of availability of human hepatocytes and cardiomyocytes and the technical challenges of culturing these cells. Here we generated induced pluripotent stem cells (iPSCs) from an ABL patient carrying a R46G mutation in the MTTP gene. iPSC-derived hepatocytes from this patient recapitulated multiple aspects of the pathophysiology of ABL, including hepatic lipid accumulation, high levels of cellular triglyceride and total cholesterol, and absence of APOB expression. Importantly, most of these molecular changes were reversed after the correction of the R46G mutation by CRISPR/Cas9 mediated gene editing. We next examined the role of the MTTP gene in cardiomyocyte metabolism, a cell type previously shown to secrete apoB possibly as a means of protection against toxic effects of lipid excess. We found that iPSC-derived cardiomyocytes harboring the R46G MTTP mutation are hypersensitive to cellular metabolic stress such as hypoxia, high dose of oleic acid, and pharmacological induction of apoptosis as compared with control iPSC-derived cardiomyocytes. These data suggest that MTTP plays a protective role against lipid toxicity in human cardiomyocytes. Taken together, patient specific iPSC derived hepatocytes and cardiomyocytes recapitulate abetalipoproteinemia phenotype in a dish and establish a tool to dissect disease-relevant defects and cellular mechanisms.

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W1094

DIRECTED DIFFERENTIATION OF PLURIPOTENT STEM CELLS REVEALS A CRITICAL ROLE FOR WNT SIGNALING IN THE PROXIMODISTAL PATTERNING OF THE HUMAN LUNG EPITHELIUM

Benson, Katherine[1], Hawkins, Finn[1], Jacob, Anjali[2], Serra, Maria[1], Morrisey, Edward[3], Ikonomou, Laertis[1] and Kotton, Darrell[1], [1]Boston University School of Medicine, Boston, MA, U.S., [2]Boston University, Boston, MA, U.S., [3]University of Pennsylvania, Philadelphia, PA, U.S.

Many common and incurable pulmonary diseases involve perturbations in airway epithelial cell fate. Improved understanding of the signals involved in the normal development of the lung epithelium will therefore provide insight into the mechanisms by which dysregulation of airway cell fate occurs and results in disease. One pathway of particular interest is canonical Wnt signaling, due to prior evidence that stage-dependent oscillations in this pathway are critical for normal lung development. To further elucidate the role of this signaling pathway in human lung cell fate, new studies of purifiable populations at high resolution are required to understand the developmental stage-specific effects and downstream consequences of this signaling. We have therefore differentiated a knock-in NKX2-1^{GFP} human pluripotent stem cell (hPSC) line to NKX2-1+ endodermal lung progenitors to study the effects of Wnt signaling on these cells post-lineage specification. These lung progenitors are heterogeneous and express early markers of both the proximal and distal lung epithelium. Modulation of canonical Wnt signaling in these cells led to rapid changes in the NKX2.1+ population consistent with altered airway patterning. Specifically, withdrawal of the GSK3B inhibitor CHIR99021 resulted in decreased canonical Wnt signaling, as measured by a lentiviral TCF reporter, and in gene expression changes consistent with increased proximal and secretory lung fate and loss of distal identity. Manipulation of Wnt in purified NKX2.1+ cells resulted in similar patterning changes, suggesting that this effect is intrinsic to the epithelium. Taken together, our findings suggest that sustained canonical Wnt signaling inhibits the proximal airway program in developing human lung progenitors consistent with in vivo murine models. These results have allowed us to generate improved protocols for the differentiation of three-dimensional proximal lung organoids from hPSCs. Ongoing studies seek to understand the mechanisms driving this inhibition to elucidate the key signals that regulate airway cell fate in development and disease. This novel system will provide the basis for the development of in vitro models of airway disease in patient-derived hPSCs, a crucial barrier to applications of cell-based therapy and in vitro drug testing.

W1096

A PREDICTIVE MODELING OF HEPATIC DIFFERENTIATION PROPENSITY IN HUMAN PLURIPOTENT STEM CELLS

Fukuda, Takayuki, Laboratories of Stem Cell Cultures, Department of Disease Bioresources Research,, Ibaraki, Japan

Hepatocyte-like cells differentiated from human pluripotent stem cells (hPSCs) are expected to be utilized as a tool for screening for hepatotoxicity in the early phase of pharmaceutical development. However, previous studies demonstrated that hPSCs have individually distinct potential to differentiate into specific lineages. Selection of suitable hPSC lines or clones by predicting differentiation propensity of undifferentiated hPSCs for hepatocytes would substantially accelerate developing application using hPSC-derived hepatocyte-like cells for pharmaceutical research. In this study, we have compared the expression levels of genes related with hepatic differentiation and cell signaling in five hPSC lines to predict differentiation propensity of hPSC lines for hepatocytes. Gene expression patterns of undifferentiated hPSCs, hPSC-derived hepatocyte-like differentiated cells and hepatocytes were grouped by two way clustering analysis. Then, three genes were selected to distinguish characteristics of undifferentiated hPSCs among the cells studies here. To predict differentiation propensity, expression of the genes in 21 hPSC lines were examined. In fact, the hPSC line showed lower hepatic differentiation propensity, suggesting that our analytical approach would be used to predict the differentiation propensity.

W1098

CROSS-TALK BETWEEN CELL CYCLE AND ACINAR DIFFERENTIATION IN PANCREATIC CANCER REPROGRAMMING

Kim, Heejung[1,2], Lahmy, Reyhaneh[2] and Itkin-Ansari, Pamela[2], [1]California State University, San Marcos, San Marcos, CA, U.S., [2]Sanford Burnham Presby Medical Discovery Institute, La Jolla, CA, U.S.

Pancreatic ductal adenocarcinoma (PDA) is the 4th leading cause of cancer death in the US. Despite current treatments including surgery, radiation or chemotherapy, the 5 year survival rate is less than 5%. There have been tremendous efforts to find effective ways to combat this lethal disease but without significant progress in the past 30 years. Thus novel approaches to treating PDA are needed. Our lab queried whether altering acinar transcriptional networks could reprogram PDA cells. PDA arises from acinar cells in the exocrine pancreas in response to Kras mutation. During progression to PDA there is significant dysregulation of bHLH signaling. We found that restoring bHLH activity by overexpression of the bHLH protein E47

is sufficient to reprogram conventional human PDA lines and established patient derived xenograft PDA lines into quiescent cells with acinar characteristics. E47 upregulation induces growth arrest by inducing cell cycle inhibitors, including p21. E47 induced acinar differentiation is characterized by re-expression of numerous digestive enzymes including trypsinogen. Here we began to explore possible crosstalk between the growth and differentiation pathways, by asking whether growth arrest is sufficient to induce acinar differentiation. For these studies, 3 PDA lines were exposed to nutrient deprivation to halt their growth in the absence of exogenous E47. The cells lines are routinely cultured in 10-12% fetal calf serum. For each PDA line, serum in culture media was reduced to a concentration that arrested cell growth, as determined by Ki67 and growth curves, yet did not induce significant cell death. The final serum concentrations for growth arrest ranged from 1% down to 0.1% and cells were treated for 72 to 144 hours. At the molecular level serum deprivation raised p21 levels in all cell lines and induced low levels of the exocrine enzyme trypsinogen to a level consistent with that observed by E47 overexpression. In conclusion, nutrient sensing efficiently regulates growth but does not completely control cell fate in PDA. Moreover, E47 induction of acinar cell fate is independent of its effects on growth. The data suggest that bHLH signaling may be the final common denominator between the growth and cell fate pathways, making it an attractive target for therapeutic intervention.

W1100

HETEROGENEITY OF MESENCHYMAL CELLS IN THE ADULT LUNG: IMPLICATIONS FOR EPITHELIAL REGENERATION.

McQualter, Jonathan L., Carraro, Gianni, Selvaggio, Anna and Stripp, Barry, Cedars-Sinai Medical Center, Los Angeles, CA, U.S.

Regeneration of the adult lung epithelium requires a balance between promoting stem cell proliferation while also acting to limit these responses once the tissue has been adequately regenerated. While our knowledge of epithelial stem cells and the lineage relationships of their progeny are increasing, our understanding of the heterogeneity and distinct functions of the surrounding mesenchymal cells that are essential for regulating epithelial cell fate is lacking. We have previously revealed that resident lung mesenchymal cells are both necessary and sufficient to support the proliferation and differentiation epithelial stem cells in vitro. Moreover we have established that the epithelial-supportive capacity of the mesenchymal cells as a population is transient and directly correlates with their state of differentiation. We have shown that mesenchymal cells with high proliferative potential and multi-lineage differentiation potential are able to support the proliferation of epithelial stem cells, while more differentiated myofibroblasts are not. The present study sought to elucidate the heterogeneity and associated functions of mesenchymal cell lineages in the adult lung. To address this we adapted our three-dimensional epithelial colony-forming assay to model epithelial-mesenchymal interactions involved in epithelial regeneration. Using high throughput flow cytometry-based surface marker analyses we have identified several phenotypically distinct mesenchymal cell populations from mouse and human lungs, suggesting that not all mesenchymal cells are alike. At a functional level the different cell subsets that we have identified also displayed significant differences in proliferation, mesenchymal differentiation and epithelial support. Thus, mesenchymal cells in the adult lung represent a dynamic epithelial stem cell niche in which the heterogeneity and temporal differentiation of mesenchymal cells carefully controls epithelial regeneration.

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W1102

FLUID SHEAR STRESS INDUCES CYTOCHROME ACTIVITY IN 2D- AND 3D-CULTURED HUMAN EMBRYONIC STEM CELL-DERIVED HEPATOCYTE-LIKE CELLS.

Rashidi, Hassan, University of Edinburgh, Edinburgh, U.K.

The isolation and culture of freshly isolated human adult hepatocytes is considered to be the gold standard tool to evaluate human drug metabolism and safety in vitro. However, primary hepatocyte scarcity, cell cycle arrest and the rapid loss of liver-phenotype post isolation are major limitations. Immortalized and hepatoma cell lines have therefore been employed as potential alternatives for in vitro evaluation of drug screening and toxicity, however, their poor functionality, karyotypic instability and higher tolerance to toxicological insult limits their widespread application. Human embryonic stem cells and induced pluripotent stem cells provide renewable resources to obtain hepatocyte-like cells (HLCs) in vitro. Despite the use of various differentiation protocols, derived hepatic cells, like primary human hepatocytes, exhibit unstable and poor phenotype *i*n vitro. It has been shown that the functional capacity can be improved by adding back elements of human physiology, such as cell co-culture or through the use of different extracellular matrices, such as laminin 111 and 521. Here the effect of fluid shear stress on HLC phenotype was investigated. The data obtained demonstrates that fluid shear stress did not compromise cell viability and induced cytochrome P450 enzyme activity. Therefore, the field might benefit from adaptation of fluid shear stress with high throughput screening plat-

forms and to develop more efficient stem cell derived liver systems.

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W1104

LIVER STEM/PROGENITOR CELLS IN THE MOUSE LIVER REGENERATION PROCESS WITH LOCAL INFLAMMATION AFTER A PHYSICAL PARTIAL HEPATECTOMY

Suzuki, Yuji[1,2], Katagiri, Hirokatsu[1], Wang, Ting[2], Kakisaka, Keisuke[2], Kume, Kohei[1,3], Nishizuka, Satoshi S.[1,3] and Takikawa, Yasuhiro[2], [1]Molecular Therapeutics Laboratory, Department of Surgery, Iwate Medical University School of Medicine, Morioka, Japan, [2]Division of Hepatology, Department of Internal Medicine, Iwate Medical University School of Medicine, Morioka, Japan, [3]Institute of Biomedical Sciences, Iwate Medical University, Yahaba, Japan

Liver stem/progenitor cells (LPCs) are thought to differentiate into both hepatocytes and biliary cells, which eventually contribute to tissue repair. In the situation of a conventional partial hepatectomy model in rodents, which removes approximately 2/3 of the liver by lobular ligation without tissue dissection, liver mass is restored by hypertrophic reaction and replication of existing hepatocytes, with or without minimal contribution of LPCs. However, a hepatectomy in humans involves physical damage (i.e., physical partial hepatectomy, PPHx). Therefore, the liver regeneration process after PPHx should involve reactions to acute local injury followed by systematic remodeling. Here, using a murine liver injury model that mimics the actual human surgical procedure, we aimed to determine the contribution of LPCs during the tissue repair process. A 20–30% PPHx was performed by transection of the left lobe of the liver, using an ultrasonically-activated scalpel, in mice. Gene expression and morphological characteristics were analyzed during the liver regeneration process. Proliferation of mouse LPCs cultured with TNF-α, NFκB inhibitor BAY11-7082, or a combination of the two were examined in vitro. Liver weight continuously increased by hypertrophic reaction of hepatocytes, while Ki67 staining showed hepatocyte proliferation. At the transected border after 48 h PPHx, CK19-positive LPCs that form bile duct-like structures were observed, suggesting ductular reactions. Gene expression of the transected border and non-damaged lobes revealed that inflammatory cytokine- and extracellular matrix-associated genes were significantly up-regulated at the transected border. Inflammatory infiltrating cells including α-SMA-positive hepatic stellate cells were expressed MMP-9 at the transected border. In vitro experiments revealed that administration of TNF-α induced LPC proliferation via the IKK/NFκB inflammatory pathway. Our results suggest that LPCs emerged from the local inflammatory proliferating cells during the tissue repair process after PPHx. These results may provide insight for clarifying the mechanisms of liver regeneration from injury and inflammation of the liver.

W1106

ANTI-ONCOGENIC FUNCTION OF HOMEOBOX HOXA13 IN HUMAN GASTRIC CANCER CELLS DERIVED INDUCED PLURIPOTENT STEM CELLS

Yokoyama, Kazunari K.[1], Tsai, Ming-ho[1], Kenly, Wuputra[1], Ku, Chia-Chen[1], Liu, Pei-Zhen[1], Lin, Wen-Hsin[1], Lin, Yin-Chu[2], Wu, Deng-Chyang[3] and Lin, Chang-Shen[1], [1]Koahsiung Medical University, Kaohsiung, Taiwan, [2]Koahsiung Medical University, Koahsiung, Taiwan, [3]Koahsiung Medical University Hospital, Koahsiung, Taiwan

Homeobox HOXA13 is known as the transcriptional regulators in cell proliferation, differentiation and development, and also have been found to be deregulated in tumorigenesis. However, less is known about the involvement of HOXA13 with gastric cancer development and its direct targets were unknown. To understand the role of HOXA13 in cancer development, we generated induced pluripotent stem (iPS)-like cells from human gastric cancer cell line CS12 and its immortalized normal cell line CSN by electroporation using stemness gene OCT4 and AP-1 repressor gene Jun dimerization protein 2 (JDP2), and examined the role of HOXA13. The knockdown experiments with shRNA against HOXA13 on xenotransplantation in SCID mice demonstrated that HOXA13 plays a critical role in development of the gastric cancer; increased expression in CS12 and further induction in CS12 derived iPS-like cells, however, the tumor formation was shut down in CS12 iPS-like cells. By studying the target genes of HOXA13, we identified bone morphogenetic protein 7 (BMP7), which is significantly induced by HOXA13 in CS12, but not in CS12 iPS-like cells, and also BMP7 is a critical factor for gastric cancer development. Moreover, the long noncoding RNA (lncRNA) HOTTIP is involved in co-recruitment of HOXA13 in BMP promoter in CS12 cells but not in CSN cells, inducing histone H3K4me3. Instead other lncRNA TOTAIR is involved in the recruitment to the BMP promoter to induce the trimethylation of H3K27me3 in BMP7 promoter. Taken together, the recruitment of HOXA13-HOTTIP and HOXA13-HOTAIR to BMP7 gene promoter is critical for decision of oncogene and anti-oncogene in human gastric cancer cells.

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ENDOTHELIAL CELLS/ HEMANGIOBLASTS

W1110

ROBUST AND EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO ENDOTHELIAL AND VASCULAR SMOOTH MUSCLE CELLS TO STUDY CORONARY ARTERY DISEASE PREDISPOSITION.

Challet Meylan, Ludivine and Cowan, Chad, Harvard University, Cambridge, MA, U.S.

Current protocols to differentiate pluripotent cells into endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) often rely on embryoid body formation and the use of fetal calf serum. As a result, the differentiation of vascular cells from human pluripotent stem cells (hPSCs) remains inconsistent, inefficient, and time consuming. Developing differentiation protocols that accurately recapitulating key events regulating cell lineage specification in the embryo has proven to be a powerful and reproducible approach for generating highly enriched populations of differentiated cells. In mammals, vascular progenitors emerge from lateral and posterior mesoderm in a Wnt dependent fashion that also requires a careful balance of BMP4 and Nodal signaling. Based on these observations, we recently published a rapid, robust and highly efficient protocol for the differentiation of vascular cells from hPSCs that utilizes the constitutive activation of Wnt signalling via a GSK-3 inhibitor (CHIR-99021). Our protocol results in the differentiation of hPSCs to ECs and VSMCs at efficiencies of 50%-80% and 85-99% respectively in only 6 days. ECs can be further enriched to more than 95% purity by magnetic-activated cell sorting (MACS). Using transcriptional profiles, we determined that our ECs and VSMCs were similar to primary cell lines (HCAECs and UASMCs) and exhibited mature functional properties including, patent tube formation wound healing, and angiogenic sprouting. We are currently using these differentiated cells and the CRISPR/Cas9 system to decipher by which mechanism(s) risk variants confer CAD susceptibility in the 9p21 locus.

W1112

CD34+ CD45- CELLS ISOLATED FROM HUMAN BLOOD HAVE MOLECULAR AND FUNCTIONAL CHARACTERISTICS OF VASCULAR ENDOTHELIAL PROGENITORS

Jiang, Yajuan[1], Meissner, Alexander[2] and **O'Neill, David[1]**, [1]PCT, a Caladrius Company, Allendale, NJ, U.S., [2]Harvard University, Cambridge, MA, U.S.

Rare CD45- cells have been described in adult human blood, bone marrow and umbilical cord blood that lack blood cell lineage markers (Lin-) but express antigens found on hematopoietic progenitors (CD34, CD133). The function of these Lin- CD45- CD34 and/or CD133+ (CD34/133+) cells remains poorly understood. To better understand and characterize the cells, we purified them from G-CSF mobilized blood by elutriation, immunomagnetic selection and FACS sorting, analyzing them using 6-color flow cytometry including dyes that distinguish live nucleated cells from dead cells, vesicles and debris. We observed that >90% of the Lin- CD45- CD34/133+ objects in mobilized human blood were extracellular vesicles, and that <10% of the objects were nucleated cells, almost all of which expressed CD34 but not CD133. This Lin- CD45- CD34+ CD133- nucleated cell population had a frequency of approximately 1 in 300,000 PBMC. Lin- CD45- CD133+ cells were variably seen but extremely rare (1 in 10 million PBMC). Similar vesicle and cell populations were found in umbilical cord blood. We then sorted single Lin- CD45- CD34/133+ cells from adult blood into wells of 384-well plates for targeted RNA sequencing (Cellular Research Precise™ assay) using a panel of 150 primers for mesodermal, pluripotent and hematopoietic-associated RNAs. CD45+ CD34/133+ hematopoietic progenitors were sorted as controls (about 400 cells analyzed for both populations). The two populations had very distinct gene expression patterns, with CD45+ cells having RNAs consistent with hematopoietic progenitors (CD44, CD133, HHEX, MYB, MYC, CD49d, FLI1) and CD45- cells having RNAs associated with the vascular endothelial lineage (TM4SF1, KLF4, CDH5, SOX7, SOX17, KDR, CD49f, H19, IGF2, CD146, Nestin). No clear evidence of heterogeneity was seen within either cell population. To assess for endothelial progenitor function, we FACS sorted umbilical cord blood into CD45-CD34-, CD45+CD34-, CD45-CD34+ and CD45+CD34+ fractions for analysis using a wellestablished bioassay (ECFC assay). Enrichment of ECFC activity (up to 3,000-fold) was observed only in the CD45-CD34+ fraction. Cells expanded from these colonies had both phenotypic (vWF, KDR, CD31, CD144, CD146) and functional (capillary-like structures in Matrigel, Ac-LDL uptake) characteristics of endothelial cells.

W1114

VASCULAR DISEASE PATHOGENESIS RECAPITULATED WITH INDUCED PLURIPOTENT STEM CELLS (IPSCS) FROM HEREDITARY HAEMORRHAGIC TELANGIECTASIA (HHT) PATIENTS

Orlova, Valeria[1], Freund, Christian[1], van den Hil, Francijna[1], Petrus-Reurer, Sandra[2], Gkatzis, Konstantinos[1], Hawinkels, Lucas[1], Ploos van Amstel, Hans Kristian[3], Mager, Hans-Jurgen[4], ten Dijke, Peter[1] and Mummery, Christine L.[1], [1]Leiden University Medical Center, LEIDEN, Netherlands, [2]Karolinska Institute, Stockholm, Sweden, [3]Utrecht University Medical Center, Utrecht, Netherlands, [4]St Antonius Hospital, Nieuwegein, Netherlands

Patient-specific induced pluripotent stem cells (iPSCs) possess unprecedented ability to model genetic diseases, as they can serve as a source of virtually any specialised differentiated cells. Therefore, they can be useful to model complex genetic diseases with non-cell autonomous phenotype. This is, in particular, true for vascular disease hereditary haemorrhagic telangiectasia (HHT) that results in fragile blood vessels with defective endothelial cell (ECs)-mural cell coverage. Affected individuals develop small telangiectasia and are suffer from nosebleeds due to unstable vessels with excessive angiogenesis. Arteriovenous malformations (AVMs) are the major clinical manifestations in HHT patients and can be a potentially life-threatening condition. HHT is caused primarily by autosomal-dominant mutations in members of transforming growth factor (TGF)-β signalling pathway, and can be classified into HHT1 caused by mutations in ENG, and HHT2 caused by mutations in activin receptor-like kinase (ALK1). Both ENG and ALK1 are expressed in endothelial cells (ECs), and their mutations in mouse models result in defective ECs sprouting and EC-mural cell interactions. Here we demonstrated that ECs from patient-specific HHT1-iPSCs recapitulate the disease pathogenesis. ENG expression in ECs from HHT1-iPSCs was reduced relative to controls to a similar extent as on primary peripheral blood monocytes from HHT1 patients. This ENG deficiency, however, did not affect TGFβ-mediated signal transduction, or EC function in standard EC assays but co-culture of HHT1-iPSC ECs with pericytes from control iPSCs revealed multiple abnormal EC functions. These included atypical sprouting, altered proliferation and reduced competence to induce a contractile phenotype in control pericytes, recapitulating defective endothelial-pericyte cell interactions believed to contribute to the disease pathology in patients. In addition, HHT1-iPSC ECs showed reduced secretion of soluble ENG in culture, correlating with reduced ENG levels in the blood of HHT1 patients. We thus demonstrated that iPSC-derived ECs model this genetic vascular disease, providing a platform for revealing underlying disease mechanisms.

EPITHELIAL CELLS (NOT SKIN)

W1116

ODONTOGENIC ABILITY OF HUMMAN AMNIOTIC EPITHELIAL CELLS

HAN, LU, West China college of stomatology, Cheng du, China

Abstract Recently, tooth tissue engineering has become a promising new therapy for tooth loss or absence. Previous studies have demonstrated that finding a suitable source of epithelial stem sells for making teeth is an important issue.The Amniotic Epithelial cells (AECs) have been proved to possess enormous regenerative potential. The present study focuses on the AECs for making teeth in vivo and their relative efficiency. In this study, we induced AECs which were extracted from the lining of the inner membrane of the placenta by Tooth Germ Cell Conditioned Medium (TGC-CM). The induced AECs displayed similar features related to morphology, proliferation rates, expressions of various cell surface markers and differentiation potentials into tooth. DSP,DMP-1 demonstrated significant levels of staining in an immunofluorescence analysis at 28days after the induced AECs were implanted under the renal capsule of test mice. Our in vivo data confirms the ability of AECs can be induced to differentiate toward odontoblast-like cells with TGC-CM and provide a novel strategy for tooth regeneration research.

W1118

DEPENDENCE OF MOUSE LUNG EPITHELIAL STEM CELL PROLIFERATION AND DIFFERENTIATION ON FIBROBLAST-SPECIFIC FGF2 SIGNALING

Hegab, Ahmed E.M.[1], Ozaki, Mari[1], Guzy, Robert[2], Ornitz, David M[3] and Betsuyaku, Tomoko[1], [1]Keio University School of Medicine, Tokyo, Japan, [2]The University of Chicago, Chicago, IL, U.S., [3]Washington University, St. Louis, MO, U.S.

Idiopathic pulmonary fibrosis (IPF) affects thousands of patients worldwide. Pathological and genetic association studies have implicated epithelial injury and aberrant repair in IPF pathogenesis. Fibroblast Growth Factors (FGFs) are involved in the repair of injured alveolar epithelium and induction of fibroblast proliferation in IPF. We have reported that FGF2 is essential for epithelial repair and for maintaining epithelial integrity after bleomycin-induced lung injury (Guzy, AJRCMB, 2015). In addition, FGF2 activated lung epithelial progenitors to proliferate and differentiate into alveolar lineage (Hegab, Stem Cell Res, 2015).

The purpose of this study is to elucidate the requirement of FGF2 for the proliferation and differentiation of lung epithelial progenitor cell, and to identify the mechanism by which FGF2 promotes lung epithelial repair after injury. Fibroblasts are an essential component of the lung stem cell niche. Lung fibroblasts were isolated from $Fgf2^{-/-}$ and their littermate wild type (WT) mice then co-cultured with WT whole lung epithelium in the 3D colony formation stem cell assay. We have previously established this assay and described its exploitation as an assay to study lung stem cells and their niche (Hegab, Stem Cell Res, 2015). Growing colonies from both fibroblast genotypes were compared for number, type and size. Then colonies were collected, and histologically analyzed for effect on differentiation. Additional sets of wells were treated with rFGF2. In the Fgf2-/- fibroblast co-culture, colonies were smaller in size and fewer in number compared to those growing in the WT co-culture. The alveolar colonies (Type C colonies) were the most affected by the absence of FGF2 from the fibroblasts, indicating the importance of FGF2 for alveolar cell proliferation and colony formation. rFGF2 treatment to the Fgf2-/- fibroblast co-culture wells rescued the colonies to a lever comparable to the WT fibroblasts co-culture wells. rFGF2 treatment to the WT fibroblasts co-culture wells produced colonies that were bigger in size and more in number, mostly alveolar colonies. However, no significant effect on differentiation profile was observed in any of the different colony types.

Lung Fibroblasts FGF2 plays a critical role in supporting lung epithelial progenitor activation and proliferation.

W1120

IMMUNO-PRIVILEGED CORD LINING EPITHELIAL CELLS FOR ADVANCED WOUND REPAIR - A PILOT IN VIVO STUDY

Li, Yanzhe[1], Chua, Alvin W.C[2], Kua, Jonah E.H.[2], Phan, Toan Thang[1,3], Lim, Fui Ping[1], Masilamani, Jeyakumar[3], Chan, Sui Yung[1] and Cheong, Han Hui[1,4], [1]National University of Singapore, Singapore, Singapore, [2]Singapore General Hospital, Singapore, Singapore, [3]CellResearch Corporation Pte Ltd, Singapore, Singapore, [4]KK Women's and Children's Hospital, Singapore, Singapore

Wound healing is a complex and dynamic process resulting in the restoration of normal function and structure after injury. In larger, more severe wounds such as trauma or burn wounds, skin grafting is necessary to prevent complications like dehydration and infection following debridement. Human cadaveric allograft skin is commonly used as a temporary biologic dressing. However, this standard practice has considerable limitations which immunological rejection poses a prominent obstacle to effective wound repair due to the potent inflammatory responses elicited in its course. Epithelial stem cells isolated from the umbilical cord have been characterized to have immuno-privileged properties, making it an attractive candidate for stem cell therapy in advanced wound repair. The objective of this study is to compare the safety and efficacy of human cord lining epithelial cells (CLEC) versus cadaveric skin treatment for advanced wound repair. Animal excisional wound models were created on two Yorkshire pigs. Each pig was randomly allocated to either CLEC topical treatment or human cadaveric skin treatment. Human cadaveric skin graft or CLEC at a density of 1.0 x 10^5 cells per cm^2 was spread evenly over each wound bed. Topical wound treatments were performed on Day 0, 4, 14, 28 and 42. Wounds were inspected weekly; experimental endpoint was reached upon the achievement of complete wound repair. Both wound and blood analysis were carried out throughout the course of treatments to evaluate the extent of immunological rejection and rate of wound repair. It was found that the final wound contraction was similar between CLEC- and cadaveric skin- treated wound. However, CLEC-treated wound demonstrated a faster rate of wound closure and achieved complete wound healing 6 days earlier as compared to cadaveric skin-treated wound. This significantly shorten duration of wound healing is clinically critical for survival of patients with severe wounds. In addition, serum proinflammatory cytokines were found to be lower in CLEC-treated wounds. CLEC is easily accessible, in abundance and ethically acceptable, and therefore the great potential of CLEC in clinical application of wound healing is apparent.

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W1122

BMP REGULATES NEPHRON PROGENITOR CELLS THROUGH SMAD DEPENDENT AND INDEPENDENT SIGNALING

Oxburgh, Leif, Muthukrishnan, Sree Deepthi, Brown, Aaron and Sarkar, Prasenjit, Maine Medical Ctr Research Institute, Scarborough, ME, U.S.

The nephron progenitor cell (NPC) gives rise to all of the cells of the nephron with the exception of the vasculature. Importantly, new nephron formation is limited to the embryonic period, and there is therefore a need for tissue engineering approaches to the treatment of kidney disease in the adult. Recent work has shown that the NPC can be isolated from developing mouse kidneys and propagated *ex vivo* with maintained differentiation potential. Furthermore, several directed differentiation protocols have been developed to derive this cell type from human ESC and iPSC. Our goal is to develop in vitro niches in which naturally occurring and stem cell derived NPCs can be expanded in their undifferentiated state or differentiated to epithelial structures in a controlled manner, enabling

us to form large quantities of laboratory-grown tissue with predictable differentiation properties. This requires a detailed understanding of the dynamic cell signaling environment in which NPCs are located within the developing kidney. BMP, FGF, and WNT signaling act in concert to control both maintenance of the progenitor cell state, and commitment to epithelial differentiation. We show that BMP signals through TAK1, MYC, and JUN to promote G1-S transition in NPCs. However, we also show that BMP primes NPCs for epithelial differentiation in response to local WNT signaling. This signaling event is mediated through Smad signaling and removes cells from the NPC pool through differentiation. Interestingly, BMP-TAK signaling inhibits BMP-Smad signaling, and the BMP pathway thus maintain an intrinsic balance between NPC maintenance and differentiation. We show that the amplitude of BMP signaling influences the choice of TAK or Smad signaling in NPCs, and we present a model for how BMP, FGF, and Wnt signaling may integrate.

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W1124

ALVEOLAR EPITHELIAL CELL THERAPY RESCUES THE LUNG PHENOTYPE IN A MOUSE MODEL OF SURFACTANT PROTEIN C DEFICIENCY

Shafa, Mehdi[1], Vadivel, Arul[1], Xu, Liqun[1], Collins, Jennifer[2], de Caen, Genevieve[1], Xu, Xin[1] and Thébaud, Bernard[1], [1]Ottawa Hospital Research Institute, Ottawa, ON, Canada, [2]Ottowa Hospital Research Institute, Ottowa, ON, Canada

Pulmonary surfactant protein C (SP-C) is synthesized by type II Alveolar epithelial cells (AEC-II) as a precursor and is processed to form the functional secreted protein. Mutations in the SP-C gene (Sftpc) are inherited as an autosomal dominant trait and lead to a misfolded protein and subsequent cellular stress in AEC-II. Sftpcmutations are linked to respiratory distress in term newborn infants, hereditary forms of idiopathic interstitial pneumonitis in older children, and chronic pulmonary fibrosis and emphysema in adults. Histological features due to SP-C deficiency include alveolar remodeling, airspace loss, fibrosis, cellular infiltrates and epithelial cell dysplasia in conducting and peripheral airways. The application of wild type AEC-II and human induced pluripotent stem cell (hiPSCs)-derived AECs-II to rescue a lung genetic disease has never been investigated. In this study, we hypothesized that AEC-II and hiPSC-derived AEC-II therapy can rescue the phenotypic and pathological consequences of Sftpc gene knock-out in SP-C deficient mice ($SPC^{-/-}$). Wild type AEC-II were isolated from 4-6 weeks old 129J mice by enzymatic digestion followed by Ficoll-purification and non-plastic adherence selection. We also established a highly efficient method to differentiate hiPSCs into a homogenous population of AEC-II. Both cell types were labelled by a membrane-specific fluorescent dye and were intratrachealy administered as single cells to one year old $SPC^{-/-}$ mice. Cyclosporin A was administered to mice that received human cells to prevent immune rejection. Mice were treated with two doses of AEC-II during a period of 12 days. Age matched $SPC^{-/-}$and 129J mice that did not receive cells were controls. Freshly isolated AEC-II engrafted into the distal lung, improved lung function and structure and attenuated cell infiltration in $SPC^{-/-}$ mice. AECs therapy increased the exercise capacity and improved lung mechanical properties. Our results also showed that hiPSC-derived AEC-II were retained in the distal lung, engrafted into alveolar structure and attenuated lung structural injuries. Patient-specific AEC-II therapy exerts short-term therapeutic benefit in this experimental model and may offer new therapeutic options for lung genetic disorders that affect alveolar epithelial cells.

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W1126

IN VITRO PROPAGATION AND BRANCHING MORPHOGENESIS FROM SINGLE URETERIC BUD CELLS

Yuri, Shunsuke and Yanagawa, Norimoto, UCLA VAGLAHS at Sepulveda, North Hills, CA, U.S.

With the recent advancement in kidney regeneration using stem cell biology and tissue engineering techniques, there is an increasing need to develop culture systems that can maintain and expand kidney progenitor cells in vitro with defined factors in a serum-free condition. We aimed in our present study to establish such a system for the maintenance and expansion of ureteric bud cells. We found that the survival of ureteric bud cells in a serum-free culture condition required signaling from fibroblast growth factors or retinoic acid, while the proliferation and maintenance of ureteric bud tip cells required glial cell-derived neurotrophic factor together with signaling from either Wnt-β-catenin pathway or retinoic acid. We also found that R-spondin ligand-receptor relationship is conserved between metanephric mesenchyme and ureteric bud, and R-spondins are required for the activation of β-catenin signaling in ureteric bud cells by endogenous Wnt proteins. We further found that Rho kinase inhibitor was required for the survival and expansion of dispersed single ureteric bud cells under such culture condition. With this culture system, we demonstrated the ability of single ureteric bud cells to form colonies and reconstruct ureteric bud-like structures retaining the in vivo characteristics of the original ureteric bud. In conclusion, we describe in our present study a serum-free culture system suitable for the maintenance and expansion of dispersed ureteric bud cells in vitro. The ability to re-build ureteric bud-like structures from single ureteric bud cells under our culture

condition could provide a useful tool for kidney regeneration with kidney progenitor cells.

EPIDERMAL CELLS

W2002

DUAL ROLE OF THE ANAPHASE PROMOTING COMPLEX/CYCLOSOME IN REGULATING STEMNESS AND DIFFERENTIATION IN THE HUMAN SKIN EPITHELIUM

Quek, Ling Shih, Grasset, Nicolas, Tan, Chye Ling and **Bellanger, Sophie**, A*STAR, Singapore, Singapore

The Anaphase Promoting Complex/Cyclosome (APC/C) activates the transition from M to G1 when bound to Cdc20 but negatively regulates the G1/S transition when bound to Cdh1. Although a direct role of APC/C in stemness maintenance - or loss - has never been established in the embryo or in adult tissues, a low APC/C-Cdh1 activity in human embryonic stem cells (hESC) correlates with fast proliferation and a short G1 phase, whereas APC/C-Cdh1 activity increases upon differentiation. Using the skin epithelium (which contains both adult stem cells and differentiated cells) as a model to study the fate of adult stem cells, we addressed the role of APC/C in both stemness maintenance and differentiation of human primary keratinocytes. Keratinocyte stem cells are characterized by high proliferation capabilities. When cultured on 3T3-J2 feeders *in vitro*, they give rise to large undifferentiated proliferating colonies (holoclones), whereas cells committed to differentiation appear as small colonies (paraclones). In a heterogeneous population of human primary keratinocytes, we found that while inhibiting APC/C-Cdc20 drastically inhibits holoclone growth and enhances differentiation, Cdh1 silencing reproducibly increases the holoclones/paraclones ratio. Molecular biology approaches allowed us to determine that Cdc20-silenced cells express high level of early (e.g. Keratin 1 and Keratin 10) and late (e.g. Involucrin and Filaggrin) keratinocyte differentiation markers. In contrast, silencing Cdh1 significantly decreases expression of these markers. In conclusion, our data show that in the human skin epithelium the APC/C plays a critical role in deciding on the fate of adult stem cells.

Funding Source: This work is funded by A*STAR Singapore.

W2004

AXIN2 MARKS QUIESCENT HAIR FOLLICLE BULGE STEM CELLS THAT ARE MAINTAINED BY AUTOCRINE WNT/β-CATENIN SIGNALLING

Lim, Xinhong[1], Tan, Si Hui[1], Yu, Ka Lou[1], Lim, Sophia Beng Hui[1] and Nusse, Roeland[2], [1]Institute of Medical Biology, Singapore, Singapore, [2]Howard Hughes Medical Institute, Department of Developmental Biology, Stanford, CA, U.S.

How stem cells maintain their identity and potency as tissues change during growth is not well understood. In mammalian hair, it is unclear how hair follicle stem cells can enter an extended period of quiescence during the resting phase but retain stem cell potential and be subsequently activated for growth. Here, we use lineage tracing and gene expression mapping to show that the Wnt target gene Axin2 is constantly expressed throughout the hair cycle quiescent phase in outer bulge stem cells that produce their own Wnt signals. Ablating Wnt signaling in the bulge cells causes them to lose their stem cell potency to contribute to hair growth and undergo premature differentiation instead. Bulge cells express secreted Wnt inhibitors, including Dkks and Sfrp1. However, the Dkk3 protein becomes localized to the Wnt-inactive inner bulge that contains differentiated cells. We find that Axin2 expression remains confined to the outer bulge while Dkk3 continues to be localized to the inner bulge during the hair cycle growth phase. Our data suggest that autocrine Wnt signaling in the outer bulge maintains stem cell potency throughout hair cycle quiescence and growth, while paracrine Wnt inhibition of inner bulge cells reinforces differentiation.

Funding Source: This work was supported by the HHMI, California Institute of Regenerative Medicine grant TR1-01249, and the Agency for Science, Technology and Research in Singapore.

W2006

HUMAN LONG TERM DEREGULATED CIRCADIAN RHYTHM IN VIVO ALTERS CLONOGENIC PROPERTIES OF HAIR FOLLICLE KERATINOCYTES

Deshayes, Nathalie, Genty, Gaïanne, Dimitrov, Ariane and **Paris, Maryline**, L'Oreal R&I, Aulnay-sous-Bois, France

In humans, epidermal stem cell function in vitro is regulated by circadian oscillations, the deregulation of which may contribute to skin aging. Circadian arrhythmia of hair follicle precursor cells (keratinocytes located in the outer root sheath, ORS) contributes to age-related hair cycling defect, in mice. Despite the well-described impact of circadian oscillation, involving a CLOCK gene pathway

feedback loop, on keratinocyte function, little is known about changes in the clonogenic potential of human Hair Follicle (hHF) keratinocytes after a long-term alteration of the circadian rhythm *in vivo*. This study assessed the properties of hHF keratinocytes through a CLOCK pathway alteration due to long-term deregulated circadian rhythm. First, the physiological relevance of the study was validated. Using a 3D Spinning disk imaging approach on micro-dissected hHF's, we demonstrated the expression of Per1 and Bmal1 (two CLOCK pathway proteins) in keratinocytes of the upper and lower ORS. Second, we compared the CLOCK pathway protein expression and hHF keratinocyte properties in two groups of women: shift workers and diurnal workers. Cell culture characterization, measurement of colony area and immunostaining were performed. We demonstrated that long term circadian rhythm deregulation affected CLOCK pathway protein expression and was correlated with alteration in hHFK clone-forming efficiency. This study, for the first time in humans, provides evidence that *in vivo* alterations of the CLOCK gene pathway affect the clonogenic properties of hHF precursor cells and circadian protein expression.

EYE OR RETINAL CELLS

W2010

HIGH EFFICIENCY DIFFERENTIATION OF NEURAL RETINA FROM MOUSE PLURIPOTENT STEM CELLS IN THREE-DIMENSIONAL RETINAL ORGANOID CULTURE

Chen, Yu Holly, Dong, Lijin and Swaroop, Anand, National Institutes of Health, Bethesda, MD, U.S.

Mouse embryonic or induced pluripotent stem cells (ESCs or iPSCs) can be differentiated into three-dimensional (3D) retinal organoids with stratified neural retina. However, differentiation efficiency of SFEBq (serum-free floating culture of embryoid body-like aggregates with quick reaggregation) method for 3D retinal culture is low for generating immature neural retina (NR). While NR differentiated in vitro has been examined for a few cell type-specific markers, its precise molecular description in relation to the retina in vivo is unclear. We have developed a 3D culture system that mimics in vivo retinogenesis with high efficiency and reproducibility by modifying oxygen level, growth factors and various nutrients. We show that hypoxia condition in the early stage of differentiation (<D10) greatly improves the efficiency of formation of optic vesicles (>85%) at D7 and optic cup (>75%) at D10. The 3D retina culture can be maintained until D35, with mature Müller glia and elongated cilia in photoreceptors. Taking advantage of ESCs and iPSCs derived from the *Nrl*-GFP mice, where GFP labels all rod photoreceptors at birth, we purified developing rod photoreceptors from 3D retina by flow cytometry. RNA-seq is being performed to examine the gene expression profiles of flow-sorted rod photoreceptors and dissected NR and these profiles would be compared to purified rods from mouse retina at distinct developmental stages. Our study should provide new insights for the application of 3D organoid cultures to investigate retinal organogenesis and pathogenesis of degenerative diseases.

Funding Source: This work was supported by intramural research program of the National Eye Institute, National Institutes of Health.

W2012

PHERIPHERAL INTRAVENOUS INJECTION OF HUMAN PLACENTA MESENCHYMAL STEM CELLS PROMOTE AXON SURVIVAL FOLLOWING OPTIC NERVE COMPRESSION BY ACTIVATION OF NF-

Park, Mira, Kim, Hyungchul and **Lew, Helen**, CHA university, Seongnam, Korea, South

We investigated compressed rat optic nerve following transplantation with human placenta-derived MSCs (hPMSCs) isolated from placenta. hPMSCs have a function to recover neuronal damages by up-regulating expression of genes associated with axon regeneration. After making rat models of optic nerve compression crush(ONC), hPMSCs (1x10^6), hPMSCs (1x10^6) with G-CSF (0.2ml/kg) , G-CSF (0.2ml/kg) were injected into the tail vein for each group. The axon survival ratio and immunohistochemical evaluation of normal right side and ONC left side in each rat. The expression markers for inflammation, apoptosis and optic nerve regeneration such as Gap43, Hif-1$^{\alpha}$and Gapdh and Thy-1, Nf-l ad Brn-3b as a specific marker for retinal ganglion cell (RGC) analyzed by Western blot and real-time PCR with axon survival rate by direct counting. To determine the recovery pathway by hPMSCs in damaged retinal precursor cell line (R28) cells, cell viability, immunoblot analysis, immunofluorescence analysis and electrophoretic mobility shift assay (EMSA) were conducted. Increased axon survival rate ($p = .009$) were observed in hPMSCs injection compared to controls. At 2 weeks, the expression level of GAP43 was decreased in injured eye groups than normal eye of hPMSCs injection group and recovered in injured eye of hPMSCs injection group. The expression level of Hif-1α was decreased in injured eye of hPMSCs. We have shown that 30% of the R28 cells exposed to Cocl$_2$ which induced hypoxia underwent apoptosis. After co-cultured incubation with hPMSCs, we found death was reduced in R28 cells exposed to hypoxia by recovery of disturbed levels of neuronal regeneration related proteins (GAP43, ERMN and OCM) and decrease of activated caspase3 protein. In addition, we found NF-κB protein mediates neuroprotection pathway via up-regulation of target proteins by hPMSCs. Confocal result showed decreased NF-κB expression of R28 cells exposed to Cocl$_2$ by co-culture with hPMSCs. And also,

we demonstrated that loss of NF-κB DNA-binding affinity recovered by hPMSCs. In conclusion, hPMSCs have abilities to recover neuronal damages by up-regulating expression of genes induced axon regeneration. Our results can provide the better understanding of hPMSCs function in retinal damage.

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W2014

EFFECTS OF EXOSOMES FROM HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS ON COLONY FORMATIONS OF HUMAN LIMBAL EPITHELIAL STEM CELLS

Okubo, Toru[1,2], Hayashi, Ryuhei[2], Shibata, Shun[1,2], Honma, Yoichi[1,2] and Nishida, Kohji[2], [1]Rohto Pharmaceutical Company Limited, Osaka, Japan, [2]Osaka University Graduate School of Medicine, Suita, Japan

Exosomes from mesenchymal stem cells (MSCs) have been recently reported to participate in therapeutic processes, including tissue repair and immunomodulation. Human limbal epithelial stem cells (hLESCs), that are located in basal layer of corneal limbus, differentiate into transient amplifying cells and corneal epithelial cells eventually, as they proliferate and migrate towards central cornea. An impairment of hLESCs results in limbal stem cell deficiency (LSCD). In this study, we investigated effects of exosomes from human adipose-derived (hAD-) MSCs on the proliferation and the differentiation of hLESCs. Exosomes were isolated from cell culture media of hAD-MSC using ultracentrifugation method. For colony-forming assay, hLESCs were seeded on MMC-treated NIH/3T3 cells at 200 cells/well in 24 well plates and exosomes were added simultaneously to cell culture media. After 10 days culture, colonies were stained with rhodamine B and the numbers were counted. Gene expression of Keratin (K)12 and K3, markers for differentiated corneal epithelial cells, was assessed by real-time-RT-PCR. Protein expression of K12 was examined by immunofluorescence staining.Exosomes treatment increased colony formations of hLESCs by 1.48-fold, compared to that in untreated control. Expression levels of K12, K3 mRNAs were reduced in the colonies treated with hAD-MSCs exosomes. Immunofluorescence staining showed that expression of K12 protein was also decreased in the hAD-MSCs exosomes-treated colonies. In conclusion, exosomes from hAD-MSCs inhibited corneal differentiation, but promoted colony formations of hLESCs. Therefore, exosomes derived from hAD-MSCs are the promising candidate for treatments of LSCD.

W2016

TROPHIC EFFECT OF MESENCHYMAL STEM CELLS ATTENUATES TGF-BETA-INDUCED EPITHELIAL-MESENCHYMAL TRANSITION IN HUMAN CORNEAL LIMBAL EPITHELIAL CELLS

Shibata, Shun[1,2], Hayashi, Ryuhei[3], Okubo, Toru[1,2], Honma, Yoichi[1,2] and Nishida, Kohji[2], [1]Rohto Pharmaceutical Co., Ltd., Osaka, Japan, [2]Osaka University Graduate School of Medicine, Suita, Japan, [3]Osaka Univ, Osaka, Japan

Epithelial-mesenchymal transition (EMT) is a process of converting from polarized epithelial characteristics to migratory mesenchymal properties. In ocular surface, some evidences suggest that EMT is involved in scarring, pterygium, and limbal stem cell senescence, however, detailed mechanism of EMT has not been elucidated. On the other hand, some reports indicated that secreted factors from mesenchymal stem cells (MSCs) suppressed EMT. In the present study, we investigated effects of MSCs on EMT in human limbal epithelial cells (hLECs). hLECs were isolated from the limbus of research human corneal tissues and expanded *in vitro*. Treatment of transforming growth factor (TGF) -β, a main inducer of EMT, induced fibroblast-like morphological changes and decrease of cell-cell contacts in hLECs. The results of qRT-PCR and immunostaining showed that TGF-β increased the expression of mesenchymal markers, Slug, Vimentin, and Fibronectin, but decreased the expression of epithelial markers, E-cadherin and Claudin-1 in hLECs. Next, hLECs were treated with TGF-β and then co-cultured with human adipose-derived MSCs (hAD-MSCs) using cell culture insert. EMT-related genes induced by TGF-β in hLECs were attenuated by the co-cultivation with hAD-MSCs. In addition, in spite of the presence or absence of TGF-β, the co-cultivation with hAD-MSCs promoted terminal differentiation of hLECs which was marked by an increase of Keratin12 expression and the bloated cell morphology. Finally, to determine a factor in MSCs conditioned medium (MSC-CM) responsible for EMT inhibition, we performed LC-MS/MS for MSC-CM following SDS-PAGE. The proteome analysis successfully identified a protein correlated with the EMT-inhibiting activity in MSC-CM. Our findings suggest that trophic effect of MSCs attenuates EMT and promotes terminal differentiation of hLECs and provide a novel candidate protein marker of EMT-inhibiting function in MSC-CM.

W2018

IMPROVING OUR UNDERSTANDING OF AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA USING PRPF31 PATIENT SPECIFIC INDUCED PLURIPOTENT STEM CELLS

Zhu, Lili[1], Buskin, Adriana[1], Mellough, Carla[1], McKibbin, Martin[2], Inglehearn, Chris[3], Steel, David[1], Johnson, Colin Anfimov[3], Armstrong, Lyle[4], Collin, Joseph[1] and Lako, Majlinda[4], [1]Newcastle University, Newcastle upon Tyne, U.K., [2]St James University Hospital, Leeds, U.K., [3]University of Leeds, Leeds, U.K., [4]Newcastle University, Newcastle, U.K.

Retinitis pigmentosa (RP), a common cause of blindness, is a group of inherited diseases characterized by progressive degeneration of the mid-peripheral retina, leading to night blindness, visual field constriction, and eventual loss of visual acuity due to loss of photoreceptor cells of the retina. RP, affecting more than 1.5 million people worldwide, accounts for 13% of all blind registrations in children and 16% in adults of working age. Mutations in one key gene, PRPF31, shown to be involved in pre-mRNA splicing, account for approximately 5.5% of autosomal dominant RP cases. PRPF31 is ubiquitously expressed in all tissues of the adult organism, but its mutations cause only retina-specific disease. Currently, the molecular mechanisms by which ubiquitous pre-mRNA splicing genes confer a tissue-specific pathogenesis are not clear. The direct study of retinal tissue affected by RP is challenging, as it is difficult to obtain. Animal models and patient-specific lymphoblastoid cell lines have failed to identify retinal-specific pathogenic splice variants, hence in vitro disease models of retinal specific diseases are necessary and timely. In our study, we have generated a patient-specific induced pluripotent stem cells (iPSC) model of PRPF31-RP and we are in the process of correcting the PRPF31 mutation by CRISPR-Cas9. PRPF31-iPSCs show typical expression of pluripotency markers, in vitro differentiation capacity and the ability to generate teratomae comprised of all three germ layers in vivo. These iPSCs were successfully differentiated into a large number of patient-specific photoreceptor and retinal pigmented epithelial (RPE) cells, which enabled us to study the features, and RNA splicing defects of these retinal cells. Alongside the benefits of a greater understanding the disease mechanisms of RP disease, our findings could also help develop effective treatments to restore sight in affected patients.

Funding Source: FIGHT FOR SIGHT

NEURAL CELLS

W2020

FUNCTIONAL CHARACTERIZATION OF IPSC-DERIVED NEURONS ON MICROELECTRODE ARRAYS AND THEIR APPLICATION TO PHENOTYPIC DISEASE MODELING AND NEUROTOXICITY ASSESSMENT

Bader, Benjamin M.[1], Jügelt, Konstantin[1], Hess, Dietmar[2], **Kfoury, Elena**[3], Luerman, Gregory[3] and Schröder, Olaf H. -U.[1], [1]NeuroProof GmbH, Rostock, Germany, [2]Axiogenesis AG, Cologne, Germany, [3]Axiogenesis Inc, Plymouth Meeting, PA, U.S.

Primary cultures are widely used for testing drug candidates in phenotypic in vitro models. Moreover, they serve as the gold standard and are used to evaluate human induced pluripotent stem cell-derived (hiPSC) neuronal cultures to transfer current models into the human background. The goal is to increase predictability, sensitivity and specificity. We cultured different hiPSC-derived CNS neurons including TH+/dopaminergic hiPSC neurons on MEAs and recorded the spontaneous electrical network activity over weeks in culture using micro electrode arrays (MEAs). All neuronal cultures demonstrated spontaneous electrical activity which partly matures over time. The neurons demonstrated burst-like activity for > 3 weeks in culture and demonstrated sensitivity to multiple well characterized neurotoxic agents. Moreover, we compared the phenotypic functional activity patterns with those recorded in primary mouse cultures from different brain regions and observed a phenotypic similarity e.g. between primary midbrain and iPSC-derived cultures containing ventral dopaminergic neurons. Toxin-induced activity changes were also comparable between primary and hiPSC neurons and the effects of known in vitro challenge/rescue models on both human and mouse neuronal networks were shown. In conclusion, well-characterized functional human iPSC-derived neuronal in vitro systems and comparison to known primary models increase the predictive value for disease modeling, neurotoxicity assessment and compound screening.

W2022

TRANSCRIPTIONAL MAPPING OF TRANSPLANTED HUMAN NEURAL STEM CELLS IN ISCHEMIC STROKE USING TRANSLATING RIBOSOME AFFINITY PURIFICATION

Azevedo-Pereira, Ricardo L.[1], Manley, Nathan[1], Winge, Marten[1], Berry, Jack[1], Sun, Guohua[1], Bliss, Tonya[2] and Steinberg, Gary[3], [1]Stanford University, Palo Alto, CA, U.S., [2]Stanford University, Stanford, CA, U.S., [3]Stanford School of Medicine, Stanford, CA, U.S.

Human neural stem cell (hNSC) transplantation is being explored as a potential therapy for ischemic stroke (IS). Although research has shown the efficacy of NSCs in stroke little is known about the mechanisms involved in NSC-induced functional recovery. Identifying what the transplanted NSCs express in vivo will help elucidate their mechanism of action. To date, the challenge has been resolving factors expressed by the transplanted cells from that of surrounding host cells. We have overcome this critical barrier by adapting the novel TRAP (Translating Ribosome Affinity Purification) approach to enrich mRNA from transplanted hNSCs. Nude rats were subjected to permanent distal cerebral artery occlusion, 7 days later hNSCs expressing GFP under an RPL10a promoter were transplanted into the cortex of IS and non-ischemic (NI) rats. Seven days post-transplantation the graft site was dissected and processed to isolate ribosome-bound RNA from the transplanted hNSCs using TRAP. RT-qPCR analysis of a human housekeeping gene panel revealed that the average enrichment of human transcripts was 22-fold (range: 17.5-31.80) and 101-fold (range: 57.27-154.94) in IS and NI rats, respectively. TRAP-isolated RNA was subjected to HiSeq-2500 sequencing and a total of 25,272 NSCs genes were found differentially expressed between NSCs in IS and NI rats. Of these genes, 630 showed statistical significant differential expression (i.e. $p<0.05$, $q<0.05$) plus at least a 2-fold difference in expression between the two groups. Gene ontology analysis of this signature revealed that these genes clustered into biological process significantly ($p\leq0.05$) enriched for Regulation of Cell Death (7.1%), Cell-cell Signaling (6.9%) and Neuron Differentiation (5.9%). Our data demonstrate the feasibility of the TRAP procedure to enrich for the transcriptome of hNSCs transplanted into rat brain, making analysis of their differential expression profile in the stroke brain possible. This sets the stage to probe the molecular mechanism of action of transplanted stem cells.

W2024

A HUMAN NEURONAL MODEL FOR TUBEROUS SCLEROSIS

Blair, John[1], Hockemeyer, Dirk[1] and Bateup, Helen[1], [1]University of California, Berkeley, Berkeley, CA, U.S.

Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder associated with epilepsy, intellectual disability, and autism spectrum disorder. TSC is caused by loss of function mutations in either the TSC1 or TSC2 genes whose protein products form a complex that is a key negative regulator of the mTOR pathway. Mouse models of TSC have provided insights into possible disease mechanisms but do not recapitulate all aspects of the disease, specifically the focal cortical malformations that are observed in patients. To generate a novel human neuronal model for TSC we have used CRISPR/Cas9-mediated gene editing to engineer loss of function mutations in TSC1 or TSC2 in human pluripotent stem cells. Differentiating these cells into neurons revealed a distinct morphology associated with TSC1 or 2 loss, including increased cell body size and altered dendritic arborization. We also observed differential protein expression, particularly in downstream targets of mTOR that control cellular metabolism and protein translation. In addition to two-dimensional neuronal differentiation, we have created three-dimensional “cortical organoids” to model the early steps of human cortical development. We are using this system to investigate the molecular mechanisms by which mutations in TSC1 or 2 affect human neuronal differentiation and migration. Our approach provides a novel, genetically controlled platform from which to elucidate the cellular and molecular basis of TSC and related neurodevelopmental disorders.

W2026

MODELING HUNTINGTON'S DISEASE USING ASTROCYTES DERIVED FROM TRANSGENIC HD MONKEY IPSCS-DERIVED NEURAL PROGENITOR CELLS

Cho, In Ki[1,2], Qian, Lu[3], Phung, Phung T.[3] and Chan, Anthony W.S.[1,3], [1]Emory University School of Medicine, Atlanta, GA, U.S., [2]Emory University, Atlanta, GA, U.S., [3]Yerkes National Primate Research Center, Atlanta, GA, U.S.

Huntington’s disease (HD) is a devastating neurodegenerative disease caused by the expansion of CAG repeats in exon 1 of the huntingtin (HTT) gene IT15 that results in an extended poly-glutamine residue in N-terminus of the HTT protein. The accumulation of mutant HTT (mHTT) leads to the formation of nuclear inclusions and oligomeric aggregates causing neuronal cell dysfunctions and death. Various cell and animal models have been made produced, but there still is a strong demand for experimental models that can recapitulate the progress and development of

the disease phenotypes. In addition to our effort in establishing transgenic HD monkey model, we have generated neural progenitor cells (rNPCs) from induced pluripotent stem cell (iPSC) of HD monkey as a model for studying HD pathogenesis. As the most abundant cell type in the brain, astrocytes play an important role in the development and maintaining homeostasis of the central nervous system (CNS). There are increasing evidences supporting the involvement of astrocytes in the onset and progression of neurodegenerative diseases such as Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). In this study, we report the in vitro derivation of astrocytes from rNPCs at high homogeneity confirmed by immunocytochemistry (ICC) and fluorescence-activated cell sorting (FACS). rNPC-derived astrocytes were positive for canonical astrocyte-specific markers (ALDH1L1 and GFAP), and they were negative for NPC markers (MSI1 and PAX6) and neuronal marker (MAP2). RT-PCR showed up-regulation of the astrocyte-specific transcripts GFAP, S100B, APOE, and LCN2 while down-regulation of NPC-specific transcript SOX2. Compared to NPC, there was no difference in MSI1, TUBB3, and TH expressions, which are neural crest and neuronal restricted progenitor population makers. Furthermore, astrocytes derived from HD-rNPC developed cytosolic mHTT aggregates and nuclear inclusions. The HTT and GRM1 up-regulation and the PPARGC1A, SLC1A2, and SOD2 down-regulation in HD-rNPC-derived astrocytes compared to WT were consistent with prior reports in HD. In summary, we report a novel primate astrocyte model for studying HD pathogenesis, a cell source for cell therapy, and a platform or the discovery of novel treatments for HD.

Funding Source: NINDS/NIH (NS084163), NIMH/NIH (MH100670)

W2028

FUNCTIONAL PHENOTYPIC CHARACTERIZATION OF IPSC-NEURONS FROM ALZHEIMER'S DISEASE PATIENTS CARRYING PS-1 MUTATION IN DRUG SCREENING AND DISEASE MODELING

Winkler, Maria[1], **Dutta Passecker, Priyanka**[2], Gibbons, George[2], Wessel-Carpenter, Naomi[2], Schröder, Olaf[1] and Bader, Benjamin M.[1], [1]NeuroProof GmbH, Rostock, Germany, [2]Axol Bioscience Ltd., Cambridge, U.K.

Adult cells from human individuals carrying disease-associated gene mutations can be reprogrammed into induced pluripotent stem cells (iPSCs) and can then be differentiated into a variety of cell types including human neural stem cells (hNSCs) and cerebral cortical neurons (hCCNs). Our aim was to phenotypically investigate patient iPSC-derived neurons carrying the presenilin-1 (PS-1) mutation (supplied by Axol Bioscience) and to compare them with cells from healthy controls (supplied by Axol Bioscience). Transcriptome analysis revealed an up-regulation in the expression of neuronal genes and a decrease in pluripotency markers in hCCNs. Immunocytochemistry showed the appropriate neural cell morphology in hCCNs, with both cell types expressing markers typically associated with the corresponding developmental stage. Whole patch clamp and multi-electrode arrays (MEAs) successfully established electrical activity in these cells. We differentiated these neural progenitor cells into spontaneously active neuronal networks using a xeno-free differentiation protocol and recorded spontaneous activity during neuronal differentiation using micro-electrode array (MEAs). Multi-parametric phenotypic analysis was used to identify specific differences of functional activity patterns during development into mature neuronal networks within 4-5 weeks. Moreover, we investigated the effects of neurotoxins on mutant and control neurons. We have identified a range of characteristics in the patient-derived and control-derived iPSC-neurons that establishes them as an ideal tool for use in numerous applications such as disease modeling, drug screening and toxicology and other assays.

W2030

MODELING NEUROBLASTOMA THROUGH PRIMARY MOUSE NEURAL CREST CELL TRANSFORMATION

Garcia Lopez, Jesus, St. Jude Children's Research Hospital, Memphis, TN, U.S.

Neuroblastoma (NB) is the most common extracranial solid tumor in the pediatric population and arises from the sympathoadrenal lineage of trunk Neural Crest Stem Cells (NCSCs). Two common genetic features of neuroblastomas are allelic deletion of 1p36 locus and MYCN amplification which together are involved in about 30% of all cases. Deletion of chromosome 1p is frequently found in MYCN amplified tumors suggesting that MYCN regulatory mechanisms and tumor suppressor genes may be lost in 1p36 deletion. Since all the genetic aberrations which are associated with NB occur during NCSC differentiation, to model NB disease, we have developed an ex-vivo system which allows us to collect and transform primary mouse NCSCs to screen novel neuroblastoma oncogenic drivers. Among the different 1p36 candidate genes we are targeting, ARID1A and CHD5 are promising candidates that are involved in chromatin remodeling. Through CRISPR/CAS9 technology, we suppressed ARID1A and CHD5 expression on primary mouse NCSCs. We are currently combining ARID1A and CHD5 deletion with MYCN overexpression, to emulate the genetic disorders observed in high risk NB tumors. Our results suggest that primary

NCSCs manipulation is a useful tool to identify early oncogenic events and key oncogenic drivers in neuroblastoma.

Funding Source: Funding was provided by ALSAC and a grant from the U.S. Department of Defense

W2032

INVESTIGATING THE EFFECTS OF STEM CELL POSITIONAL IDENTITY ON BRAIN TUMOR DEVELOPMENT IN THE MOUSE

Rushing, Gabrielle, Leelatian, Nalin, Brockman, Asa, Jurewicz, Amanda, Fu, Cary, Ess, Kevin, Irish, Jonathan and **Ihrie, Rebecca**, Vanderbilt University, Nashville, TN, U.S.

The ventricular-subventricular zone is the largest stem cell niche in the postnatal brain, and is a likely site of origin for many brain tumors. Cells in this niche have a positional identity: a stem cell's position predicts the types of progeny that will be generated, and several transcription factors are preferentially expressed in localized domains. However, it is not known whether the intrinsic patterning of these microdomains corresponds to measurable differences in proliferative signaling, or whether this patterning may influence tumor development or other disease states. We are using a mouse model of a neurodevelopmental disorder, Tuberous Sclerosis Complex (TSC), to test the effects of positional identity on brain tumor development. TSC patients have mutations in either the TSC1 or TSC2 genes, and may develop two benign tumor types within the brain - small, ubiquitous and asymptomatic subependymal nodules or large, location-restricted and potentially lethal subependymal giant cell astrocytomas (SEGAs). The mechanism for larger, location-specific tumor development is unknown. We hypothesized that SEGAs originate from a specific, ventral population of stem cells that is more susceptible to the effects of TSC1/2 gene mutation. We developed phospho-specific flow cytometry assays in cultured mouse V-SVZ cells to simultaneously measure the per-cell levels of multiple relevant phospho-proteins affected in this disease, including p-S6, p-STAT3, and p-Akt. Preliminary data suggest that ventral progenitors have elevated mTOR pathway activity both in the wild-type and TSC2-heterozygous state, and that these progenitors are less effectively inhibited by rapamycin. Complementary experiments in mouse models likewise suggest that localized loss of TSC2 protein results in SEGA-like symptoms in a subset of mice. Collectively, these results suggest a novel model for the development of localized tumors in TSC and illustrate the utility of single-cell approaches in dissecting signaling within this niche.

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W2034

DYNAMIC TRANSCRIPTION OF ENDOGENOUS RETROVIRUSES DURING HUMAN BRAIN DEVELOPMENT

Brattås, Per Ludvik[1], **Jönsson, Marie**[1], Fasching, Liana[1], Falk, Anna[2], Parmar, Malin[1] and Jakobsson, Johan[1], [1]Lund University, Lund, Sweden, [2]Karolinska Institutet, Stockholm, Sweden

How the complexity of the mammalian brain is achieved is largely unknown but factors such as epigenetic modifications, non-coding RNAs and transposable elements (TEs), have been emerging as important players in the control of gene regulatory networks. More than half of the human genome is composed of TEs and it is becoming increasingly clear that TEs can act as gene regulatory elements and may play an important role in controlling and fine-tuning gene networks. Recent studies indicate that endogenous retroviruses (ERVs) participate in the control of gene expression during early mouse and human development and there is also a remarkable cell-type specific expression of these elements during different stages of early human development. Still, if and how ERVs act as regulatory elements in somatic cells including the brain is poorly explored. In this study, we characterize the expression profile of HERVs in the human nervous system during embryonic development as well as in human neural progenitor cells (hNPCs) and upon their neuronal differentiation. The data shows that HERVs are dynamically expressed in the developing human nervous system, based on both regional and age related differences. The expression patterns of HERVs in hNPCs and upon their differentiation towards mature neurons revealed a dynamic control also in this in vitro model of human neural development. In addition, we show that in hNPCs a selected number of HERVs are repressed by H3K9me3 recruited by the epigenetic co-repressor TRIM28. These data demonstrate that HERVs have the capacity to act as regulatory RNAs as well as epigenetic hubs that attract repressive histone marks, which may mediate transcriptional silencing of nearby genes. Taken together, these results establish a dynamic transcriptional control of HERVs during brain development and indicate that these elements play a critical functional role in regulating transcriptional networks in human cells which in turn influence the progeny they produce. Considering that the genomic composition of HERVs varies considerably between species, this mechanism has the potential to mediate human-specific gene regulatory network. These experiments will open up for further studies on the role of ERVs in driving human brain evolution and their role in contribution to individual variation.

W2036

IMAGE-BASED ANALYSIS OF A HUMAN NEUROSPHERE STEM CELL MODEL FOR THE EVALUATION OF POTENTIAL NEUROTOXICANTS

Larson, Brad[1], Sherman, Hilary[2], Gitschier, Hannah[2], Wolff, Alexandra[3] and Luty, Wini[3], [1]BioTek Instruments, Inc., Winooski, VT, U.S., [2]Corning Incorporated, Life Sciences, Kennebunk, ME, U.S., [3]Enzo Life Sciences, Farmingdale, NY, U.S.

Developmental neurotoxicity (DNT) of environmental chemicals has long been identified as a threat to the health of the human population, as the developing nervous system is particularly susceptible to toxicant exposure. The resulting neurological deficits can have long-term effects on families and society both financially and emotionally. Current DNT testing guidelines involve the use of animal models; primarily rodents. The testing strategy incorporates large numbers of animals, which can be extremely time- and cost-intensive; particularly due to the backlog of chemicals needing to be tested (Lein *et al.*, 2005). This demand, in addition to current and future proposed regulations on the use of animals for testing makes it imperative that new models be found to reduce animal experimentation while providing a suitable method to test new chemicals. Three-dimensional cell models, which incorporate human neural stem cells (hNSCs) aggregated into neurospheres, have been proposed as a viable alternative for DNT testing. The in vitro system has the ability to recapitulate the processes of brain development, including proliferation, migration, differentiation and apoptosis (Salma *et al*., 2015). Inclusion of human cells, as opposed to murine, also meets recommendations to circumvent the drawback of species differences between in vivo testing and actual exposure effects. Here we will demonstrate the use of a 3D neurosphere model, composed of hNSCs, to conduct toxicity testing of potential neurotoxicants. A spheroid microplate was used to create and maintain cells in the 3D model. 3D neurosphere proliferation, multipotency, along with the continued capacity to differentiate into neurons, astrocytes, and oligodendrocytes was initially validated. Neurotoxicity testing was then performed using neurospheres maintained in the 3D spheroid plate. Detection of induced levels of oxidative stress, apoptotic, and necrotic activity within treated neurospheres, compared to negative control spheres, was evaluated. Monitoring of cell proliferation, differentiation, multipotency and experimental testing was performed using a novel cell imaging multi-mode reader.

W2038

GENERATION OF BRAINSTEM SEROTONERGIC NEURONS FROM HUMAN iPSCs TO STUDY POTENTIAL SUDEP MECHANISMS

Liu, Yu[1], Glanowska, Kasia[1], Richerson, George[2] and Parent, Jack[1], [1]University of Michigan, Ann Arbor, MI, U.S., [2]University of Iowa, Iowa, IA, U.S.

Sudden unexpected death in epilepsy (SUDEP) is the most common cause of death in intractable epilepsies, but pathophysiological mechanisms that lead to SUDEP are poorly understood. Proposed SUDEP mechanisms include cardiac arrhythmia, brainstem dysfunction, obstructive apnea, or autonomic instability. Dravet syndrome (DS) is an infantile-onset intractable epilepsy caused by heterozygous loss-of-function mutations in the SCN1A gene, which encodes the neuronal voltage-gated sodium channel $Na_v1.1$. The incidence of SUDEP in DS appears even higher than in other intractable epilepsies, and a greater understanding of this high risk is urgently needed. Serotonin-producing neurons in the brainstem raphe nuclei are implicated in regulating crucial physiological processes such as breathing and body temperature. Defective serotonin networks are associated with disorders such as sudden infant death syndrome (SIDS), and have been proposed to play a role in SUDEP. To test this idea, we sought to differentiate induced pluripotent stem cells (iPSCs) from controls and subjects with DS into brainstem-like serotonergic neurons. We first generated neural progenitors using small molecules through dual SMAD inhibition, and then sonic hedgehog, Fgf8/Fgf4, and Wnt agonists were used to posteriorize neural progenitors and regionalize them into midbrain/hindbrain identities. After 5 weeks of differentiation, the neural progenitors were dissociated into single cells and then differentiated into neurons without growth factors. Over 80% of human iPSC-derived neurons expressed PET-1, an early and specific marker of central serotonin neurons. After further differentiation, they also expressed tryptophan hydroxylase 2 et al mature serotonin neuron molecules markers and displayed mature electrophysiological properties. Future work aims to compare the function of DS patient and control serotonin neurons. These cells will provide a valuable in vitro model to study SUDEP mechanism of DS.

Funding Source: U01 NS090364 Center for SUDEP Research, Project 6: iPSC and Mouse Neurocardiac Models

W2040

ANTI-AGING: EFFECT OF STEM CELLS ON AGING & STEM CELL AGING

Moon, Jisook, Park, Ji-min, Bae, Sang-hun, Kong, Tae-Ho, Choi, Yuri, Park, Jae-Hyun and Shin, Seo-Jung, CHA Univ, Seoul, Korea, South

The aging process is characterized by gradual, cumulative damages to structure and function of stem cells which exist during the lifespan of organisms. Aging can be conceived of as a process that a pool of endogenous stem cells loses progressively its ability to replenish the damaged cells over age. Many studies with OMICS on aging heavily rely on blood samples which consist of different cell types, thereby placing obstacles in the way of interpretation on the phenomenon. An alternative approach can be to focus on stem cell aging among diverse hallmarks of the process using OMICS technology. In consistent with the strategy, our study investigated gene expression changes of neural stem cells during differentiation into dopaminergic cells and with increasing passages in a proliferation state, both of which can be seen as aging: differentiation as a part of "chronological aging" and increasing passage as "replicative aging". Neural stem cells showed cell stage-specific patterns of gene expression during differentiation and specific genes participated in neurogenesis by forming molecular co-expression network. Also, we determined the effects of hypoxia or normoxia on the placenta-derived cells with increasing passage based on the transcriptome data. Genes that are differentially expressed under hypoxia are enriched for nucleosome assembly and chromatin organization, suggesting the involvement in epigenetic regulation. These findings add weight to the notion that study of stem cell aging with OMICS is an efficient means for elucidating the biological basis of the aging process. In this regard, the maintenance of stem cell pool or stem cell rejuvenation holds great therapeutic promise for age-related impairments. Human placenta derived MSCs (hpMSCs) has been used as a candidate for anti-aging treatment. Our animal studies exhibited better cognitive functions measured 12 weeks after hpMSCs injection. Taken together, we will discuss here the integrative studies of the stem cell aging and a therapeutic effect of adult stem cells with OMICs technology and the underlying mechanisms of the complex process at diverse molecular levels, with the final goal of practically applying stem cell treatment to the aged for maintaining health over time.

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W2042

REGULATION OF TRANSCRIPTION IN ACTIVITY-DEPENDENT OLIGODENDROCYTE PRECURSOR CELL DIFFERENTIATION

Nagaraja, Surya, Lennon, James, Gibson, Erin and Monje, Michelle, Stanford University, Stanford, CA, U.S.

Myelin development continues through several decades of human life and is shaped by experience and environment. Work by our lab and others have shown that neuronal activity modulates the behavior of myelin-forming precursor cells, but the molecular mechanisms mediating activity-dependent proliferation and subsequent differentiation of oligodendrocyte precursor cells (OPCs) remain to be elucidated. Using an in vivo optogenic mouse model of elevated premotor circuit activity, we have previously shown that physiomimetic neuronal activity promotes OPC proliferation, followed by differentiation to mature oligodendrocytes and remodeling of myelin microstructure in a manner that improves function of that circuit. These adaptive changes in myelin-forming cells establish plasticity of myelin as a dimension along which experience modulates brain structure and function. Using this optogenetic model, we have now described a time course of OPC differentiation following elevated neuronal activity. Using quantitative measurements of global histone 3 lysine 9 trimethylation (H3K9me3) levels in OPCs within the active premotor circuit at various time points within the first 24 hours following optogenetic stimulation, we have characterized the temporal dynamics of initiation of OPC differentiation and have used this time course to guide a careful examination of the early transcriptional response controlling entry to OPC differentiation. Thy1:ChR2 mice and paired WT littermate controls were optogenetically stimulated and the stimulated premotor circuit was microdissected 1 hour after stimulation. OPCs from this circuit were collected via FACS isolation of Pdgfra+ cells. We then performed RNA-seq using the published Smart-seq2 protocol. This has allowed us to identify previously uncharacterized gene expression dynamics in OPCs in response to neuronal activity prior to the onset of global chromatin remodeling associated with differentiation. This allows for potential identification of genes and transcriptional regulators that control OPC proliferation and differentiation in adaptive myelination.

W2044

DISSECTION OF CELL TYPE-SPECIFIC CAG REPEAT LENGTH EFFECTS USING AN NOVEL ISOGENIC HUNTINGTON DISEASE (ISOHD) HESC ALLELIC PANEL

Ooi, Jolene[1], Xu, Xiaohong[2], Utami, Kagistia H.[2], Tay, Yi Lin[2], Sim, Bernice[2], Huang, Yihui[2], Yip, Lian Yee[3], Tan, Wei Shan[3], Sobota, Radoslaw M.[4], Ginhoux, Florent[5], Chan, Eric[6], Ho, Ying Swan[3], Hayden, Michael R.[2,7] and Pouladi, Mahmoud[1,8], [1]Agency for Science, Technology and Research, Singapore, Singapore, [2]Translational Laboratory of Genetic Medicine, Singapore, Singapore, [3]Bioprocessing Technology Institute, Singapore, Singapore, [4]Institute of Molecular and Cell Biology, Singapore, Singapore, [5]Singapore Immunology Network, Singapore, Singapore, [6]Department of Pharmacy, National University of Singapore, Singapore, Singapore, [7]Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute, University of British Columbia, Vancouver, BC, Canada, [8]National University of Singapore, Singapore, Singapore

Huntington disease (HD) is a monogenic, autosomal dominant, and progressive neurodegenerative disorder where patients exhibit motor dysfunction, cognitive impairment, and psychiatric features. The disease is caused by an expansion of CAG trinucleotide repeats in exon 1 of huntingtin (HTT) to more than 36 repeats. Two aspects of the genetics and pathology of HD have remained a source of intrigue. The first is the inverse correlation between the length of the pathogenic CAG repeat and the rate of disease onset. The second is the differential susceptibility of tissues and cell types to HD pathology, despite widespread expression of mutant HTT. To better understand the molecular basis of these features of HD, we used TALEN-mediated homologous recombination to engineer an allelic panel of isogenic HD (IsoHD) human embryonic stem cell (hESC) lines carrying 30, 45, 65 and 81 CAG repeats in the first exon of HTT. To explore tissue-specific effects in HD, the IsoHD panel will be differentiated into lineages-of-interest for functional analyses. In order to dissect CAG repeat length-dependent and cell type-specific molecular phenotypes, the transcriptome and metabolome of resultant cell types will be analysed. We anticipate that the IsoHD panel will serve as a versatile resource to dissect the molecular factors that contribute to the pathogenesis of HD and highlight potential approaches of therapeutic relevance.

W2046

IDENTIFICATION OF BIOMARKERS FOR CLINICAL TRIALS IN FRIEDREICH'S ATAXIA

Petrosyan, Lina[1], Soragni, Elisabetta[2], Lai, Jiun-I[2] and Gottesfeld, Joel[2], [1]The Scripps Research Institute, La Mesa, CA, U.S., [2]The Scripps Research Institute, La Jolla, CA, U.S.

The genetic defect in 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 (iPS) cells, we identified the HDAC inhibitor 109 that increases FXN mRNA levels and frataxin protein. A phase I clinical trial with 109 in FRDA patients showed increase of FXN mRNA in blood from patients treated with the drug and demonstrated that HDAC inhibitors hold promise as FRDA therapeutics. Derivatives of this molecule with improved brain penetration and acid stability are being developed in collaboration with Biomarin Pharmaceutical and have proven most effective in restoring FXN transcription. To aid subsequent Phase II/III efficacy trials in FRDA patients, we set to identify surrogate biomarker genes that respond to FXN levels in the cell. While frataxin (mRNA or protein) provides a valid biomarker, increases in frataxin do not necessarily indicate reversal of mitochondrial dysfunction. To identify gene expression signatures that are common to cells that are affected in FRDA (neurons and cardiomyocytes) and circulating lymphocytes (PBMCs), we derived a set of isogenic iPS cell lines that differ only in the length of the GAA·TCC repeats, using helper-dependent adenovirus mediated homologous recombination. Gene expression analysis of isogenic neuronal cells revealed several pathways affected by the level of FXN transcription, namely mitochondrial respiration, cholesterol biosynthesis, neuronal differentiation, transcriptional regulation, and cell junction biology. RNA-seq analysis of PBMCs isolated from blood of FRDA patients and healthy individuals pointed at lipid metabolism and cell adhesion as pathways similarly affected in iPSC-derived neurons and peripheral blood cells. Validation of several of these genes in both cell types and in peripheral sensory neurons and cardiomyocytes is currently underway and could shed light on the pathophysiology of FRDA, and provide biomarkers for future clinical studies.

W2048

CHARACTERIZING THE STEM CELL PROPERTIES OF EPENDYMAL CELLS IN VITRO AND AFTER INJURY

Shah, Prajay T., Stratton, Jo Anne, Koblinger, Kathrin, Stykel, Morgan, Abbasi, Sepideh, Hagner, Andrew, Whelan, Patrick and Biernaskie, Jeff, University of Calgary, Calgary, AB, Canada

Exploiting the brain's endogenous repair system to enhance recovery of function following brain injury and disease requires a comprehensive understanding of the biology of neural stem cells (NSCs) and the factors that regulate their function. One component of the adult NSC niche that has received particular interest recently is the ependymal cell, an epithelial cell that creates an interface between the ventricular system and brain parenchyma. It is believed that ependymal cells are highly differentiated and decidedly post-mitotic under normal conditions, but intriguingly exhibit exceptional plasticity, re-enter the cell cycle and generate new neurons in response to stimuli such as injury. However, these claims remain controversial and difficult to prove due to the lack of inducible fate-mapping techniques to study ependymal cells that exclude neighboring Type-B NSCs also residing within the adult subventricular zone. As a resolve, we have developed a novel transgenic system that allows for specific, inducible and permanent fluorescent labelling of ependymal cells within the adult brain. We validated this system using thorough immunohistochemical and fate-mapping analysis to demonstrate its robust specificity to target ependymal cells while excluding Type-B NSCs. Interestingly, following prospective isolation of labelled ependymal cells by FACS and subsequent growth in NSC-proliferation media, we found that ependymal cells failed to generate self-renewing colonies, compared to control NSCs. Moreover, in vivo labelled ependymal cells did not incorporate EdU (proliferation), or migrate away from the subventricular zone, or give rise to DCX^{+ve} neuroblasts following ischemic or hemorrhagic injury. Thus, our preliminary results suggest that ependymal cells do not acquire NSC-like functional properties in culture or after injury.

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W2050

D-SERINE CONTROLS THE ONSET OF ASTROGENESIS VIA STARTING NMDA RECEPTOR ACTIVATION IN THE DEVELOPING MOUSE HIPPOCAMPUS

Sugimori, Michiya and Mori, Hisashi, Graduate School of Medical and Pharmaceutical Sciences for Research, University of Toyama, Toyama, Japan

During neural development, neural stem/progenitor cells (NSPCs) generate neurons, oligodendrocytes and astrocytes in specific temporal orders. Switches of neural fates from neurons to glia occur at specific timings. Accumulating evidence suggest that NSPCs are pharmacologically and/or electrically able to response to neural activities and change their differentiating properties. Here, we provide an evidence that D-serine, a regulator of NMDA receptor signaling, plays crucial roles in time specific neuro-gliogenesis in the developing hippocampus. D-serine, which is generated from L-serine by serine racemase (SR), starts to act at postnatal day 7 (P7) when SR starts to express in the developing nervous system of mouse. Ca imaging revealed that P8 SR$^{+/-}$ derived NSPCs responded to NMDA administration while the response was down-regulated in SR$^{-/-}$, suggesting that NMDA receptors are functionally expressed in SR$^{+/-}$ NSPCs and that the activity of NMDA receptors is regulated by SR expression. We then found that P8 SR$^{-/-}$ multi-potent NSPCs were biased to generate neurons and oligodendrocytes rather than astrocytes compared with wild type (WT)/SR$^{+/-}$ NSPCs. Clone assay of NSPCs revealed that SR$^{+/-}$ NSPCs were mainly astrogenic and lineage-restricted astrocyte progenitors, while SR$^{-/-}$ NSPCs were mainly neurogenic and oligodendrogenic. The phenotype in SR$^{-/-}$ NSPCs was rescued by administration of D-serine, suggesting that D-serine/SR are involved in the control of neural fate specification. The rescue of D-serine was disturbed by administration of MK801, an inhibitor of NMDA receptor, supporting an idea where D-serine controls astrogenesis via activating NMDA receptor signaling. Consistently, we found increase in the number of both immature and mature oligodendrocytes at P13 and P21 in the developing hippocampus of SR$^{-/-}$ mouse, suggesting D-serine/SR negatively control both specification and maturation of oligodendrocytes. We further found significant number of immature astrocytes positive for Glutamine synthase at P13 in WT, while few in SR$^{-/-}$. We quantitatively confirmed 70% reduction in the percentage of immature astrocytes in SR$^{-/-}$ by dissociation of the P14 hippocampus. Thus, D-serine/SR control both neural fate specification and the timing of the onset of astrogensis in the developing hippocampus.

W2052

DEVELOPMENT OF A HIGH-CONTENT SCREENING ASSAY WITH HUMAN NEURONS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

Wang, Chao[1,2], Ward, Michael[3], Chen, Robert[4], Liu, Kai[1,2], Tracy, Tara[2,5], Sohn, Peter[2,5], Xie, Min[2,5], Meyer-Franke, Anke[2,5], Ding, Sheng[2,5] and Gan, Li[2,5], [1]J. David Gladstone Institute, San Francisco, CA, U.S., [2]University of California San Francisco, San Francisco, CA, U.S., [3]National Institute of Neurological Disorders and Stroke, Bethesda, MD, U.S., [4]Washington University School of Medicine, St. Louis, MO, U.S., [5]J David Gladstone Institute, San Francisco, CA, U.S.

Intraneuronal aggregations of the microtubule-associated protein tau in neurofibrillary tangles are a hallmark of Alzheimer's disease and other neurodegenerative tauopathies. Therapeutic strategies targeting hyperphosphorylated and aggregated tau have been widely explored but are of unclear clinical efficacy. Reduction of total tau protein levels has emerged as an attractive therapeutic strategy. However, screens to identify tau-lowering compounds have utilized neuroblastoma or neuroglioma cell lines that are not physiologically and pathologically relevant to tauopathies. Use of human induced pluripotent stem cells (iPSCs) derived neurons would facilitate high-throughput screening in a disease-relevant system. However, drug discovery with these cells has been hampered by the high cost, cumbersome differentiation procedures, variability, and low yield of homogenous neurons. We engineered a stable iPSC line that harbors a single copy of tetracycline-inducible neurogenin 2 (NGN2), a transcription factor that rapidly converts iPSCs to glutamatergic neurons, at the AAVS1 locus. Using a simple two-step glia-free differentiation protocol, we could differentiate this inducible NGN2 iPSC line into electrophysically active cortical glutamatergic neurons with minimal well-to-well and experiment-to-experiment variability. We optimized a protocol for large-scale production of induced neurons and developed a robust high-content screening assay to quantify neurite tau levels and monitor neurotoxicity. Using this assay, we validated several compounds previously shown to lower tau levels, including salicylate and YM-01. We then screened 2400 compounds from LOPAC and TOCRIS libraries and identified 28 additional tau-lowering compounds, which are in the process of validation by conventional total tau ELISA and western blot assays. Our techniques for engineering stable NGN2 iPSCs and for large-scale production of homogenous human neurons will pave the way for automated high-throughput screening of novel drugs for specific neurodegenerative disorders and neurological diseases

W2054

MODELING ANESTHETIC-INDUCED DEVELOPMENTAL NEUROTOXICITY USING HUMAN STEM CELL-DERIVED NEURONS: THE ROLES OF MIR-21 AND MITOCHONDRIAL FISSION

Yan, Yasheng, Twaroski, Danielle, Zaja, Ivan, Bosnjak, Zeljko and Bai, Xiaowen, Medical College of Wisconsin, Milwaukee, WI, U.S.

Every year in the United States approximately 4 million children are administered anesthetics for imaging or surgical purposes. Propofol is a commonly used agent in pediatric anesthesia, and like other anesthetics, has been linked to neurodegeneration and cognitive dysfunction in animal models, with very limited direct evidence of human neurotoxicity. Human embryonic stem cells (hESCs) are capable of differentiating into any cell type and hESC-derived neurons represent a promising model to study mechanisms governing anesthetic-induced neurotoxicity in humans. MicroRNAs, which are endogenous, small, non-coding RNAs, have been implicated to play important roles in many different disease processes. Increases in mitochondrial fission have been shown to induce cell death in many models. We hypothesized that propofol will induce toxicity in hESC-derived neurons and that this toxicity will occur via a microRNA-21(miR-21)-mediated mitochondrial fission pathway. To perform this experiment, hESCs were differentiated into neurons through a four-step differentiation protocol. Two-week-old neurons were subjected to 6 hours of exposure to 20 μg/mL propofol. TUNEL staining was used to assess cell death. miR-21 was overexpressed using a miR-21 mimic and was quantified by qRT-PCR. Mitochondrial fission was assessed using TOM20 staining and confocal microscopy. The expression of the miR-21 targets of interest and mitochondrial fission-related proteins was assessed by Western blot. The differentiation protocol is 90-95% efficient in the generation of neurons. Exposure to propofol induced cell death, downregulated miR-21 expression, upregulated Sprouty 2 (the direct target of miR-21), and increased mitochondrial fission in the neurons. Pretreatment of the neurons with mdivi-1 (a selective inhibitor of mitochondrial fission) rescued the propofol-induced neuron death and mitochondrial fission. Overexpression of miR-21 and knockdown of Sprouty 2 attenuated the increases in propofol-induced neuron death, Sprouty 2 level, and mitochondrial fission. These data demonstrate for the first time that propofol-induced toxicity in human neurons occurs through a miR-21/Sprouty 2/mitochondrial fission-mediated pathway. miR-21 supplementation may be promising in counteracting the harmful effects of propofol.

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W2056

INVESTIGATION OF ABERRANT CHROMOSOME FOLDING AND GENOME ORGANIZATION IN FRAGILE X SYNDROME

Zhang, Ai and Loring, Jeanne F., The Scripps Research Institute, San Diego, CA, U.S.

Fragile X Syndrome (FXS) is the most common inherited form of intellectual disability and the leading monogenic cause of autism. It is caused by expansion of a trinucleotide repeat in the 5' untranslated region of the fragile X mental retardation-1 (FMR1) gene. The expanded CGG triplet repeats are hypermethylated and the expression of the FMR1 gene is repressed, resulting in the loss of the fragile X mental retardation protein (FMRP). While current FXS research focuses on loss of FMR1 expression as the primary cause of synaptic dysregulation and circuit malformation, we propose that aberrant epigenome and chromosome architecture plays a major role in disrupting normal neuronal development in FXS. We have generated human induced pluripotent stem cell lines (iPSCs) from male individuals afflicted with FXS and have established methods to differentiate them into cortical neurons. Our genome wide analysis of DNA methylation in these cells has shown that aberrant methylation is not restricted to FMR1 locus but is genome-wide. We hypothesize that aberrant methylation in FXS leads to altered genome architecture during neural development, which in turn leads to aberrant neuronal growth and synaptogenesis. Specifically, we are investigating the chromosome folding, chromatin accessibility, and gene expression regulation that are altered in FXS. Identifying such dysregulation may help identify novel disease-causing pathways and suggest potential therapies for FXS and autism.

REPROGRAMMING

W2060

EFFICIENT GENERATION OF FOOTPRINT-FREE, FEEDER-FREE, VIRUS-FREE, INDUCED PLURIPOTENT STEM CELLS (IPS) USING A REPROGRAMMING COCKTAIL

Kamath, Anant[1], Ternes, Sara J.[1] and Moy, Alan B.[1,2], [1]Cellular Engineering Technologies Inc, Coralville, IA, U.S., [2]John Paul II Medical Research Institute, Coralville, IA, U.S.

A fundamental challenge in consistently generating IPS cells has been their extremely low programming efficiency. This difficulty has been compounded by the use of varied target cells, some of which are high in passage number or nearly senescent. Overcoming these problems is central to creating patient-specific IPS lines, many of which might be derived from patients with chronic illnesses that affect target cell health or cells that might be collected from geriatric donors. Equally importantly, the use of viral systems including both Sendai virus and Lentivirus have their own limitations in creating clinically relevant IPS cells that could be potentially reintroduced into human patients. Regardless of the type of reprogramming system used, efficiencies approaching 0.1% are considered to be excellent. This percentage assumes that target cells are in excellent health, actively dividing and expressing high levels of endogenous myc. However, this is often not the case, and this bottle-neck has stifled the creation of IPS lines. Here, we describe a novel reprogramming cocktail consisting of the following components: 1) A histone deactylase inhibitor, 2) A water soluble vitamin, which mitigates oxidative stress and increases cellular division, 3) An Alk-5 (Activin/Nodal Receptor Kinase) inhibitor and 4) A small molecule that activates PDK-1 (Phosphoinositide-dependent protein kinase-1), which facilitates a conversion from mitochondrial oxidation to glycolysis. Addition of this cocktail during the first fourteen days of the reprogramming process enhances the efficiency of IPS generation at least 10-fold and allows for IPS generation from previously resistant cells.

W2062

NUCLEAR AND MITOCHONDRIAL GENOMIC/ EPIGENOMIC GENE THERAPY : WILD-TYPE AND NUCLEASE-NULL CRISPR-CAS9 AS BREAKTHROUGH BOOSTERS FOR AN UPGRADED STEM CELL GENE THERAPY PLATFORM

Bertolotti, Roger, Gene Therapy & Regulation, Faculty of Medicine, University of Nice - Sophia Antipolis, Nice, France

Directed to their ~20bp genome target by an easily customizable short guide-RNA, CRISPR-Cas9 endonuclease and nuclease-null dCas9 drive a double genome/epigenome editing breakthrough, thereby boosting next generation stem cell gene therapy and our relevant platform devised in 2005. Aimed at reversing both nuclear/mitochondrial inherited diseases and acquired/aging disorders by reestablishing healthy genomic/epigenomic homeostasis, our platform relies on 1) endonuclease-boosted gene targeting (gene repair/genome editing), 2) transient epigenetic gene therapy (stable epigenome editing mediated by the transient action of an epigenetic effector), 3) transient regenerative gene therapy and 4) transmitochondrial cybridization. The iPS cell breakthrough provided the long-sought autologous pluripotent drive of our platform and is now magnified by the CRISPR-Cas9 breakthrough that promotes 1) easy personalized genome editing thanks to its digital short guide-RNA (≠ analogic zinc-finger/TALE

nucleases) and 2) straight epigenome editing driven by DNA-targeting dCas9 modules fused to epigenetic effectors (*eg* DNA/histones methylases/demethylases). In its 2005 design, transient epigenetic gene therapy was 1) coined as a translational application of human promoter-specific siRNA-mediated transcriptional gene silencing and promoter demethylation mediated by non-coding antisense RNA, and 2) aimed at long-term transcriptional gene silencing/activation through the transient action of promoter-specific siRNA/antisense RNA. Unlike canonical RNA interference mediated by siRNAs that target cytoplasmic mRNAs for post-transcriptional gene silencing, promoter-specific small RNAs/oligos induce a long-lasting epigenetic process involving DNA methylation/demethylation and post-translational histone modifications. Thus, chimeric dCas9:epigenetic-effector proteins broaden our initial design, in particular for cell reprogramming. Our platform is discussed in terms of 1) clinical geno/epigenosafety, 2) translational synergy for iPS cell-based therapy, 3) *in vivo* genome/epigenome editing (*eg* gene disruption/silencing) and 4) transmitochondrial iPS cybrids for homoplasmic mtDNA diseases (allotopic gene therapy substitute & complement to heteroplasmic iPS cell segregation).

W2064

USE OF NON-MODIFIED RNAS FOR THE DERIVATION OF CLINICALLY RELEVANT IPS CELL LINES FROM HUMAN BLOOD, URINE AND SKIN CELLS USING GMP-COMPLIANT REAGENTS

Eminli-Meissner, Sarah[1], Poleganov, Marco[2], Beissert, Tim[2], Yi, Kevin[1], Moon, Jung-Il[1], Huang, Chris[3], Morrell, Nick[3], Rana, Amer[3] and Hamilton, Brad[1], [1]Stemgent, a Reprocell Group Company, Lexington, MA, U.S., [2]BioNTech RNA Pharmaceuticals GmbH, Mainz, Germany, [3]University of Cambridge, Cambridge, U.K.

Human fibroblasts can be reprogrammed with a cocktail of mRNAs into integration-free human induced pluripotent (iPS) cells. Human blood provides easy access to adult human cell types for reprogramming purposes. Notably, blood-outgrowth endothelial progenitor cells (EPCs) can be clonally isolated from only 10 mL of fresh or frozen mononuclear cell (MNC) preparations from both human peripheral and cord blood. The adherent nature and high proliferative capacity of EPCs makes them highly desirable for repeated transfection with RNA when compared to commonly isolated hematopoietic suspension cell types. Recently, we published the unique application of non-modified RNA technology to the reprogramming of human blood-derived EPCs. Furthermore, urine sampling provides perhaps the most non-invasive form of cell procurement. Renal epithelial cells can be highly reproducibly isolated from only 30 mL of urine. Here we present a flexible, yet powerful, RNA-based reprogramming method that combines a novel cocktail of synthetic, non-modified reprogramming [OCT4, SOX2, KLF4, cMYC, NANOG and LIN28 (OSKMNL)] and immune evasion mRNAs [E3, K3, B18-R] with reprogramming-enhancing mature, double-stranded microRNAs from the 302/367 cluster. This unique combination of different RNAs results in a highly efficient (up to 4%) and robust reprogramming protocol using only GMP-compliant substrates (vitronectin and laminin-511), media compositions (xeno-free or human serum), and RNA to produce clinically relevant iPS cells from blood-derived EPCs, neonatal as well as adult fibroblasts, and for the first time renal epithelial cells derived from urine.

W2066

THE NEW BMP DOWN-STREAM MOLECULE ANKS1B IS CONTROLLING HISTONE H3 METHYLATION WITH ABNORMAL BMP SIGNALING.

Hamasaki, Makoto[1], Kiboku, Takayuki[1], Soga, Minami[1], Shinojima, Naoki[2], Furuya, Hirokazu[3] and Era, Takumi[1], [1]Department of Cell Modulation, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan, [2]Department of Neurosurgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan, [3]Department of Neurology, Kochi Medical School, Kochi University, Nangoku, Japan

Fibrodysplasia ossificans progressiva (FOP) is a rare congenital disorder characterized by progressive ossification of soft tissues. FOP is caused by mutations in activin receptor-like kinase 2 (ALK2) that cause its constitutive activation and result in dysregulation of BMP signaling. Here, we show that abnormal signaling inhibit Histone H3K4 and H3k27 tri-methylation(me3) during reprogramming. As a BMP signal mediator SMADs form complex with Histone H3 K4me3 demethylase (LSD1) and K27me3 demethylase KDM6B).SMAD/LSD1 complex inhibit Histone H3K4me3upregulation of OCT3/4 and NANOG promoter region and these gene expression were decrease during reprogramming. SMAD/KDM6B complex inhibit Histone H3K27me3 upregulation of Osteo-transcriptional factors genome locus and these gene expression were elevated during reprogramming. These complexes dysregulate Histone methylation during reprogramming, result in Abnormal BMP signaling inhibit iPSC generation. We searched the abnormal BMP signaling and dysregulated Histone H3 K27me3 then we identified ANKS1B not only FOP, but also in a glioma.ANKS1B controls H3K27 methylation via the regulations of methyltransferase and demethylase activities in both diseases, and suppressing ANKS1B expression can rescue iPSC generation, inhibit osteogenesis in FOP and tumor growth in glioma. Moreover, we have demonstrated the involvement of ANKS1B in the pathogenesis of both FOP and gliomas carrying ALK2 R206H mutation

and Histone3.3K27M mutations. Our findings suggest that FOP and gliomas share common pathogenesis represented by the dysregulation of K27 demethylation in histone H3 via aberrant BMP signaling, this is, ANKS1B as a new therapeutic candidate to treat these diseases.

W2068

DIRECT REPROGRAMMING OF FIBROBLASTS INTO SPECIFIC CELL TYPES USING INTEGRATION-FREE METHODS

Kim, Sungmin[1], Kim, Jonghun[1] and Han, Dong Wook[2], [1]Konkuk university, Seoul, Korea, South, [2]School of Medicine Konkuk University, Seoul, Korea, South

The viral vector-mediated overexpression of the defined factors could induce the direct conversion of somatic fibroblasts into various types of cells. However, viral vectors may be randomly integrated into the host genome thereby increasing the risk for undesired genotoxicity, mutagenesis, and tumor formation. Here, we describe that mouse fibroblasts can be converted into specific cell types such as induced-hepatocytes (iHeps) or -neural stem cells (iNSCs) using a different set of transcription factors (Gata4, Hnf1a, and Foxa3 or Brn4/Pou3f4, Sox2, Klf4, and c-Myc) based on integration-free vector systems. Integration-free iHeps or iNSCs closely resemble their in vivo counterparts in morphology, gene expression profile, epigenetic status, and functionality. Therefore, our study provides a novel concept for generating functional iHeps or iNSCs using a non-viral, non-integrating, plasmid-based system that could facilitate their biomedical applicability.

Funding Source: Grant from the Next-Generation BioGreen21 program (PJ011311), Rural Development Administration, Republic of Korea.

W2070

RAPID SELECTION OF EXOGENE-FREE INDUCED PLURIPOTENT STEM CELLS IN EPISOMAL VECTOR-BASED REPROGRAMMING

Lee, Minhyung[1,2], Ahn, Hyunjun[1,2], Ha, Jeongmin[2,3], Sim, Hyuna[1,2] and Kim, Janghwan[1,2], [1]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea, [2]Korea University of Science & Technology, Daejeon, Korea, [3]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea, South

The reprogramming with episomal vectors suggest an easy and cost-effective method to generate induced pluripotent stem cells (iPSCs). However, isolation of exogene-free iPSC colonies requires more than ten passages of culture. Moreover, the frequency of exogene-integration to host genome is relatively very high than expected. Because integration of reprogramming factors can cause insertional inactivation of important genes for differentiation or tumor suppression, selection of exogene-free iPSCs is a critical requirement for any application in regenerative medicine. Thus, we sought to develop a new reprogramming system to eliminate the residual episomal vectors rapidly and to remove iPSCs which have integrated copies of episomal vectors to their genome. We inserted a suicide gene (SG), which can convert a non-toxic pro-drug into a toxic drug to cells, to each episomal vectors. We assumed that SG harboring cells may experience retarded growth or death when the pro-drug is treated so this may lead to enhancing the removal process of exogene-harboring cells. We were able to generate rapidly the exogene-free iPSCs with SG-episomal vectors than with conventional episomal vectors and the generated iPSCs showed good marker expressions and differentiation potentials in vitro and in vivo. When we integrated the SG to embryonic stem cells (ESCs), we found the SG-integrated ESCs were negatively selectable under the pro-drug treated condition. Thus, we suggest that our new episomal vector sets with SG will reduce the time and cost in generating clinically applicable safe iPSCs.

Funding Source: This research was supported by Stem Cell Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Science, ICT and Future Planning(NRF-2015M3A9C7030128, NRF-20

W2072

OVOL2 MAINTAINS THE TRANSCRIPTIONAL PROGRAM OF CORNEAL EPITHELIAL CELLS

Masui, Shinji[1], Kitazawa, Koji[1,2] and Kinoshita, Shigeru[2], [1]CiRA, Kyoto University, Kyoto, Japan, [2]Kyoto Prefectural University of Medicine, Kyoto, Japan

In development, embryonic ectoderm differentiates into neuroectoderm and surface ectoderm using poorly understood mechanisms. Here we show that the transcription factor OVOL2 maintains the transcriptional program of corneal epithelial cells (CECs), a derivative of surface ectoderm, and that OVOL2 might regulate the differential transcriptional programs between the two lineages. A functional screening identified OVOL2 as a repressor of mesenchymal genes to maintain CECs. Transduction of OVOL2 with several other transcription factors induced the transcriptional program of CECs in fibroblasts and produced a specific chromatin landscape. Moreover, neuroectoderm derivatives were found to express mesenchymal genes, and OVOL2 alone could induce the transcriptional program of CECs in neural progenitors by repressing these genes while activating epithelial genes. Our data suggest that the difference between the transcriptional programs of some neuroectoderm- and surface ectoderm-derivatives cells may be regulated in part by a reciprocally-repressive mechanism between epithe-

lial and mesenchymal genes, as seen in epithelial-to-mesenchymal transition.

Funding Source: CREST, Japan Science and Technology Agency (JST), Japan

W2074

NEURAL REPROGRAMMING OF HUMAN BONE MARROW MESENCHYMAL STROMAL CELLS

Ramos-Mandujano, Gerardo[1], Aguilera-Castrejón, Alejandro[1], Montesinos, Juan José[2], Castro-Manrreza, Marta E.[2] and Pasantes-Morales, Herminia[1], [1]Instituto de Fisiología Celular,UNAM, Mexico City, Mexico, [2]Mesenchymal Stem Cells Laboratory, Oncology Research Unit, Oncology Hospital, National Medical Center, IMSS, Mexico City, Mexico

Human mesenchymal stromal cells (hMSCs) upon grafting have shown positive inflammatory and immune-modulatory actions, secretion of trophic factors, and lower risk of tumor formation. Furthermore, it has been proposed that hMSCs are able to transdifferentiate into lineages other than the tissue of origin without the introduction of ectopic genes. Several studies have reported that mesenchymal stromal cells (MSCs) generate cells with neural stem/progenitor properties that are named mesenchymal stem cell derived neural stem/progenitor-like cells (MSC-NSPs). In the present study, human bone marrow MSCs were cultured with a specific serum-free media that contained hEGF and hFGF on low attachment, standard or fibronectin-coated plates. In low attachment plates, MSCs generated neurosphere-like structures formed by nestin$^+$ cells with increased expression of the neural progenitor genes Nestin, Sox2, Sox1, and Pax6. The neural progenitor properties were similar in the MSC-NSPs obtained as neurosphere-like structures or in monolayers on standard or fibronectin-coated plates. When proliferative capacity was analyzed, MSC-NSPs seeded on low attachment or standard plates showed a minimal cell number increase as well as few BrdU$^+$ or Ki67$^+$ cells. On fibronectin-coated plates, cell number increased 4-7-fold; the percentage of BrdU$^+$ and Ki67$^+$ cells was 42-45% and 16-19%, respectively. After neuronal differentiation induction, the MSC-NSPs generated βIII tubulin$^+$ cells; however, MAP2$^+$ cells were not detected even in the fibronectin-coated condition. This indicates that the MSC-NPs have not the potential to differentiate into mature neurons. To induce mature and functional neurons, we are testing a chemical induction approach, using compounds that modulate epigenetics modifiers and signaling pathways relevant for neuronal differentiation and in cell reprogramming. Preliminary results indicated that cell derived from MSC-NSPs show more neuronal properties that those derived from hMSC. These results can contribute to understand the neural reprogramming of MSC in vitro to making them a suitable option for neural replacement strategies.

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W2076

FGF12 IS A NOVEL REGULATOR OF VASCULAR SMOOTH MUSCLE CELL PLASTICITY AND FATE

Suh, Wonhee, Chung-Ang University, Seoul, Korea

Vascular smooth muscle cells (VSMCs) modulate their phenotypes between synthetic and contractile states in response to environmental changes, which plays a crucial role in the pathogenesis of restenosis and atherosclerosis. Here, we identified fibroblast growth factor 12 (FGF12) as a novel key regulator of the VSMC phenotype switching. We used murine models and human specimens and found that FGF12 was highly expressed in contractile VSMCs of normal vessel walls but was downregulated in synthetic VSMCs of injured and atherosclerotic vessels. In human VSMCs, FGF12 expression was inhibited at the transcriptional level by platelet-derived growth factor-BB. The gain- and loss-of-function experiments showed that FGF12 was necessary and sufficient for inducing and maintaining the quiescent and contractile phenotypes of VSMCs. The FGF12-induced phenotypic changes in VSMCs were mediated by the p53 pathway. Moreover, FGF12 upregulated the key factors involved in VSMC lineage differentiation, such as myocardin and serum response factor. Indeed, FGF12 promoted the differentiation of mouse embryonic stem cells and transdifferentiation of human dermal fibroblasts into SMC-like cells. Further, adenoviral infection of FGF12 substantially decreased neotintima hyperplasia in a rat carotid artery injury model. In general, FGF family members induce a synthetic VSMC phenotype. Interestingly, the present study showed the unanticipated finding that FGF12 belonging to FGF family, strongly induced the quiescent and contractile VSMC phenotypes and directly promoted VSMC lineage differentiation. These novel findings suggested that FGF12 could be a new therapeutic target for treating restenosis and atherosclerosis.

W2078

EFFICIENT GENERATION OF INTEGRATION-FREE IPS CELLS FROM ADULT PERIPHERAL BLOOD MONONUCLEAR CELLS BY OPTIMIZED EPISOMAL VECTORS

Wen, Wei[1], Zhang, Jian-Ping[2], Xu, Jing[2], Ji, Guangzhen[2], Su, Ruijun Jeanna[3], Neises, Amanda[3], Yuan, Weiping[2], Cheng, Tao[2] and Zhang, Xiaobing[3], [1]State Key Laboratory of Experimental Hematology Institute of Hematology, Tianjin, China, [2]Institute of Hematology and Blood Disease Hospital, Tianjin, China, [3]Loma Linda University, Loma Linda, CA, U.S.

Integration-free iPS cells generated from adult peripheral blood (PB), the most available and non-invasive cell source, hold great promise for clinical regenerative medicine. We have reported that an improved episomal vector (EV) reprograming system (*Molecular Therapy*. 2012;20(2):408-16; *PLoS One*. 2013;8(5):e64496), in which a strong promoter SFFV and an additional factor BCL-CL (B) are used, leads to a 10- to 100-fold increase in PB reprogramming compared to similar systems developed by other labs. However, EV is still less efficient than another popular integration-free reprograming vector system, Sendai virus (SV). After a systemic investigation of factor combinations in the EV system, we find that balanced expression of OCT4 (O) and SOX2 (S), by using a 2A self-cleavage peptide sequence to link the 2 factors, and expression of MYC (M) and KLF4 (K) using 2 individual vectors, lead to an additional ~100-fold increase in PB reprogramming than we previously reported (OS+MK+B). The reprogramming efficiency of the new combination (OS+M+K+B) is even slightly higher than the SV system, making the affordable EV reprogramming approach more attractive. Other combinations such as OM+S+K+B, OK+S+M+B, or OSK+M+B show a significant decrease in reprogramming efficiency compared to OS+M+K+B, highlighting the importance of vector design. All the 5 factors are critical: without O or S, virtually no iPS cells are observed; omitting MYC or KLF4 reduces reprogramming efficiencies by a factor of 200 and 10, respectively; absence of BCL-CL leads to an ~70% decrease in reprogramming efficiency. The iPSCs are indistinguishable from those generated with the previous protocol in expression levels of pluripotency markers and teratoma formation ability. In addition, the iPSC lines show no residual plasmids and a normal karyotype after 2-3 months of culture. Finally, using optimized EV and plasmids to express the reprogramming factors and Cas9, sgRNA and a donor template, we achieve >10% knockin efficiency in established iPSC lines. In summary, our optimized EV reprogramming system is highly efficient: one may obtain thousands of iPSC colonies from one ml of PB. Combination of PB reprogramming and CRISPR-mediated genome editing in a single step should have broad applications in replacement therapy and disease modeling.

IPS CELLS

W2082

THE DNA GLYCOSYLASE NEIL3 MAINTAINS PLURIPOTENCY AND DIFFERENTIATION CAPACITY IN IPS CELLS

Askeland, Georgina Adeline[1], Wang, Wei[2], Scheffler, Katja[2], You, Panpan[2], Suganthan, Rajikala[2], Sullivan, Gareth[1], Bjoras, Magnar[2] and Eide, Lars[1], [1]University of Oslo, Oslo, Norway, [2]Oslo University Hospital, Oslo, Norway

DNA glycosylases play an important role in DNA repair, keeping at bay the accumulation of DNA damage and mutation. Neil1 and Neil2 of the Neil family have been well characterized and participate in the base excision repair pathway, removing damage caused by oxidation. Neil3 has shown to be a functional DNA glycosylase in vitro, with a proliferative tissue-specific expression, although its biological function remains unknown. Our lab has previously established Neil3-deficient iPS cells, a valuable tool to study proliferation and differentiation. Methods included qPCR and immunocytochemistry for analysis of stem cell and differentiation marker genes, an in-house developed DNA damage assay, ATP assay and oxidant/anti-oxidant treatment. Characterization of Neil3-/- iPS cells revealed stronger stem cell characteristics through elevated activity of the alkaline phosphatase and more than 10-fold higher expression of Rex1; two pluripotency markers. Defects were also seen in expression of lineage marker genes upon differentiation to embryoid bodies and oxidant treatment caused a reduced ability for embryoid body formation. Intracellular ATP level was reduced in Neil3-deficient iPS cells, in parallel with upregulation of mtDNA-encoded genes. Our results indicate that Neil3 plays a role in the pluripotency and differentiation ability of iPS cells by maintaining bioenergetic capacity.

W2084

CHCHD2 PRIMES THE DIFFERENTIATION POTENTIAL OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO NEUROECTODERMAL LINEAGES

Armstrong, Lyle[1], Zhu, Lili[1], gomez-Duran, Aurora[1], Saretzki, Gabriele C.[2], Jin, Shibo[1], Tilgner, Katarzyna[3], melguizo-Sanchis, Dario[1], Anyfantis, Georgios[1], al-Aama, Jumana[4], Vallier, Ludovic[5], Chinnery, Patrick[1] and Lako, Majlinda[1], [1]Newcastle University, Newcastle, U.K., [2]Institute for Aging and Health, Newcastle Upon Tyne, U.K., [3]Cambridge University, Cambridge, U.K., [4]King Abdulaziz University, Jeddah, Saudi Arabia, [5]Cambridge Stem Cell Institute, Cambridge, U.K.

Human induced pluripotent stem cells (hiPSC) utility is limited by variations in their ability to undergo lineage specific differentiation. We have undertaken a transcriptional comparison of human embryonic stem cell lines (hESC) and hiPSC lines and have shown that hiPSC are inferior in their ability to undergo neuroectodermal differentiation. We have identified *CHCHD2*, whose expression is much lower in hiPSC when compared to hESC and can be used as a marker to predict the neuroectodermal differentiation potential of hiPSC. We provide evidence that the hiPSC variability with respect to *CHCHD2* expression and differentiation potential is caused by clonal variation during the reprogramming process and that CHCHD2 primes neuroectodermal differentiation of hESC and hiPSC through regulating the activity of the TGFβ signalling pathway. The potential utility of using CHCHD2 as a marker for assessing and comparing the hiPSC clonal and/or line differentiation potential provides a tool for large scale differentiation and hiPSC banking studies.

Funding Source: Biotechnology and Biosciences Research Council

W2086

DIRECTING LUNG DIFFERENTIATION IN HUMAN IPS CELLS

Carraro, Gianni, Konda, Bindu, McQualter, Jonathan L. and Stripp, Barry, Cedars-Sinai Medical Center, Los Angeles, CA, U.S.

Human lung epithelium is composed of specialized cell types that vary in composition and function along the proximo-distal axis. The process of cellular differentiation that occurs during human lung development is still largely unknown. However, spatially restricted expression of transcription factors such as NKX2.1, SOX2 and ETV5 are known to define specification of lung endoderm and specialized proximal or distal lineages, respectively. In order to obtain a better understanding of the remodeling process typical of many lung diseases, it would be highly beneficial to identify the molecular cues that regulate formation and maintenance of specialized cell types. Methods were developed for the regulated differentiation of human induced pluripotent stem (iPS) cells into NKX2.1-expressing lung endoderm. Reporter lines were developed using the CRISPR/Cas9 system to introduce fluorescent protein reporters 3' to the open reading frame of transcription factors that define proximo-distal patterning of lung endoderm. Through regulated differentiation of human iPS cells, we generated NKX2.1+, PAX8- (thyroid differentiation) and TUJ1- (neural differentiation) lung endoderm. A karyotypically normal human iPSC line (83i-SOX2nYFP) was developed harboring a nuclear localized YFP reporter downstream of the SOX2 coding sequence, allowing normal expression of the SOX2 RNA in combination with the YFP reporter. Regulated differentiation of 83i-SOX2nYFP was associated with the dynamic regulation of nYFP leading to the generation of NKX2.1+ lung endoderm that was composed of a mixed population of nYFP+ and nYFP- cells. There is a strong need to develop therapies that allow for the restoration of the epithelial microenvironment. We are using human iPS cells that hold great potential for cell based-therapy. Using the Sox2-YFP line in combination with other reporters that we are creating will allow us to analyze human lung proximal and distal differentiation in an in vitro setting. Furthermore, the current results suggest that the CRISPR/Cas9 system is a valuable tool to introduce specific reporters in human iPS cells. Advancement of these studies will help to improve the differentiation of specific epithelial lineages that could be used as in vitro models for lung diseases and for drug screening.

Funding Source: Funding by CIRM and NHLBI

W2088

N-ACETYLCYSTEINE RESCUE MITOCHONDRIAL DYSFUNCTION IN MERRF-hiPSC-DERIVED CARDIOMYOCYTE

Chou, Shih-Jie[1], Lai, Yu-Fen[2], Chien, Chian-Shiu[3], Lee, Hsin-Chen[1], Wei, Yau-Huei[4] and Chiou, Shih-Hwa[2], [1]Pharmacology NYMU, Taipei, Taiwan, [2]Department of Med Res & Education, Taipei Veterans General Hosp, Taipei, Taiwan, [3]Taipei Veterans General Hospital, Taipei City, Taiwan, [4]Mackay Medical College, New Taipei City, Taiwan

Myoclonus epilepsy associated with ragged-red fibers (MERRF) is an A to G mutation at 8344th nucleotide of mitochondrial DNA (mtDNA), leading to disruption of the mitochondrial gene for tRNA(Lys). MERRF syndrome is characterized by myoclonus epilepsy, generalized seizures, ataxia and myopathy. Apart from the well-characterized syndrome in neural system, around 53% of MERRF patients also suffered from cardiomyopathy. However, the mechanism by which this tRNA(Lys) mutation causes

mitochondrial dysfunction in cradiomyocyte remains unclear. In this study, we established human induced pluripotent stem cells (hiPSC) carrying mtDNA A8344G mutation and mutation-free isogenic hiPSC from patients with MERRF syndrome (MERRF-hiPSC). We induced MERRF-hiPSC differentiation into cardiomyocyte (MERRF-CM) which exhibited reduced mitochondrial mass and lower expression level of mitochondria-associated genes. Moreover, the extracellular flux analyzer demonstrated decreased ATP synthesis and oxygen consumption in MERRF-CM due to impaired mitochondrial function. The mitochondria dysfunction also induced higher level of reactive oxygen species (ROS) and expression of alternative antioxidant gene. Respectively, we demonstrated that the low mitochondrial mass, excess ROS and poor ATP production were rescued by the treatment of N-acetylcysteine (NAC), a precursor of glutathione that acts as the first-line intracellular antioxidant in MERRF-CM. Taken together, these investigations are valuable in dissecting MERRF pathogenesis in patient's cardiomyocyte and providing a suitable platform for drug screening.

W2090

EFFECT OF CELL MIGRATION BEHAVIOR ON DEVIATION FROM THE UNDIFFERENTIATED STATE IN COLONY OF HUMAN INDUCED PLURIPOTENT STEM CELLS

Eri, Shuzui, Kim, Mee-Hae and Kino-oka, Masahiro, Osaka University, Suita, Japan

In culture of human induced pluripotent stem cells (hiPSCs), the cell deviation from undifferentiated state is well-observed in the central or peripheral region of the colony. In the present study, the spatio-temporal observation was conducted to estimate the migration rate through the cultures with SNL or MEF feeder cells, and the retrospective analysis was made to understand the locational dependency of cell deviation in colony as well as the colony-size dependency. The deviation from undifferentiated state occurred at the center and periphery of the colony in cultures with SNL or MEF feeder cells, respectively. And the migration rates of undifferentiated iPSC at the peripheral region in colony were 1.9 and 1.7 times higher than those of the central region, respectively. The colonies including the deviated cells from the undifferentiated state on MEF feeder cells show higher migration rate at the peripheral region compared with those in culture with SNL feeder cells, whereas the colonies including the deviated cells from the undifferentiated state on SNL feeder cells have lower migration rate at the central region compared with those in culture with MEF feeder cells. The further experiments of exposure to migratory (Rac1) inhibitor (NSC 23766) and activator (HMG-1) revealed that the inhibitor generated the deviated cells at the center of colony in culture with MEF feeder cells and the activator generated the deviated cells at the periphery of colony in culture with SNL feeder cells, showing the opposite positions of cell deviation in the colony by changing the migratory activity. Thus, the cell deviation is considered to have migration dependency that excess reduction and enhancement of migration lead to cell deviation at the central and peripheral region of colony, respectively, without distinction of feeder cells.

W2094

TARGETED GENE EDITING IN NON-HUMAN PRIMATE IPSC USING CRISPR/CAS9 SYSTEM

Hong, So Gun[1], Zou, Jizhong[1], Choi, Kyujoo[2], Yada, Ravi Chandra[2], Carpentier, Arnaud[2], Liang, Jake[2], Merling, Randall[2], Sweeney, Colin[2], Malech, Harry[2], Jung, Moonjung[2], Corat, Marcus[2], Liu, Huimin[1], Lin, Yongshun[2], Tunc, Ilker[2], Wang, Xujing[2], Palisoc, Maryknoll[2], Pittaluga, Stefania[2], Winkler, Thomas[2] and Dunbar, Cynthia[2], [1]NHLBI/NIH, Bethesda, MD, U.S., [2]National Institutes of Health, Bethesda, MD, U.S.

Non-human primate (NHP) induced pluripotent stem cells (iPSCs) offer the opportunity to investigate the safety, feasibility and efficacy of proposed iPSC-derived cellular delivery in clinically relevant models. However, there is need for stable, robust, and safe labeling methods for NHP iPSCs and their differentiated lineages to study their survival, proliferation, tissue integration, and biodistribution following transplantation. Typically, randomly integrating viral vectors have been used to deliver and permanently integrate marker genes in target cells. However, genotoxicity as well as silencing of transgenes, particularly in pluripotent stem cells, hinders their utility for tracking and eventual clinical applications. Site directed integration of marker genes into potentially safe genomic regions could be an alternative approach. Adeno-associated virus integration site 1 (AAVS1) has been suggested to be such a safe harbor for human cells. Here, we investigate its utility in our rhesus macaque iPSCs (RhiPSCs) model. Knock-in of human truncated CD19 (hΔCD19), the clinically relevant marker gene or green fluorescent protein (GFP) at the AAVS1-like site in RhiPSCs was highly efficient using the clustered regularly interspaced short palindromic repeats /CRISPR-associated nuclease 9 (CRISPR/Cas9) system. Genetically modified RhiPSCs maintained normal karyotype and pluripotency, demonstrated by teratoma formation. CRISPR-edited clones were able to further differentiate towards granulocytes or hepatocytes. More importantly, transgene expression was stable in CRISPR-edited RhiPSCs but also differentiated cell types derived from the RhiPSCs, in contrast to prior experience with viral vector delivery. We have established a computational platform to evaluate efficiency and off-target effects of guide RNAs in the rhesus genome. Off-target analysis in CRISPR-edited clones is ongoing and result will be presented at the meeting. Genetically marked RhiPSCs will

be useful to further advance clinically relevant models for iPSC-based cell therapies.

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W2096

MATERNAL RNAs DEGRADATION IS ESSENTIAL FOR IPS REPROGRAMMING

Kami, Daisuke[1], Kitani, Tomoya[2] and Gojo, Satoshi[1], [1]Kyoto Prefectural University of Medicine, Kyoto, Japan, [2]Kyoto Prefectural University of Medicine, Kyoto City, Japan

Non-coding RNAs are involved in the regulation of most biological processes such as cancer, embryonic development, and cellular reprogramming. However, little is known about their roles in iPS reprogramming. We previously reported that non-coding RNA, RNY1 (Y1, 113 nt), transiently expressed in iPS reprogrammed fibroblasts at early stage, and inhibition of Y1 expression was failed iPS reprogramming. It is known that the Ro60 protein and Y1 in cytoplasm form protein and RNA complex, so-called ribonucleoprotein (RNP), and the RNP prevents Y1 degradation. However, much remains unknown about Ro60-Y1 RNP function in iPS reprogramming. The objective of this study is to identify a non-coding RNA to regulate iPS cell reprogramming, and analyze the effect to the processes. We analyzed the other interactions protein with Ro60 protein by immunoprecipitation, and mass spectrometry-analysis. We identified the unique protein, called X, which is one of the proteins in Processing body (P-body) component, which is distinct foci within the cytoplasm of the eukaryotic cell consisting of many enzymes involved in mRNA turnover. Furthermore, we analyzed that Ro60 antibody-immunoprecipitated proteins co-localized with X-protein by western blotting, and immunocytochemistry methods. X gene-knockout cells by CRISPR technology cannot form iPS colonies. In Addition, the interaction ratio between X and Ro60 proteins using siRNA for Y1 were significantly increased than these using siRNA for negative control.Our results showed that Ro60-Y1 RNP regulated the behavior of X protein in P-body. These data indicate that it might be an important rule to be regulated the RNA-metabolic turnover in iPS reprogramming cells.

W2100

USING PARKINSON' S DISEASE (PD) MOUSE MODEL TO RESCUE ALPHA-SYNUCLEIN MUTATION-INDUCED PD BY USING IPS CELLS TECHNOLOGY AND GENE THERAPY

LIU, Shih-Ping, Lin, Guan-Cyun, Huang, Yu-Chuen and Chen, Shih-Yin, China Medical University, Taichung, Taiwan

Parkinson's disease (PD) is the most common neurodegenerative disease and it has no effective therapeutic method. The symptoms of PD include resting tremor, bradykinesia and muscle rigidity. PD pathogenesis is characterized by loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). α-synuclein (SNCA) which has three types of missense mutations, A53T, A30P, and E46K, which play an important role in pathogenesis of Parkinson's disease. The SNCA-A53T mutation transgenic mice model (PD mouse model from The Jackson Lab) developed PD around 8 months of age. Stem cells provide hope for PD. In all type of stem cells, cells (including iPS cells) are the most powerful cells that could differentiate into all-three kinds of germ layers, including DA neurons. In our experiment, we will create iPS-SNCA from SNCA-A53T mutation transgenic mouse fibroblast. The iPS-SNCA cells will silence the SNCA-A53T mutation gene by using shRNA system to achieve therapeutic effect and differentiate into neural stem cell (NSC) to treat SNCA-A53T mice to confirm that the NSC is useful therapeutically. Finally, the behavior test data used to evaluate the therapy efficiency of the NSC transplanted by locomotor activity, beam walking and rotarod. In summary, we demonstrated that the IPS is a powerful technology to rescue Parkinson patients.

W2102

DROPTECH(R) - A HANGING DROP BASED AUTOMATED STEM CELL TECHNOLOGY PLATFORM FOR PRODUCTION AND TESTING

Meiser, Ina[1], Stracke, Frank[1], Gentile, Luca[1], Neubauer, Julia C.[1], Gorjup, Erwin[1], Koutsouraki, Eirini[2], Gardner, John O.[2], De Sousa, Paul[3], Keminer, Oliver[4], Claussen, Prof. Carsten[5], Gribbon, Philip[4], Pless, Ole[5] and Zimmermann, Prof. Heiko[1,6], [1]Fraunhofer IBMT, Sulzbach, Germany, [2]Roslin Cellab LTD, Midlothian, U.K., [3]Roslin Cells Limited, Edinburgh, U.K., [4]Fraunhofer IME Screening Port, Hamburg, Hamburg, Germany, [5]Fraunhofer IME ScreeningPort, Hamburg, Germany, [6]Saarland University, Saarbruecken, Germany

Cell-based screening assays are a necessary tool for pre-clinical compound development approval. A major challenge concerning these assays is the supply of homo-

geneous and physiological model systems based on patient-specific human induced pluripotent stem cells (hiPSCs). Standard 2D cultivation techniques in flasks, or bulk suspension cultures with high medium consumption and undefined environmental parameters around the resulting inhomogeneous cell aggregates are not feasible for this purpose. Hence research focuses on 3D models. The hanging drop (HD) cultivation technique is a method to achieve homogeneous 3D cell aggregates: HDs from cell suspensions act as micro-volume single bioreactors (20-40µl). Due to gravity cells are gently forced to aggregate on the bottom and connect only to each other or to an intentionally added growth surface. Since HDs are usually still processed manually, the technique is not ready for high throughput. To improve this, we introduce the DropTech® approach, an automated platform for the cultivation and manipulation of HDs with an integrated readout system. Using HD plates with access from above (i.e. 96 well format), pipet robots are able to dispense HDs, exchange media, add specific factors, or harvest the drops, in parallel. To prove the flexibility as well as the reliability of the DropTech® platform, the Embryonic Stem Cell Test (EST) to predict embryotoxicity and cytotoxicity in vitro, has been implemented and translated to workflows completely in HDs. The EST has been established by Seiler et. al. (Nature Protocols, 2011) using murine stem cells and has been validated as an alternative to animal testing by the European Centre for the Validation of Alternative Methods (ECVAM). It consists of the cytotoxicity endpoint, where the cell viability is evaluated after 7d cultivation in the presence of the compound, and of the differentiation endpoint. Here, the differentiation potential of stem cells into cardiomyocytes in presence of compounds is analyzed. We show here that both workflows, using murine embryonic and human induced pluripotent stem cells, are fully automatable in HDs using the DropTech® platform. In addition, a novel readout system has been developed, which is capable of non-contact contraction detection in differentiated cardiac cluster in HDs.

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W2104

CRISPR/CAS9 CORRECTION OF SICKLE CELL ANEMIA MUTATION IN PATIENT-DERIVED IPSC

Molina Estevez, Francisco Javier[1,2], Park, Seonmi[1,2], Sommer, Cesar[1,2], Gianotti-Sommer, Andreia[1,2], Steinberg, Martin [2], Murphy, George[2] and **Mostoslavsky, Gustavo**[1,2], [1]Section of Gastroenterology, Department of Medicine. Boston University, Boston, MA, U.S., [2]Center for Regenerative Medicine, Boston University School of Mecidine, Boston, MA, U.S.

Sickle cell disease (SCD) is a genetic disease with a carrier's frequency as high as 3/1000 births in the Afro-American population. All SCD patients share a common mutation affecting the HBB gene, which encodes the β-chain that is assembled into the adult hemoglobin tetramer. The sickle hemoglobin (HbS) aggregates forming long filaments that change the characteristic erythrocyte's shape and flexibility. This results in disseminated hemolysis when sickle cells endure capillary hydraulic forces. Maintained over time, the body response to this situation results into a complex syndrome featuring diverse degrees of chronic pain, anemia, intravascular hemolysis, systemic endothelial inflammation and toxic iron overload in the liver. Currently, the only cure for patients is bone marrow transplantation. However, as little as 5-10% of healthy RBC chimerism has been documented to diminish the symptoms in SCD patients. Our goal is to test the feasibility of the use of SCD patients' own cells as the starting point for the generation of disease-free red blood cell progenitors. To achieve this goal, we took advantage of two major novel methodologies: cell reprogramming, which allows the generation of induced pluripotent stem cell lines (iPSC) from adult somatic cells; and the CRISPR/CAS9 system, which allows the quick generation of engineered nucleases targeting the sickle mutation in the HBB locus. We use the described STEMCCA cassette to generate a SCD iPSC bank. Then, we designed and assembled a set guide RNAs directed against de vicinity of the SCD mutation and combined them with ssODNs homologous to the targeted region. Our tools successfully corrected the SCD mutation in SCD patient derived iPSC. We show evidence that after the correction, cells retained a normal karyotype and all their pluripotent features, including their ability to be differentiated into erythroid progenitors under controlled conditions. Ours, together with other studies, have demonstrated control over generation, therapeutic gene editing and deterministic differentiation of patient-specific iPSC; contributing to set the foundations for the claims to consider this cells as a real future cell therapy alternative for SCD patients, currently hurdled by chronic non-curative treatments.

W2106

GENERATION OF INTERSPECIES CHIMERA BETWEEN CYNOMOLGUS MONKEY INDUCED PLURIPOTENT STEM CELLS AND PORCINE EMBRYOS

Nowak-Imialek, Monika[1,2], Wunderlich, Stephanie[2], Herrmann, Doris[1], Klein, Sabine[1], Baulain, Ulrich[1], Lucas-Hahn, Andrea[1], Petkov, Stoyan[1], Mall, Eva Maria[1,2], Petersen, Björn[1], Martin, Ulrich[2] and Niemann, Heiner[1,2], [1]Friedrich Loeffler Inst (FLI), Neustadt, Germany, [2]Hannover Medical School, Hannover, Germany

The use of porcine viable cells, tissues and organs for pig-to-human transplantation is considered the most promising alternative to overcome the growing allograft shortage. The generation of human iPSCs paves the way to generate organs from patient's own pluripotent stem cells. The production of chimeric piglets carrying organs that have a high contribution of human cells or tissue, could be used as a new source for therapeutic tissue or organ replacement. Here, we optimized an interspecific chimera assay using cynomolgus monkey induced pluripotent stem cells (cyiPSCs) and porcine embryos to evaluate the feasibility of a potential use of human iPSCs to produce human stem cell-derived organs in pigs. First, we identified effective in vitroculture system for porcine parthenogenetic blastocysts and monkey cyiPSCs. We compared porcine blastocyst rates cultured in eight different monkey stem cell media and porcine zygote medium 3 (PZM-3). The number of blastocysts on day 8 cultured in iPS 20% medium was significantly higher (91%) than in the commonly used porcine PZM-3 medium (65%). We found significantly fewer degenerated embryos on day 8 after culture in iPS 20% medium (9%) compared to PZM-3 (35%). Thereafter, we injected clusters of 10-15 Venus-marked cyiPSCs into porcine parthenogenetic embryos from days 4 and 6. Injection of cyiPSCs into porcine embryos day 4 resulted in significant higher number of Venus-positive blastocysts (74.5%) compared to the injection into blastocysts from day 6 (43%). We observed 26% (41/155) blastocysts with Venus-expressing cyiPSCs exclusively in the porcine ICM, 53% (82/155) in trophectoderm and 21% (32/155) in both the ICM and trophectoderm. However, chimeric blastocysts plated onto dishes exhibited outgrowth with separate porcine- and cyiPSCs colonies. Next we generated chimeras between cyiPSCs and vivoderived porcine embryos (4-16-cells and morulae). Of 93 chimeric embryos transferred to two gilts 34 were recovered seven days later. Unfortunately, only single cyiPSCs was found in the trophectoderm. In summary we optimized culture conditions for interspecies chimera in which monkey cyiPSCs are able to integrate and survive in porcine embryos in vitro. However, cyiPSCs injected into porcine vivo embryos did not integrate into the epiblast of host embryos.

W2108

PLURIPOTENCY POTENTIAL OF EQUINE MESENCHYMAL CELLS OBTAINED FROM DIFFERENT SOURCES SUBJECTED TO GENETICALLY INDUCED REPROGRAMMING (IPS CELLS).

Pessôa, Laís Vicari de Figueiredo[1], Fernandes Bressan, Fabiana[1,2], Ratto Lisboa Pires, Pedro[1], Recchia, Kaiana[2] and Vieira Meirelles, Flávio[1,2], [1]University of São Paulo, São Paulo, Brazil, [2]University of São Paulo, Pirassununga, Brazil

iPS cells and its use on regenerative medicine have a great potential on both human and veterinary medicine. Large mammals are suitable models for studying human diseases and cell therapy is highly desirable in the horse due to common orthopedic and joint injuries. The influence of the origin of somatic cell used in iPS production is currently discussed. Considering that equine mesenchymal cells obtained from different tissues present variable pluripotent characteristic and in vitro differentiation capability due to their derivation source; this proposal aimed to generate equine iPS (eiPS) cells through viral transduction of human and murine pluripotency factors (hOSKM and mOSKM respectively) into mesenchymal cells derived from bone marrow (BM), umbilical cord blood (UCB), umbilical cord tissue (UCT), adipose tissue (eAdMSC) and adult fibroblasts (aFIBRO). These cells were analyzed regarding their morphology, in vitro pluripotency maintenance potential through alkaline phosphatase (AlkPhos) detection and in vitro spontaneous differentiation. The aFIBRO and mesenchymal cells were isolated, in vitro cultivated, had their doubling time calculated in hours (BM 46±12; UCB 66±18; UCT 58±14, eAdMSC 23±11 and aFIBRO 29±9). From these cells, 84 iPS clonal cell lines have been produced (30, 33 and 21 cell lines derived from aFIBRO, eAdMSC and UCT, respectively using hOSKM and 1 cell line derived from eAdMSC using mOSKM). Induction of BM and UCB cells did not result on eiPS formation. Preliminary results show that hOSKM seem more effective to reprogram equine cells than mOSKM. While mOSKM were able to produce only one iPS cell line derived from eAdMSC, hOSKM were capable of reprograming multiple equine cell lines and colony formation was more efficient for eAdMSC cells (48±19, $P<0.01$) than for aFIBRO (7±3.5) and UCT cells (9±8.4), which did not differ ($P=0.95$). Although in vitro spontaneous differentiation was not achieved, eiPS cells obtained were positive for AlkPhos detection and embryoid body formation. While this discerned behavior among reprogrammed cell lines can be explained once it has been shown that even after reprogramming, iPS cells may maintain a residual epigenetic memory that can affect its reprogramming and dif-

ferentiation capacity, further studies are needed for fully understanding this process.

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W2110

THE ROLE OF INSULIN IN CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELLS

Shahbazi, Mohammad[1], Cundiff, Paige[2], Hoffman, Gabriel[3], Pandy, Gaurav[3], Lee, Philip[1], Patel, Achchhe[2], D'Souza, Sunita[2], Carlos Carcamo Oribe, Ivan[1], Abbasi, Fahim[1], Schadt, Eric[3], Lemischka, Ihor[2], Quertermous, Thomas[1] and W Knowles, Joshua[1], [1]Cardiovascular Research, Stanford, CA, U.S., [2]Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, U.S., [3]Institute of Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, CA, U.S.

The presence of either insulin or IGF1 has been found essential for culture of iPSCs. Studying the role of insulin in culture of pluripotent stem cells can potentially improve our understanding of molecular mechanism of pluripotency and differentiation protocols. This information will also reveal the potentials and limitations of iPSCs for modeling relevant conditions including insulin resistance and type-2 diabetes. iPSCs were derived from erythroblasts using integration free Sendai virus with Yamanaka factors. Glucose uptake was measured through uptake of 3H-Deoxy-glucose. Crystal violet staining was used to study the adhesion and expansion of iPSCs. Flow cytometry based cell cycle and apoptosis assays were performed via PI staining. RNA samples after 24 and 72 hrs of insulin starvation were used for RNA-Sequencing. Protein profiler array was used to monitor receptor tyrosin kinases. Insulin significantly improves the seeding of iPSC cells in culture. In addition, iPSC colonies maintain their pluripotent morphology and expression of Sox2, Oct4 and Tra1-60 after three days of insulin starvation. iPSC colonies have significantly higher expansion in presence of insulin. In addition, we observed increase in cell death and percentage of the cells in G2 in absence of insulin which hints of G2/M arrest. However, we did not observe any significant increase in glucose uptake in iPSCs after insulin stimulation. Transcriptional changes after 24 and 72hrs of starvation were similar but more intensified in the later. IPA analysis showed “Cellular Growth and Proliferation” and “Cell Death and Survival” as the top two pathways in “Molecular and Cellular Functions” category after 24hrs of starvation. Using protein array we detected higher levels of tyrosine phosphorylated ERK1/2, AKT, WNK1 after insulin stimulation. Presence of insulin significantly improved the seeding and expansion of iPSCs. This expansion is correlated with higher cell number and reduction of cell death in presence of insulin. These observations are in line with activation of ERK1/2 and AKT. Collectively our data shows that insulin mainly exerts its effect in maintenance of iPSCs through its mitogenic and anti-apoptotic effect.

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W2114

GLYCAN-TARGETED ELIMINATION OF TUMORIGENIC HUMAN PLURIPOTENT STEM CELLS BY AN ENGINEERED LECTIN-TOXIN FUSION PROTEIN DRUG

Tateno, Hiroaki[1], Onuma, Yasuko[2], Ito, Yuzuru[2] and Hirabayashi, Jun[1], [1]National Institute of Industrial Science and Technology, Tsukuba, Japan, [2]AIST, Tsukuba, Japan

Human pluripotent stem cells (hPSCs) such as human pluripotent stem cells (hiPSCs) and human embryonic stem cells (hESCs) are considered to be ideal cell sources for regenerative medicine, but their clinical and industrial application is hindered by their tumorigenic potential. Previously, we identified a human pluripotent stem cell-specific lectin, called rBC2LCN, by comprehensive glycome analysis using high-density lectin microarrays. Here we developed a recombinant lectin-toxin fusion protein of rBC2LCN with 23 kDa of a catalytic domain of Pseudomonas aeruginosa exotoxin A, termed rBC2LCN-PE23 for the targeted elimination of hPSCs. rBC2LCN-PE23 could be produced as a soluble form in E. coli at 10 mg/l culture and purified to homogeneity using one-step affinity chromatography. hPSCs were eliminated after 24 hr culture in the presence of 10 ug/mL of rBC2LCN-PE23, although no effect was observed for retinoic acid (RA)-treated hiPSCs, human dermal fibroblasts (hFibs), and human adipose-derived mesenchymal stem cells (hADSCs). Recently, we could successfully increase the killing efficiency up to 1,000-fold by conjugating rBC2LCN with 38 kDa of Pseudomonas aeruginosa exotoxin A, which is capable of eliminating hPSCs even at a concentration of 10 ng/mL. One possible concern could be the toxicity of residual toxins when transplanting cells, although they could be washed away easily. Even if residual lectin-toxin conjugates exist, there should be no effect on differentiated cells, since rBC2LCN-PE23 and rBC2LCN-PE38 cannot bind to and be internalized by differentiated cells. Outside of the cells, P. aeruginosa exotoxin A exhibits no toxicity predicted from the intoxication pathway of the toxin. rBC2LCN-PE23 and rBC2LCN-PE38 could be stably supplied and are cost-effective, and should be applicable to various experiments requiring the elimination of hPSCs, including the generation of hPSC-derived cells for drug screening as well as therapy. Thus, the lectin-toxin conjugates could be used as safe and efficient protein drugs to eliminate tumorigenic hPSCs from hPSC-derived cell

therapy products. Reference: Tateno et al. Stem Cell Reports 4(5):811-20, 2015

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W2116

TRANSCRIPTIONAL REACTIVATION OF FMR1 AS A THERAPEUTIC STRATEGY FOR FRAGILE X SYNDROME

Vershkov, Dan[1], Halevy, Tomer[2] and Benvenisty, Nissim[2], [1]Hebrew University, Jerusalem, Rishon Lezion, Israel, [2]Hebrew University, Jerusalem, Israel

Fragile X syndrome, the leading cause of inherited intellectual disability and autism, stems from the deficiency of a single protein, FMRP, encoded by the gene FMR1. In most patients, expansion of a repetitive CGG sequence in the 5'UTR of the gene leads to hypermethylation and silencing of the FMR1 promoter. We have previously generated models for fragile X syndrome in both ESCs and iPSCs, and showed that the DNA methyltransferase inhibitor 5-azactytidine is able to reactivate the silent FMR1 locus, inducing FMRP expression. However, 5-azacytidine is chemically unstable and has strong secondary effects such as genome wide hypomethylation and incorporation into DNA and/or RNA. The establishment of epigenetic modifications as therapeutic targets in various disorders promoted the search for new compounds targeting different epigenetic regulators. In this study, we analyzed the ability of additional epigenetic modulators to reactivate the transcription of FMR1 in fragile X patient derived iPSCs. Testing a series of nucleoside and non-nucleoside DNMT inhibitors demonstrated the ability of additional demethylating agents to reactivate FMR1 expression in both mRNA and protein levels. Subsequently, an immunostaining based imaging assay was established in order to test a larger array of epigenetic active molecules with various mechanisms. Identification of additional epigenetic modifiers able to reactivate the expanded FMR1 locus can shed light on the mechanisms mediating its transcriptional silencing, and might suggest a novel therapeutic strategy for the treatment of fragile X syndrome.

W2118

CLINICAL-GRADE CULTURE MEDIUM FOR EXPANSION AND LARGE-SCALE 2D AND 3D SUSPENSION CULTURE OF HUMAN PLURIPOTENT STEM CELLS

Wessberg, Fredrik, Takara Bio Europe, AB, Gothenburg, Sweden

Here we present the development of a defined, feeder-free medium, without human- or animal-derived components. Human pluripotent stem cells (hPSC) that are cultured in this medium for an extended period of time express expected stem cell markers, remain diploid, and can differentiate into cell types from the three germ layers. Using this complete, clinical-grade culture medium, eight different hPSC lines that were expanded as a 2D monolayer (2D culture) maintain high expression of pluripotent stem cell markers and lack any expression of differentiation markers over 12 - 20 passages tested. In addition, no karyotype abnormalities were reported for any of the tested cell lines. In order to generate clinically-relevant quantities of hPSCs - 10^9 and beyond - it is essential to develop efficient, yet robust 3D suspension cultures that maintain the same stability as that described for 2D monolayer cultures. Previous reports in the literature of suspension cultures have typically described a reduced growth rate compared to monolayer cultures with a final cell concentration of 1-2 million cells per milliliter. We demonstrate that our culture system supports large-scale, 3D, non-adherent expansion of hPS cells in suspension culture in a perfusion bioreactor. Furthermore, by optimizing perfusion rates and dissolved oxygen levels, we were able to expand hPSCs by 800-fold within 3 passages over 11 days to a final concentration of 5 million cells per milliliter using our 3D suspension culture system. In summary, our clinical-grade culture system allows for efficient, robust and scalable production of hPS cells, thus facilitating the use of hPSCs for research as well as in large-scale 3D suspension clinical applications.

W2120

ENHANCEMENT OF HOMOLOGY DIRECTED REPAIR VECTOR INSERTION EVENTS OF THE SICKLE CELL ANEMIA MUTATION IN HUMAN INDUCED PLURIPOTENT STEM CELLS (iPSCs) BY TALENS

Suzuki, Shingo[1], Suzuki, Shingo[1], Gruenert, Luke[1], Xie, Fei[2], Ye, Lin[2], Kan, Yuet Wai[1,2] and Gruenert, Dieter C.[1], [1]University of California, San Francisco, San Francisco, CA, U.S., [2]University of California, San Francisco, San Francisco CA, U.S.

Sickle cell anemia (SCA) in the US affects approximately 70,000 to 100,000 individuals, mostly in the African American and Hispanic communities. The lifespan, and

quality of life, for SCA patients has improved with advances in clinical management of, the disease, although, bone marrow transplantation still appears to be the most effective treatment. The recent developments in generation and editing of human iPSCs, has opened the door to other potential therapeutic routes. One potential approach for treatment of genetic diseases like SCA utilizes patient-derived iPSCs in which the underlying disease-causing mutations have been corrected prior to differentiation into engraftable progenitor cells. The studies reported here demonstrate that homology directed repair (HDR) of the SCA A>T transversion mutation in exon 1 of human β-globin can be facilitated by sequence-specific transcription activator-like effector nuclease (TALEN) enhancement of HDR with gene-targeting vectors that contain the wild-type β-globin donor sequences. Analysis of homologous recombinant cells revealed a novel class of products consistent with vector insertions resulting in partial duplication of human β-globin gene sequences that can be used to generate footprint-free homologous recombinant cell lines. These studies were supported by NIH grants DK088760 and DK104681.

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IPS CELLS: DIRECTED DIFFERENTIATION

W2128

DIFFERENTIATION OF HUMAN iPSC INTO OSTEOBLASTS BY USING SMALL MOLECULE INDUCERS UNDER FULLY DEFINED, XENO-FREE CONDITIONS.

Zujur, Denise Carolina[1], Kanke, Kosuke[2], Chung, Ung-il[3] and Ohba, Shinsuke[3], [1]The University of Tokyo, Tokyo, Japan, [2]The University of Tokyo, Graduate School of Medicine, Tokyo, Japan, [3]The University of Tokyo, Graduate School of Engineering, Tokyo, Japan

Induced pluripotent stem cells (iPSCs) have emerged as a promising option for studies of tissue development, drug screening, and cell-based therapies. From the clinical perspective, it is prerequisite to develop efficient methods to direct iPSCs into specific lineages. Production of mixed cell population, persistence of undifferentiated cells, and the use of animal-derived components are common limitations of current methods. Here, we developed a strategy to efficiently differentiate hiPSCs into osteoblasts under fully defined, xeno-free conditions. The osteogenic induction of two independent hiPSC lines was successfully achieved by small molecule-mediated manipulation of signaling pathways that had been identified as key players in osteoblast development. CHIR99021 was used to activate canonical Wnt signaling. Smoothened agonist (SAG) and cyclopamine (Cyc) were used to activate and repress hedgehog (Hh) signaling, respectively. BMP-dependent osteogenic molecule (TH) was also used. hiPSC differentiation was examined by gene expression analyses with RT-qPCR, protein expression analyses with immunostaining, and calcification analyses with von Kossa staining. The initial 3-day treatment with CHIR and Cyc strongly induced mesodermal differentiation of hiPSCs as indicated by the suppression of pluripotency markers (REX1, NANOG, POU5F1, and SOX2) and the specific up-regulation of mesoderm markers (T and MIXL1). Osteoblast specification was then induced by a 7-day treatment with SAG and TH, with cells strongly expressing RUNX2 and COL1A1. Osteoblast maturation was subsequently achieved by the stage-specific manipulation of Hh signaling and the activation of canonical Wnt signaling, as evidenced by the expression of mature osteoblast markers (SP7, IBSP, and BGLAP) as well as by matrix calcification on day 21. Thus, we generated a robust system to differentiate hiPSCs into osteoblasts by properly manipulating signaling pathways involved in osteoblast commitment and maturation. The xeno-free condition, the short period of treatment, and the stability of the inducers in the developed protocol will facilitate its application to the clinical context for skeletal regenerative therapies.

W2130

CRISPR-CAS9 MEDIATED GENERATION OF HUMAN PLURIPOTENT STEM CELL LINES TO ENABLE NLRP3 INFLAMMASOME STUDIES

Blake, William L.[1], Stock, Jeffrey L.[2], Adusumilli, Krishna[3], Haskell, Keith M.[2], Lee, Wilson[2], Engle, Sandra[4], Bowman, Michael[5] and Lin, Lih-Ling[5], [1]Pfizer/PDM-NCE, Groton, CT, U.S., [2]Pfizer, Groton, CT, U.S., [3]Primed Workforce Solutions, Groton, CT, U.S., [4]Vertex Pharmaceuticals, San Diego, CA, U.S., [5]Pfizer, Cambridge, MA, U.S.

Nucleotide-binding oligomerization domain-like receptor pyrin containing domain 3 (NLRP3) is a cytosolic receptor that is involved in the formation of a multiprotein complex, the NLRP3 inflammasome. The NLRP3 inflammasome is activated in response to infectious stimuli or to cellular stress that can be associated with a disease state, leading to release of pro-inflammatory IL-1 family cytokines. NLRP3 inflammasone activation has been implicated in a variety of diseases such as atherosclerosis, metabolic syndrome, Alzheimer's disease, rheumatoid arthritis, irritable bowel disease and kidney disease. To better understand the role of NLRP3 in inflammasome-mediated inflammation NLRP3-knockout (KO) human pluripotent stem cells (PSC) were generated. To accomplish the NLRP3 KO we utilized the novel CRISPR-Cas9 genome editing system. Editing was directed to exon 3, a region common to all forms of the NLRP3 protein. Custom CRISPR-Cas9 was transfected into PSC. We subsequently screened for

PSC clones in which the Cas9 nuclease had altered exon 3. Ultimately, sequencing analysis of DNA from single cell clones revealed multiple clones with frameshift causing deletions in exon 3 on both alleles. Two of these putative NLRP3 KO clones, along with wild-type PSC, were then differentiated in vitro to macrophage-like cells, a relevant cell type. To demonstrate loss of NLRP3 protein expression in KO macrophages, Western analysis was conducted on macrophage lysates. Macrophages from both KO lines had no detectable NLRP3 protein as compared to wild-type control. Absence of NLRP3 protein is expected to lead to a reduction in IL-1β release. To assess IL-1β release, KO and wild-type macrophages were first stimulated with lipopolysaccharide followed by activation with nigericin. Media was collected and IL-1β levels were determined. LPS/nigericin treatment resulted in extensive IL-1β release from wild-type macrophages, whereas in NLRP3 KO macrophages IL-1β release was dramatically reduced, thus confirming loss of function. In summary, human PSC NLRP3 KO lines have been generated utilizing CRISPR-Cas9 genome editing technology, and when differentiated in vitro to a physiologically relevant cell type (macrophage), display a functional phenotype (reduced Il-1β release), providing a tool to explore the biology around NLRP3.

W2132

GENERATION OF HIGH PURITY AND QUALITY PHOTORECEPTOR CELLS TO TREAT RETINAL DEGENERATION DISEASES

Duong, Khanh Linh, Baranov, Petr and Young, Michael, Harvard Medical School, Boston, MA, U.S.

Retinal degeneration (RD) diseases, hereditary or age-related, are among the leading cause of blindness, affecting millions of people worldwide. There is no effective therapy to prevent or treat RD, and there is a lack of RD disease models that accurately capture the phenotypes and pathologies found in patients. Due to lack of endogenous regeneration, replacing lost cells with functional ones appears promising. Transplantation of photoreceptor precursors derived from induced pluripotent stem cells (iPSCs) improves vision in rodent hosts. Unlike murine iPSCs, differentiation of human iPSCs toward a retinal cell fate requires an extensive time in culture [~90 days], with only a small percentage of rods (30%)/cones (0.1%) produced. The imbalance in gene dosage of the reprogramming factors and incomplete resetting of epigenetic memory in parent cells contribute to variation in quality among iPSC lines. Our goal is to identify molecular and cellular conditions that enhance the yield of photoreceptors, particularly cones, from hiPSCs differentiation. Here, we employed human retina progenitor cells (hRPCs), that our lab has isolated and established, as parental cell source for iPSC generation. We demonstrated that the use of four human reprogramming factors co-expressing with specific fluorescence proteins [GFP-OCT4, mOrange-SOX2, mKate-CMYC, and TagBFP-KLF4] allowed us to track the reprogramming kinetics. Unlike fibroblasts, at low MOI (<1) hRPCs are much more efficient and exhibited significant molecular and cellular changes. Interestingly, in addition to ES-like colonies expressing all four fluorescence colors [Nanog, OCT4/SOX2/CMYC/KLF4 positive], there were colonies that sprouted neuronal-like processes. Quantitative PCR showed significant upregulation in early neuronal markers including DCX, NeuroD1, Neurofilament M, FGFR3H, and MAPII. Importantly, Opsin genes were also upregulated. These events taken place within four weeks post transduction with OSMK. We also observed some neuronal-like colonies exclusively express SOX2 transgenes. This work suggests that our trackable system and usage of hRPCs as parental cell could provide significant tools for retinal regeneration studies.

Funding Source: Bertarelli Foundation

W2134

INVOLVEMENT OF G-COUPLED RECEPTOR-DEPENDENT CYCLIC AMP/ PKA SIGNALING PATHWAY ON NEURAL DIFFERENTIATION OF MOUSE IPS CELLS

Ishizuka, Toshiaki, Ozawa, Ayako, Katsuura, Mieko and Satoh, Yasushi, National Defense Medical College, Tokorozawa, Japan

Previous reports showed that activation of cyclic AMP (cAMP) / protein kinase A (PKA) signaling pathway may enhance neural differentiation in mouse mesenchymal stem cells. Although activation of 5-hydroxytryptamine 4 receptor (5-HT$_4$R) increases cAMP formation, activation of muscarinic acetylcholine receptors (mAchR) decreases it. The present study determined involvement of 5-HT$_4$R, mAchR, or the cAMP/PKA signaling pathway on neural differentiation of mouse induced pluripotent stem (iPS) cells. The differentiation which was initiated by embryoid body formation was stimulated with all trans retinoic acid (ATRA; 1 or 3 μM), serotonin (0.01~0.3 μM), or carbachol (a mAchR agonist; 1~10 μM) for 4 days and then transferred to matrigel-coated dishes. The differentiation potential of mouse iPS cells into neural progenitor cells was evaluated by Nestin expression using immunofluorescence staining or western blot analysis. Treatment with serotonin (0.03 μM) significantly enhanced ATRA-induced Nestin expression and cAMP response element binding protein (CREB) phosphorylation. Although the pretreatment with cholera toxin (an activator of stimulatory alpha subunit of guanine nucleotide-binding protein (Gs); 3~30 nM) significantly enhanced the effect of serotonin, the pretreatment with H89 (a PKA inhibitor; 1 μM) significantly inhibited it. On the other hand, the treatment with carbachol (10 μM) significantly inhibited ATRA-induced Nestin expression and CREB phosphorylation. Pretreatment with either pertussis toxin (an inhibitor of inhibitory alpha subunit of guanine

nucleotide-binding protein (Gi); 2~20 nM) or forskolin (an activator of adenylate cyclase; 1 μM) significantly reversed the effect of carbachol. These results suggest that the neural differentiation of mouse iPS cells may be modulated by the G-coupled receptor-dependent cAMP/ PKA signaling pathway.

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W2136

INDUCED PLURIPOTENT STEM CELLS DERIVED FROM IDIOPATHIC PARKINSON'S DISEASE PATIENTS IMPROVE MOTOR FUNCTION OF PARKINSON'S DISEASE MODEL MONKEYS

Kikuchi, Tetsuhiro[1], Morizane, Asuka[1], Doi, Daisuke[2], Okita, Keisuke[1], Nakagawa, Masato[1], Inoue, Haruhisa[1], Takahashi, Ryosuke[3], Mizuma, Hiroshi[4], Takara, Sayuki[4], Onoe, Hirotaka[4], Hayashi, Takuya[4] and Takahashi, Jun[1], [1]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [2]Center for IPS Cell Res & Application, Kyoto City, Japan, [3]Kyoto University Graduate School of Medicine, Kyoto, Japan, [4]RIKEN Center for Life Science Technologies, Kobe, Japan

Induced pluripotent stem cells (iPSCs) are promising source for cell replacement therapies. The first transplantation of iPSC-derived retinal pigment epithelium cells for Macular degeneration was performed in 2014, and Parkinson's disease is thought to be one of the next targets. In this study, we evaluated growth, differentiation, and function of iPSC-derived dopaminergic (DA) neuron progenitor in primate PD models. We generated iPSCs from idiopathic PD patients using an episomal vector method, produced dopamine neuron progenitors from these cells and transplanted them into brains of PD model monkeys. MPTP-treated cynomolgus monkeys are used as primate PD model animals. We carried out 12 months observation of monkey PD scores and observed significant improvement of PD scores. Some monkeys are subjected to long-term analysis for tumor formation up to 24 months. We performed MRI analysis of monkeys at every 3 months, and observed no tumor formation. Histological analysis showed that $6.5 \times 10^4 \pm 4.9 \times 10^4$ (average ± standard deviation) tyrosine hydroxylase (TH) positive cells are survived per grafts. We also performed positron emission tomography (PET) study, and the binding potential of [^{11}C] PE2I increased after cell transplantation in some grafts, suggesting DA neuronal maturation in those grafts. In conclusion, preclinical study of cell transplantation therapy for PD revealed that dopamine neuronal progenitors from PD-iPSCs can improve PD monkey behaviors and these cells produce no tumor in the observation period.

W2138

PRODUCTION AND ENRICHMENT OF IPSC-DERIVED MIDBRAIN DOPAMINERGIC PROGENITORS AND NEURONS

Li, Aiqun[1], Campos, Brian[1], Woodard, Chris[1], Zimmer, Matthew[1], Kuo, Sheng-Han[2], Nirenberg, Melissa[3], Zhou, Hongyan[1], Paull, Daniel[1], Rubin, Lee[4], Eggan, Kevin Carl[4], Rao, Mahendra[1], Noggle, Scott[1] and Chang, Stephen[1], [1]New York Stem Cell Foundation, New York, NY, U.S., [2]Columbia University, New York, NY, U.S., [3]New York University School of Medicine, New York, NY, U.S., [4]Harvard University Department of Stem Cell and Regenerative Biology, Cambridge, MA, U.S.

The following protocol uses a modified version of the Kriks/Studer method to produce and isolate midbrain dopaminergic progenitors (mDA) and neurons via fluorescence-activated cell sorting. Through the capture of $CD56^+/CD133^+$ double-positive cells on Day 11, the purified sample, on average, contains ~90% mDA progenitors expressing SOX1, SOX2, NESTIN, FOXA2, and LMX1. Through the isolation of $CD56^+/CD24^+/CD15^-/CD184^-$ cells from Day 33 on, the purity of dopaminergic neurons is increased to ~80%, as identified by expression of TUJ1, TH, FOXA2, LMX1, PITX3, and MAP2. This cell-type specific purification method is consistent, and normalizes the amount of mDA progenitors and neurons among multiple cell lines and differentiations. A range of functional assays, including DA measurement, electrical activity recording, assessment of a-synuclein, and cell engraftment into mouse brain confirm the sorted cell properties. The isolation of mDA progenitors takes 12 days, and for neuronal cultures, the process takes another 22 days.

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W2140

A DEFINED SYSTEM FOR THE DERIVATION OF BRAIN MICROVASCULAR ENDOTHELIAL CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Qian, Tongcheng, Maguire, Shaenah, Canfield, Scott, Bao, Xiaoping, Shusta, Eric and Palecek, Sean, University of Wisconsin-Madison, Madison, WI, U.S.

The blood brain barrier (BBB) is comprised of specialized endothelial cells, which are critical to neurological health. In order to better understand the development of brain microvascular endothelial cells (BMECs) and BBB

dysfunction in neurological disease, an unlimited source of human BMECs could prove enabling. Previously, we demonstrated that brain-like endothelial cells could be derived from hPSCs through co-differentiation of neural and endothelial progenitors, followed by purification of BMECs. Here we describe a new and simplified method for BMEC derivation using a serum-free defined protocol that employs key signaling pathway activators. By quantitative-PCR and immunonstaining, we were able to confirm that hPSCs progressed through a flk-1 endothelial progenitor stage and eventually to CD31-positive endothelial cells that expressed key BBB markers such as efflux transporter Pgp and tight junction proteins occludin and claudin-5. Flow cytometry was used to quantify the purity of cell population at different stages, and at day 10 the cultures comprised nearly 100% CD31 and Pgp dual positive populations. The hPSCs-derived BMECs exhibit endothelial properties, including tube formation, acetylated low density lipoprotein uptake in addition to BMEC-specific efflux transporter activities. Notably those cells also exhibit high transendothelial electrical resistance (around 3000 $\cdot cm^2$). In summary, we describe a defined and straightforward strategy to differentiate hPSCs into BMECs that could be used for applications such as drug screening and disease modeling.

Funding Source: R21Takeda Pharmaceutical Company Limited

W2142

PREDICTING SUCCESSFUL PLURIPOTENT STEM CELL DIFFERENTIATION USING NON-INVASIVE MULTI-ANALYTE LUMINEX ASSAYS

Rinaldi, Fabrizio, Rynning, Michael, Galitz, David, Fuerstenberg, Richard and Aho, Joy L., R&D Systems, Minneapolis, MN, U.S.

The ability of pluripotent stem cells to differentiate into any tissue of the body has the potential to revolutionize medicine. To fully realize this potential, robust and standardized differentiation and characterization protocols are necessary. In this study, we describe the use of Luminex® multi-analyte technology for non-invasively characterizing stem cells undergoing differentiation, as well as for optimizing individualized differentiation protocols. Luminex® technology allows for the simultaneous quantification of up to 100 proteins within a single small-volume sample. We utilized the multi-analyte screening power of Luminex® assays to profile the levels of cytokines and growth factors in cell culture media at key stages during the differentiation of pluripotent stem cells into hepatocytes. Cytokine and growth factor expression profiles were obtained from human iPS and ES cell lines with known differences in hepatocyte differentiation efficiency. Because samples are obtained from culture media, the cells are able to continue through the differentiation process and can be analyzed for efficiency by assessing albumin expression. We hypothesize that the multi-analyte profile of cell lines with robust differentiation efficiency will differ from cell lines with lower efficiencies. Using Luminex® multi-analyte technology we will be able to identify particular analytes that are predictive of differentiation success. Additionally, this data can be used to identify alternate factors that enhance differentiation and/or maturation of the differentiated cells.

W2144

HUMAN PLACENTA-DERIVED EXTRACELLULAR MATRIX HYDROGEL FACILITATES DIFFERENTIATION OF HUMAN IPSCS TOWARDS NEURAL CELLS AND CARDIOMYOCYTES

Treadwell, Michelle, Murchison, Angela, Francis, Michael, Breathwaite, Erick, Rodriguez, Rudy, Poole, Mike, Chen, Silvia Sihui and Lee, Jung Bok, LifeNet Health, Virginia Beach, VA, U.S.

Human pluripotent stem cells (hPSCs), including embryonic and induced pluripotent stem cells (iPSCs), hold great promise for future applications in drug discovery and cell therapies. An increasing number of hPSC culture protocols including specific substrates and/or medium supplements have been developed to support cell expansion and guide the differentiation of hPSCs towards the specific types of cells of interest. However, many of these materials commonly used for the culture are of animal origin which is a major regulatory concern from translating hPSCs technologies to the clinic. The present study evaluated the use of a novel, human placenta-derived extracellular matrix hydrogel (hpECM) to support neural cell and cardiomyocyte differentiation of multiple human iPSC lines. Embryoid bodies (EBs) were created from iPSCs in suspension and plated onto hpECM, Matrigel, or gelatin, before inducing neural differentiation by N2, B27 and bFGF stimulation. Neural precursor cells and differentiated neurons were identified by flow cytometry and immunohistochemistry using the developmental expression of Nestin and A2B5 and Tuj-1, respectively. Similarly, cardiomyocytes were generated by stimulating human iPSCs in suspension with BMP4, Activin A, bFGF, and ascorbic acid before transferring cells for direct culture on hpECM hydrogel or Matrigel under continuous stimulation. The number of beating colonies was quantified and mature cardiomyocyte phenotypes was determined by flow cytometry and immunohistochemistry using SMA, cTnT, α-Actinin, and MHC protein profiling. Using conventional hPSC culture and differentiation techniques, hpECM hydrogel as a cell culture substrate effectively supported the differentiation of iPSCs toward neurons and cardiomyocytes. Animal-free reagents are essential for hPSC-based technologies in translational research, and hpECM can be considered as a suitable substrate for completely humanized hPSC culture to prevent potential risks and short-

comings of xenogeneic materials. Additionally, hpECM may also provide a valuable tool for the development of in vitro screening platforms or the successful formation of 3-dimensional cell culture environments currently under investigation.

W2146

HIGH-EFFICIENCY DIFFERENTIATION INTO FUNCTIONAL DOPAMINERGIC NEURONS FROM PARKINSON'S PATIENTS-DERIVED INDUCED PLURIPOTENT STEM CELLS

Zafar, Faria[1], Shin, Soojung[2], Derr, Michael[3], Nguyen, Andrew[1], Flierl, Adrian[1] and Schuele, Birgitt[1], [1]Parkinson's Institute and Clinical Center, Sunnyvale, CA, U.S., [2]Thermo Fisher Scientific, Frederick, CA, U.S., [3]Thermo Fisher Scientific, Frederick, MD, U.S.

The objective of the study was to compare two neuronal differentiation protocols, Pluripotent Stem Cell (PSC) Dopaminergic (DA) Neuron Differentiation Kit (Prototype, Thermo Fisher, catalog number A30416SA) and our dopaminergic differentiation protocol (Mak et al. 2012) for length of protocol, efficiency of DA neuron generation, and electrophysiological properties. Parkinson disease (PD) patient specific induced PSCs (iPSC) differentiated into DA neurons lay the foundation for exploring disease mechanism and drug discovery. However, there remain critical challenges that must be addressed to reduce variability. It is important to develop standardized and validated iPS cell laboratory practices to generate and characterize cells. Critical factors for successful neuronal differentiation are yield of desired, functional target tissue type, reproducibility of the protocol, duration of differentiation, and cost.

We tested two different protocols to achieve high yield of DA neurons using iPSCs from PD patients. Two approaches were 1. embryoid body (EB)/rosette derived neural stem cells induced with dual SMAD inhibition and NCAM sorting that are further differentiated into mature neurons (via GDNF/BDNF) (Mak et al. 2012) in ~60 days and 2. direct differentiation of iPSCs through floorplate progenitor into mature DA neurons via PSC Dopaminergic Neuron Differentiation Kit in 35 days. Higher amount of DA neuron specific enzyme, tyrosine hydroxylase, were obtained after 35 days of differentiation with the PSC Dopaminergic Neuron Differentiation Kit compared to cells derived with EB/dual SMAD inhibition protocol after 60 days. The PSC Dopaminergic Neuron Differentiation Kit showed a sharp increase in floorplate/mesencephalic markers such as FOXA2, Corin, LMX1A, and EN-1 at Day 10 (expression range of 10-10,000 fold increase in comparison to day 0). Neuronal cultures showed spontaneous activity on multielectrode arrays (MEA, Axion Biosystems) of about 3000 spikes and an average amplitude of 22μV(range of 17μV to 40μV).

In summary, dopaminergic differentiation with the ThermoFisher PSC Dopaminergic Neuron Differentiation Kit provided reproducible culture conditions, shorter differentiation time, and a high-yield of functional dopaminergic neurons.

IPS CELLS: EPIGENETICS

W2150

ANALYSIS OF CHANGES IN THE EXPRESSION OF DOPAMINE-RELATED GENES WITH EPIGENETIC CHANGES IN PARKINSON'S DISEASE-SPECIFIC IPS CELL-DERIVED DOPAMINERGIC NEURONS

Suda, Yukari[1], Kuzumaki, Naoko[1,2], Narita, Michiko[1], Igarashi, Katsuhide[3], Takeshima, Hideyuki[4], Ushijima, Toshikazu[3,4], Hattori, Nobutaka[5], Okano, Hideyuki[2,3] and Narita, Minoru[1,3], [1]Department of Pharmacology, Hoshi University, Tokyo, Japan, [2]Department of Physiology, Keio University, Tokyo, Japan, [3]L-StaR, Hoshi University, Tokyo, Japan, [4]Division of Epigenomics, National Cancer Center Res. Institute, Tokyo, Japan, [5]Department of Neurology, Juntendo University Graduate School of Medicine Tokyo, Japan

The exploration of epigenetic alterations is providing new insights into mechanisms of neural development, neurological disease and aging. In the present study, we investigated the epigenetics in Parkinson's disease (PD) by performing a genome-wide DNA methylation study of CpG sites using patient-specific iPS cell (iPSC)-derived dopaminergic neurons. We first performed the comprehensive gene expression analysis in dopaminergic neurons derived from control or PD-specific iPSCs. We found significant differences in the expression of several dopamine-related genes between control and patients. Among those, the expression level of catechol-*O*-methyltransferase (COMT) was dramatically increased in PD-specific iPSC-derived dopaminergic neurons. Subsequently, we profiled DNA methylation in PD-specific iPSC- and control iPSC-derived dopaminergic neurons using the Illumina Infinium HumanMethylation 450 BeadChips. We found differences in pattern of DNA methylation at the transcriptional start sites with the CpG island of COMT genes in PD-specific iPSC-derived dopaminergic neurons. These findings suggest that changes in the expression of COMT genes with epigenetic modification observed in PD-specific iPSC-derived dopaminergic neurons may contribute to neuronal dysfunction in PD.

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W2152

DEVELOPMENT OF AN iPSC-BASED MODEL FOR ANGELMAN SYNDROME

Stanurova, Jana, Neureiter, Anika, Hiber, Michaela, Stolp, Kristin, Klein, Diana, Bankfalvi, Agnes, Klump, Hannes and Steenpass, Laura, University Hospital Essen, University Duisburg-Essen, Essen, Germany

Genomic imprinting is an epigenetic phenomenon resulting in parent-of-origin-specific gene expression which is regulated by a differentially methylated region. Gene mutations or failures in the imprinting process lead to the development of imprinting disorders, such as Angelman syndrome. The characteristic symptoms of Angelman syndrome including intellectual disability, lack of speech and ataxia are caused by the absence of a functional UBE3A protein in the brain due to the disruption of the maternal UBE3A copy. The paternal UBE3A allele is silenced by the long non-coding RNA SNHG14, whose expression is controlled by the differentially methylation region. Therefore, allele compensation is not possible in Angelman syndrome. To create a human neuronal model for Angelman syndrome, we reprogrammed dermal fibroblasts of a patient carrying a defined three-base pair deletion in UBE3A into induced pluripotent stem cells (AS_Δ3 iPSCs). In these iPSCs, both parental alleles are present, distinguishable by the mutation, and express UBE3A. In addition, we reprogrammed dermal fibroblasts of a healthy control person. Detailed characterization of four AS_Δ3 iPSC clones and two control iPSC clones demonstrated their pluripotency by various methods. For example, we included teratoma formation assay in immunodeficient mice as the most stringent test for pluripotency. All AS_Δ3 iPSC clones tested formed teratomas and proved their ability to differentiate into derivatives of all three germ layers. Furthermore, we showed by deep bisulfite amplicon sequencing that the differentially methylated region regulating imprinted UBE3A expression is exceptionally stable in the AS_Δ3 iPSCs, as well as the control iPSCs. Neuronal differentiation of AS_Δ3 iPSCs by dual SMAD inhibition resulted in neurons staining positive for MAP2 and ß-III-tubulin. During neuronal differentiation, we noted early initiation of SNHG14 expression, whereas silencing of paternal UBE3A expression was observed later, indicating that imprinted expression of UBE3A is a late process during neuronal in vitro differentiation and possibly in development. The generated iPSCs provide a useful tool for dissecting the specific role of UBE3A protein in neurons and the consequences of its absence in Angelman syndrome.

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CHROMATIN IN STEM CELLS

W2154

ATAC-ING MONOALLELIC DNA ACCESSIBILITY: ALLELE-SPECIFIC ATAC-SEQ UNCONVERS REGULATORY ELEMENTS DRIVING ESCAPE FROM X INACTIVATION AND RANDOM MONOALLELIC GENE EXPRESSION

Carter, Ava Clayton[1], Xu, Jin[1], Attia, Mikael[2], Giorgetti, Luca[3], Gendrel, Anne-Valerie[2], Heard, Edith[4] and Chang, Howard Y.[5], [1]Stanford, Stanford, CA, U.S., [2]Curie Institute, Paris, France, [3]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland, [4]Institut Curie, Paris Cedex 05, France, [5]Stanford University School of Medicine, Stanford, CA, U.S.

The two alleles of a gene are canonically believed to be indistinguishable to the cell and are expressed in the same spatiotemporal manner in the absence of mutation. There are, however, exceptions to this rule in which differential epigenetic regulation leads to monoallelic expression. ATAC-seq is a method for identifying sites of accessible, active chromatin rapidly in a genome-wide manner. We have developed a novel method for allele-specific ATAC-seq with which we can computationally distinguish chromatin accessibility on the two parental alleles. We have performed allele-specific ATAC-seq in highly polymorphic hybrid (129S1 x CastEiJ) mouse embryonic stem cells (mESCs) and clonal neural progenitor cells (NPCs) in order to uncover the non-coding regulatory elements whose monoallelic accessibility and TF binding drive monoallelic expression. As proof-of-principle we showed that accessibility along the inactive X chromosome in NPCs is globally lost, except at sites proximal to escape gene promoters. On the autosomes, we find that the number of monoallelically accessible elements increases 6-fold during ES to NPC differentiation and then becomes stable. We classify monoallelic regulatory elements into those that are always biased toward the same allele and those whose allelic choice is random in each NPC clone. These random MA sites are highly enriched for promoter proximal elements, while those that are genetically encoded are found more often at distal regulatory elements. We performed mitotic allelic ATAC-seq and find that while accessibility is globally slightly reduced, random monoallelic sites remain monoallelically accessible during the cell cycle and across more than ten passages at the same allele. Finally, using RNA-seq data in the same NPC clones, we find that some randomly monoallelically expressed genes, many of which have been implicated in Alzheimer's and Schizophrenia, have highly correlated allelic bias at their promoters in ATAC-seq data, while others show no correlation. This indicates that there are at least two distinct mechanisms driving the maintenance of random monoallelic gene expression in NPCs.

W2156

A NEW CLASS OF TEMPORARILY PHENOTYPIC ENHANCERS IDENTIFIED BY CRISPR/CAS9 MEDIATED GENETIC SCREENING

Shen, Yin[1], Diao, Yarui[2], Bin, Li[3], Meng, Zhipeng[4], Jung, Inkyung[3], Lee, Ah Young[3], Dixon, Jesse[3,4], Maliskova, Lenka[1], Guan, Kun-Liang[4] and Ren, Bing[5], [1]University of California, San Francisco, San Francisco, CA, U.S., [2]Ludwig Institute for Cancer Research/UCSD, San Diego, CA, U.S., [3]Ludwig Instiute for Caner Research, La Jolla, CA, U.S., [4]University of California, San Diego, La Jolla, CA, U.S., [5]Ludwig Institute for Cancer Research, La Jolla, CA, U.S.

With less than 2% of the human genome coding for proteins, a major challenge is to interpret the function of the non-coding DNA. Millions of regulatory sequences have been predicted in the human genome through the analyses of DNA methylation, chromatin modification, hypersensitivity to nucleases, and transcription factor binding, but few have been shown to regulate transcription in their native contexts. We have developed a high throughput CRISPR/Cas9-based genome-editing strategy and used it to interrogate 174 candidate regulatory sequences within the 1Mbp POU5F1 locus in the human embryonic stem cells (hESCs). We identified two classical regulatory elements - including a promoter and a proximal enhancer - that are essential for POU5F1 transcription in the hESCs. Unexpectedly, we also discovered a new class of enhancers that contribute to POU5F1 transcription in an unusual way: disruption of such sequences led to a temporary loss of POU5F1 transcription that is fully restored after a few rounds of cell division. These results demonstrate the utility of a high throughput screening for functional characterization of non-coding DNA, and reveal a previously unrecognized layer of gene regulation in human cells.

GERMLINE CELLS

W2160

GLOBAL HYPER-TRANSCRIPTION IN PRIMORDIAL GERM CELLS

Percharde, Michelle and Ramalho-Santos, Miguel, University of California, San Francisco, San Francisco, CA, U.S.

The germline transmits genetic information from one generation to the next and is pivotal to species survival and evolution. During embryonic development, large-scale reprogramming occurs in Primordial Germ Cells (PGCs) at the level of both DNA demethylation and histone modifications. These reprogramming events have been the focus of major attention in recent studies. On the other hand, the transcriptional program of PGCs during this period is thought to be well understood, due to several microarray and RNA-seq studies performed over the past decade. Such previous studies have assumed a similar overall level of transcriptional output per cell between PGCs and somatic cells, such that most of the transcriptome is considered invariant ("housekeeping"). We report that cell-number normalised (CNN) approaches reveal striking differences between the transcriptional output of PGCs and neighboring somatic cells of the embryo. We find that PGCs are globally hyper-transcribing at multiple stages of development, and contain up to tenfold higher levels of primary and mature housekeeping mRNA transcripts at E13.5. Pre-ribosomal RNA levels are similarly elevated, suggesting that RNA Polymerases I and II are both hyper-active in PGCs. Fluorescent quantification of nascent RNA transcription rates in male and female E13.5 embryos reveals a marked elevation in the transcriptional output of PGCs compared to somatic cells of the gonad or limb. We found that distinct sub-populations within PGCs have varying levels of transcriptional activity, and there are also differences between male and female PGCs. Overall, the data indicate that the global level of transcription in PGCs is significantly higher than somatic cells, even at a stage when cells are entering cell-cycle arrest (E13.5). We are expanding upon these findings with analyses at other developmental stages, as well as using CNN RNA-seq. We speculate that this elevated transcriptional output may be vital for PGC expansion, transposon surveillance and/or germ cell competition. Moreover, these results reveal that standard expression profiling methods using qRT-PCR, microarrays or RNA-seq, all of which normalise to "housekeeping gene" levels, are not appropriate for experiments involving PGCs, highlighting the need for CNN approaches.

TOTIPOTENT/EARLY EMBRYO CELLS

W2164

CONVERSION OF PRIMED PLURIPOTENT STEM CELLS TO A NAÏVE-LIKE STATE AND THEIR LONG-TERM MAINTENANCE USING RSET™ MEDIUM

Hunter, Arwen Leigh[1], Chang, Wing Yean[2], Snyder, Kimberly[2], Westendorf, Kathryn[2], Yazdi, Sahar[2], Chen, Annie[2], Hadley, Erik[2], Thomas, Terry E.[2], Eaves, Allen C.[2,3] and Louis, Sharon A.[2], [1]STEMCELL Technologies, Vancouver, BC, Canada, [2]STEMCELL Technologies Inc., Vancouver, BC, Canada, [3]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Human pluripotent stem cells (hPSCs) within the developing embryo progress through a series of pluripotent states from naïve to more lineage-restricted or primed. Recapitulating these states in vitro requires spe-

cialized culture media and protocols. Recent studies have identified novel culture conditions that revert traditional primed hPSCs and maintain them in a more naïve-like or ground state. We developed RSeT™, a defined medium that supports this reversion and allows for the continuous maintenance of naïve-like hPSCs. To revert primed hPSCs cultured in mTeSR™1, colonies were dissociated into small clumps and seeded on inactivated E12.5 mouse embryonic fibroblasts (iMEFs) and cultured in mTeSR™1 for 24 h, after which the medium was changed to RSeT™ and the cells were cultured for 5 days with daily medium changes. Colonies maintained in RSeT™ were gently dissociated with TrypLE and re-seeded on freshly plated iMEFs every 3-4 days. Culture morphology was observed at each passage (P) and at specific timepoints. Cells were harvested for characterization of marker expression by qPCR, flow cytometry, and immunocytochemistry. Genomic stability was assessed at P12 or later. Transition to a naïve-like colony morphology, consisting of increased cell multi-layering, refractive edges and domed colony shape began at 48 h after exposure to RSeT™. Early passage cultures (< P5) were heterogeneous, displaying both primed and naïve-like morphologies. Typically by P5, cultures were more homogeneous with >70% of colonies displaying a domed morphology. Multiple hPSC lines that were reverted and maintained long-term (> P12) in RSeT™ retained normal karyotypes. Cells from naïve-like colonies (> P5) expressed markers associated with pluripotency such as OCT4, SSEA4, TRA-1-60 and ALP. Importantly, naïve-like hPSCs cultured in RSeT™ Medium showed > 15 fold upregulation of naïve pluripotency markers KLF2, KLF4, KLF17, TBX3 and DNMT3L1, whereas, a 2 - 4 fold increase occurred in TFCP2L1 and STELLA compared to mTeSR™1-cultured primed hPSCs. Further, TFE3 was predominantly localized to the nucleus as expected for naïve-like hPSCs. In summary, we have developed RSeT™, a defined medium that promotes robust conversion of primed hPSCs to a naïve-like state and the continuous maintenance of these cells.

EMBRYONIC STEM CELL DIFFERENTIATION

W2170

CANCER-ASSOCIATED TERT PROMOTER MUTATIONS ABROGATE TELOMERASE SILENCING

Hockemeyer, Dirk, University of California, Berkeley, Berkeley, CA, U.S.

Telomeres, the repetitive DNA sequences at the end of linear eukaryotic chromosomes, can be generated de novo by the enzyme telomerase to allow for telomere maintenance in human stem cells. Telomere function is critically linked to tumorigenisis and aging. Telomeric abnormalities and aberrant telomere shortening are associated with premature cellular senescence while telomerase activation is frequently found in tumorigenesis. Mutations in the human telomerase reverse transcriptase (TERT) promoter are the most frequent non-coding mutations in cancer but their molecular mechanism in tumorigenesis has not been established. Previous studies using cancer cell lines and GWAS studies could not provide direct answers for these questions because, with or without TERT promoter mutations, cancer cell lines display large variability in TERT expression, telomerase activity and telomere length despite the fact that they are all immortal. To overcome these limitations, we used genome editing of human pluripotent stem cells with physiological telomerase expression to elucidate the mechanism by which these mutations contribute to human disease. Surprisingly, telomerase-expressing embryonic stem cells engineered to carry any of the three most frequent TERT promoter mutations showed only a modest increase in TERT transcription with no impact on telomerase activity. However, upon differentiation into somatic cells, which normally silence telomerase, cells with TERT promoter mutations failed to silence TERT expression, resulting in increased telomerase activity and aberrantly long telomeres. Thus, TERT promoter mutations are sufficient to overcome the proliferative barrier imposed by telomere shortening without additional tumor-selected mutations. These data establish that TERT promoter mutations can promote immortalization and tumorigenesis of incipient cancer cells.

W2172

LONG-TERM IN VITRO EXPANSION OF SKELETAL MUSCLE PRECURSORS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Borchin, Bianca and Barberi, Tiziano, Texas Biomedical Research Institute, San Antonio, TX, U.S.

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) (collectively named hPSC) provide an extraordinary research tool. In vitro these cells display extensive proliferation and the ability to differentiate into all cell types of the body. In our laboratory we have previously developed a stepwise protocol to efficiently derive, from hPSCs, hypaxial migratory (LBX1+) skeletal muscle precursors that can be used for clinical applications and basic development studies. A current limitation in muscle biology is the ex-vivo expansion of satellite cells or the in vitro expansion of hPSC-derived muscle precursors. Even though hPSCs may have an advantage towards satellite cells because they can be grown in large numbers thus allowing derivation of sufficient muscle precursors for transplantations, the in vitro expansion of these precursors remain challenging. To fill this gap, we have set specific culture conditions that enabled us to expand hPSC-derived muscle precursors for more than 280

days. While a fraction of the precursors differentiate into mature myocytes able to form myotubes in vitro, we have used a FACS strategy based on AChRa chain to separate mature MYOGENIN+ myocytes from the PAX3+/PAX7+ precursors. AChRa negative cells are mainly precursors and expanded in vitro forming again some mature myocytes. Serial FACS purification of AChRa- cells was sufficient to maintain a long-term expanding pool of precursors. Parallel to this, modulation of our current differentiation protocol has allowed the derivation of skeletal muscle with alternative regional identities. This is significant as it may provide new insights to the development of different muscle groups in vitro. A shift in HOX genes and lack of LBX1 expression confirmed those findings. Lastly, long-term propagation of muscle precursors enabled us to detect AChRe transcripts on mature myocytes indicating a switch from embryonic to fetal /adult muscle in vitro. All together our results show that hPSC-derived muscle precursors can be passaged for more than 280 days retaining PAX3 and PAX7 expression. Our findings will facilitate their use for preclinical tests in animal models of muscle disease and will foster basic studies on patient-derived hiPSC or CRISPR-edited hPSCs.

W2174

ACETYLATION OF HISTONE H3-LYSINE-14 BY BRPF2-MOZ COMPLEX IS REQUIRED FOR RETINOIC ACID-INDUCED DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS.

Cho, Hyein, Yonsei University, Seoul, Korea

Embryonic stem cells (ESCs), which are derived from the inner cell mass (ICM) of a blastocyst, pass through complicated process during differentiation stage and their lineages are committed into three germ layers: ectoderm, mesoderm and endoderm. At differentiation stages, dynamic change of epigenetic landscape is occurred, suggesting the critical role of epigenetic regulation in differentiation of ESCs. Particularly, histone acetylation is regarded to be important because they activate transcription of stemness-related genes in pluripotent stem cells and also activate lineage-committed genes properly in accordance with the differentiation stage. Histone acetylation is important in both of pluripotent and differentiated states, suggesting the crucial role of histone acetyltransferase (HAT) complex. Scaffold protein, subunit of HAT complex, may help association of histone acetyltransferase with binding partner and stabilize the complex. BRPF2 (also called BRD1) as scaffold protein of MYST histone acetyltransferase, they link the interaction of histone acetyltransferase MYST2/3/4 with binding partner ING5 and EAF6 and recruit the complex to specific histone tail of target gene, inducing active transcription of target genes. In previous research, importance of BRPF2 is reported in fetal erythropoiesis through interaction with MYST2. Also, BRPF2 was reported to implicate with adult brain development as well as embryonic neurodevelopment and susceptibility of BRPF2 gene for both schizophrenia and bipolar effective disorder. These studies indicate BRPF2 is essential in embryonic development. However, function of BRPF2 in differentiation of embryonic stem cell was not fully elucidated. In this study, we investigated the role of BRPF2 in regulation of differentiation on mouse ESCs. Using BRPF2-knock down mESCs, we confirmed that depletion of BRPF2 caused the delayed differentiation of ESC, accompanied by reduced global levels of H3K14 acetylation. Our immunoprecipitation data revealed that BRPF2 can interact physically with histone acetyltransferase MOZ, suggesting that BRPF2-MOZ complex activates transcription of differentiation marker genes during differentiation. Taken together, our data suggested BRPF2 regulates differentiation through H3K14 acetylation with MORF/MOZ in ESCs.

W2176

STANDARDIZED APPROACHES FOR EVALUATION OF THE DEFINITIVE ENDODERM DIFFERENTIATION BIAS BETWEEN INDIVIDUAL HESC LINES

Dziedzicka, Dominika[1], Markouli, Christina[2], Sermon, Karen[1] and Geens, Mieke[1], [1]Vrije Universiteit Brussel, Brussels, Belgium, [2]Vrije Universiteit Brussel (VUB), Brussels, Belgium

Individual human embryonic stem cell (hESC) lines often demonstrate a differentiation bias towards one of the embryonic germ layers. It is necessary to better understand the molecular mechanisms causing this phenomenon, as it can hamper the efficiency of hESC-based biomedical applications. Moreover, knowledge of the pathways implicated may help to improve differentiation protocols. However, an accurate quantification of line-specific differentiation bias is challenging, as culture conditions also influence the differentiation outcome. In this study, we compared different standardized methods to quantify differentiation potential towards the definitive endoderm (DE) of four hESC lines (VUB01, VUB02, VUB07 and VUB14). All lines carried a balanced chromosomal content, confirmed by array comparative genome hybridization, and were cultured on laminin-521™ in Nutristem™ medium. First, we used our in-house optimized embryoid body (EB) formation protocol to generate equal-sized EBs, followed by a 21-day spontaneous differentiation in APEL medium. Gene expression analysis by real-time PCR did not show a lineage bias between differently sized EBs from the same hESC line, but we detected consistent differences between individual lines. In contrast, when performing a 7-day spontaneous EB differentiation and evaluating gene expression levels using the TaqMan® hPSC Scorecard™ Panel, we did not obtain reproducible results for biological replicates. We therefore concluded that this set-up was not applicable for our purpose. Next, we per-

formed a 3-day DE induction with defined seeding density (20000 cells/cm^2) to normalize the method. VUB14 DE samples showed statistically significant lower expression levels of SOX17, FOXA2 and GATA4 in comparison to DE samples from the other hESC lines. These results were in concordance with the data obtained from the 21-day EB spontaneous differentiation, suggesting that these two standardized methods can serve as a tool for evaluating DE differentiation bias. Our next step will be comparing our approach with an external technique to additionally confirm its accuracy.

W2178

HUMAN PLURIPOTENT STEM CELL-DERIVED NEURAL CREST: THE ROLE OF BMP

Hackland, James, University of Sheffield, CSCB, Sheffield, U.K.

The neural crest is a migratory tissue that plays a key role in vertebrate embryonic development. Pre-migratory neural crest is formed in the ectoderm before it undergoes epithelial-mesenchymal transition (EMT) and contributes to a wide range of tissues throughout the body. Using both hES and hiPS cells we have developed a robust and fully-defined protocol for the differentiation of human pluripotent stem cells into putative neural crest cells. During this process we identified variation in endogenous BMP production as the primary source of unpredictable differentiation efficiency in defined conditions and developed a method for controlling BMP activity in the system. In the developing embryos of model organisms (Xenopus laevis, Mus musculus, Gallus gallus) BMP signaling plays a key role in patterning of the ectoderm during neurulation. Manipulation of BMP signaling during in vitro human neural crest differentiation reveals a role for the growth factor analogous to that in embryonic development as studied in model organisms. This study was only possible because of the development of a fully defined neural crest differentiation protocol. Using this protocol it will now be possible to look at the role of other ectodermal signals, such as WNT and FGF, in human neural crest differentiation in vitro.

W2180

GLIS3 REGULATES ANTERIOR-POSTERIOR PATTERNING IN HPSC-DERIVED NEURAL STEM CELLS VIA THE WNT SIGNALING PATHWAY

Jeon, Kilsoo, Brown, Robert, Kang, Hong Soon and Jetten, Anton, NIEHS/NIH, Research Triangle Park, NC, U.S.

Human pluripotent stem cells (hPSCs) differentiate into neural stem cells (NSCs) and subsequently into functional neuronal subtypes, are providing great tools for investigating functional neuronal subtypes and understanding of CNS development. Neuronal induction occurs by default in the primitive ectoderm, forming anterior neural tissue and thereafter, a number of factors can posteriorize this anterior neuroectoderm. However, our knowledge of the mechanisms that control the initial anterior-posterior (A/P) patterning of neuronal development is not well understood. Here we show that Krüppel-like zinc finger transcription factor Gli-similar 3 (Glis3) can control the A/P patterning by regulating endogenous WNT signaling during neuronal induction. In addition to enhanced expression of common neural markers, induction of Glis3 expression in hPSCs inhibits the expression of anterior lineage markers and instead induces the expression of posterior lineage markers. These results indicate that Glis3 expression causes a shift in NSC differentiation pathways from the default, anterior NSCs (aNSCs) pathway to the posterior NSCs (pNSCs) pathway. This was supported by gene profiling analysis and immunohistochemistry. Microarray analysis further revealed that Glis3 induced the expression of several WNT genes and inhibited the expression of several WNT inhibitors. Glis3 particularly increased the expression of WNT3a, which has been reported to promote the induction of posterior-related genes and the pNSCs pathway. ChIP-Seq analysis showed that Glis3 binds the WNT3a promoter suggesting that it directly regulates WNT3a transcription. The WNT inhibitors (Wnt-C59 and IWP2) greatly abrogated Glis3-induced posterior NSC differentiation supporting the conclusion that Glis3 promotes the differentiation of hPSCs into posterior NSCs via the induction of WNT3a expression and repression of WNT inhibitors. Our demonstration that Glis3 regulates A/P specification and patterning may be relevant to the various neuropathologies in which Glis3 is implicated.

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W2182

THE BULK CELL DENSITY AS DECISIVE PARAMETER AND SIMPLE METHOD FOR MESENDODERMAL PATTERNING OF HUMAN PLURIPOTENT STEM CELLS

Kempf, Henning[1], Olmer, Ruth[1], Bolesani, Emiliano[1], Haase, Alexandra[1], Coffee, Michelle[1], Annika, Franke[1], Buettner, Falk[1], Dräger, Gerald[2], Pötz, Oliver[3], Joos, Thomas[3], Martin, Ulrich[1] and Zweigerdt, Robert[1],
[1]Hannover Medical School, Hannover, Germany,
[2]Leibniz University Hannover, Hannover, Germany,
[3]Natural and Medical Sciences Institute, Reutlingen, Germany

In vitro differentiation of human pluripotent stem cells (hPSCs) recapitulates early aspects of embryogenesis, but the underlying processes are poorly understood and controlled. Therefore current differentiation methodol-

ogies often lack robustness. Here we show that simple modulation of the bulk cell density (BCD) substantially alters anteroposterior patterning of primitive streak (PS)-like priming. Inducing differentiation of hPSCs at distinct BCDs in conjunction with distinct concentrations of the WNT pathway activator CHIR99021 (CHIR), we obtained efficient cardiomyogenic differentiation only at distinct CHIR–to-BCD ratios. Detailed microarray and flow cytometric analysis revealed patterning into definitive endoderm, precardiac- or presomitic mesoderm associated with distinct BCD and CHIR conditions. The patterning was manifested during the first 24h of differentiation and is a prerequisite for efficient downstream differentiation, as exemplarily shown for cardiomyogensis using the HES3-NKX2.5 reporter cell line. Notably, BCD-dependent deflection of differentiation was highly reproducible for all cell lines (i.e. 6 independent hESC / hiPSC lines) and culture platforms tested including adherent (2D) and suspension (3D) culture under static and dynamic conditions. Secretome analysis by MS/MS after 6h and 24h of differentiation indicated that the BCD effect is mediated via establishment of distinct paracrine milieus. In line with this, knockdown of secreted factors LEFTY1 and CER1 resulted in posteriorization of differentiation, suggesting that the accumulation of these TGFß family members is a key source of "anteriorizing activity" blocking CHIR-triggered posterior progression during PS-like priming. Summarizing these mechanisms a tangible model for how the BCD deflects CHIR99021-induced lineage commitment over time is presented. By demonstrating the decisive role of the BCD, we show its utility as a simple, but effective method for distinct hPSC priming at early stages of lineage commitment. Our findings have profound consequences for directed hPSC differentiation, inter-experimental comparability, process optimization and scale-up, which is of utmost importance to harness their therapeutic potential.

Funding Source: This work was funded by the DFG (Cluter of Excellence REBIRTH DFG EXC62/1 and grant no. ZW64/7-1, MA 2331/16-1) and StemBANCC (support from the Innovative Medicines Initiative joint undertaking under grant agreement n° 115439-2, resources of which are

W2184

BRAINPHYS™ NEURONAL MEDIUM: A MEDIUM OPTIMIZED TO SUPPORT THE SYNAPTIC ACTIVITY OF NEURONS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS AND PRIMARY CNS TISSUES

Lee, Vivian M.[1], Mak, Carmen K.H.[1], Chew, Leon[1], Lloyd-Burton, Sam[1], Eaves, Allen C.[1,2], Thomas, Terry E.[1] and Louis, Sharon A.[1], [1]STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Electrical activity is a hallmark of function in neuronal cultures derived from human pluripotent stem cells (hPSCs) and primary tissues, which are useful models for studying neurological disease and development. We have recently released BrainPhys™ Neuronal Medium (BrainPhys™), a defined and serum-free medium based on the published BrainPhys™ formulation (Bardy et al., PNAS, 2015) that closely replicates the physiological environment of the CNS, and generates hPSC-derived neuronal cultures with an increased proportion of synaptically active neurons. Neural progenitor cells derived from hPSCs were differentiated in BrainPhys™ or DMEM/F-12 (control) supplemented with growth factors for up to 70 days. In this study, we also tested BrainPhys™ performance for the maturation of primary neurons. E18 rat cortical cells were plated in Neurobasal Medium with NeuroCult™ SM1 Neuronal Supplement (SM1). After 5 days, half of the cultures were transitioned to BrainPhys™ with SM1 by performing half-medium changes every 3 - 4 days, while the remaining cultures were maintained in the plating (control) medium. At the indicated time-points, neurons were quantified and characterized by immunocytochemistry for neuronal marker expression and by electrophysiology for synaptic activity. After 45 days, hPSC-derived neurons cultured in BrainPhys™ expressed MAP2, class III β-tubulin and Synapsin 1, and exhibited both excitatory and inhibitory receptor-mediated synaptic activities. Although neurons cultured in BrainPhys™ and DMEM/F-12 developed a similar resting membrane potential at 60 - 70 days, neurons cultured in BrainPhys™ consistently exhibited a higher frequency and amplitude of spontaneous synaptic currents, consistent with the findings of Bardy et al. For primary neurons after 21 days, the number of neurons in BrainPhys™ and control cultures was 1977 ± 542 and 637 ± 102 (mean ± SE; n = 3), respectively. While neurons in both conditions co-expressed MAP2 and Synapsin 1, neurons matured in BrainPhys™ showed increased frequency and amplitude of spontaneous excitatory and inhibitory synaptic currents. Together, these data demonstrate that BrainPhys™ Neuronal Medium provides a physiological culture environment that supports the growth and maturation of hPSC-derived and primary neurons.

W2186

FINE-TUNING TUBES: KRT14+ PROGENITORS BUILD SALIVARY GLAND DUCTS DURING DEVELOPMENT AND REGENERATION

May, Alison[1], Cruz-Pacheco, Noel[1], Seidel, Kerstin[2], Emmerson, Elaine[1], Nathan, Sara[1] and Knox, Sarah M[1], [1]Program in Craniofacial Biology, University of California San Francisco, San Francisco, CA, U.S., [2]University of California, San Francisco, San Francisco, CA, U.S.

Salivary glands are composed of an elaborate network of secretory end units connected to an extensive ductal system that secretes saliva into the oral cavity. Formation of this structure occurs through the process of epithelial branching morphogenesis that is regulated by a diverse array of signaling pathways. This process also requires multipotent epithelial progenitor cells to proliferate and differentiate into the 3 major epithelial lineages: secretory (acinar), ductal and myoepithelial. Similarly, during regeneration after damage, the tissue must repopulate the structure through the differentiation of adult progenitor cells. Here we show that cells marked by keratin 14 (KRT14) contribute to both the acinar, ductal and myoepithelial lineages during early development, but that by embryonic day 16 and in the adult under homeostatic and regenerative conditions (mild and severe injury) KRT14+ cells contribute solely to the ductal and myoepithelial compartments. Furthermore, we show that KRT14+ cell-mediated regeneration in the adult is mediated, at least in part, by activation of HBEGF/EGFR signaling, thereby providing a mechanism by which new ducts can be generated in vivo. These findings provide a new understanding of the contribution of progenitors to tissue development and regeneration and have significant implications for regenerative therapies aimed at restoring salivary tissue after immune/radiation-mediated destruction.

W2188

EFFECTS OF HUMAN EMBRYONIC STEM CELL COLONY GEOMETRY ON TISSUE-LEVEL FORCES AND SUBSEQUENT ROLE IN DIFFERENTIATION

Muncie, Jonathon Michael[1,2], Przbyla, Laralynne[2], Lakins, Johnathon N.[2], Sunyer, Raimon[3], Trepat, Xavier[3,4] and Weaver, Valerie[2,5], [1]University of California San Francisco, Berkeley, CA, U.S., [2]Center for Bioengineering and Tissue Regeneration, University of California, San Francisco, San Francisco, CA, U.S., [3]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain, [4]Facultat de Medicina, Universitat de Barcelona and Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain, [5]Department of Anatomy and Department of Bioengineering and Therapeutic Sciences, Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research and Helen Diller Family Comprehensive Cancer Center, UCSF, San Francisco, CA, U.S.

Tissue engineering and regenerative medicine rely on reproducible, defined approaches to direct stem cell fate. The cues that influence cell fate include proteins as well as mechanical and tissue-level signals, but our understanding is limited by a lack of knowledge regarding the role physical forces play in controlling differentiation. Particularly, we are interested in germ layer formation, which occurs during gastrulation. It has been shown that cellular geometry affects cell fate at the level of single cells, so we asked whether a similar effect can be detected within cell collectives that are induced to undergo differentiation toward mesoderm. The objective of this study was to use our model system of in vitro hESC development to investigate the relationship between tissue level forces and differentiation towards the mesoderm germ layer. We cultured human embryonic stem cells (hESCs) on polyacrylamide gels with physiologically relevant stiffness, and for added physiological control we engineered 3D-printed plating guides to control the size and shape of hESC colonies. With traction force microscopy, we quantified and mapped the cell-ECM forces being exerted by the hESCs while using immunofluorescence to monitor expression of proteins of interest. We found that hESC colonies with circular geometry generate characteristic traction force patterns that correspond to regions of mesoderm formation upon application of morphogenic differentiation factors. Perturbing colony geometry altered the pattern of traction forces observed, indicative of changes in the distribution of tissue tension and regional mesoderm specification. This technique allows us to examine how mechanical signals influence hESC differentiation in a model system of development, and could be

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broadly applicable to other studies seeking to make connections between tissue-level forces and cell behavior.

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W2190

MODULATION OF EPIBLAST STEM CELL FATES BY WNT-MIXL1 ACTIVITY.

Osteil, Pierre, Children's Medical Research Institute, Wentworthville, Australia

This study aims to elucidate the molecular activity that influences lineage propensity in the embryo-derived epiblast stem cells (EpiSCs), which are developmentally similar to the epiblast of the gastrulating mouse embryo. WNT signalling activity can be modulated during the derivation and maintenance of the EpiSCs through blocking the action of Porcupine in the intracellular trafficking and the release of WNT ligands by the chemical inhibitor IWP2. While IWP2 inhibition does not affect the ability of the EpiSCs to respond to WNT signalling, the blocking of WNT signalling activity leads to a bias of differentiation of the EpiSCs towards ectoderm derivatives, in contrast to the propensity of mesendoderm differentiation of IWP2-derived EpiSCs that are further maintained in the absence of the WNT inhibitor. Analysis of the gene expression profiles revealed differences in the transcriptome between EpiSCs derived under WNT-free and WNT-active condition. Mixl1, which is expressed in the mesendoderm progenitor in the mouse primitive streak, is down regulated in the EpiSCs when WNT activity is inhibited, consistent with the bias of EpiSC towards non-endoderm cells. This shift in the trajectory of cell differentiation implicates the plasticity of cell fates of EpiSC derived in WNT-free condition and that the lineage propensity can be re-set by changes in WNT activity. In contrast, while EpiSCs that were derived without WNT inhibition but maintained under inhibition can respond to chemical inhibition by down-regulating the WNT responsive genes, they show no changes in the lineage propensity. In these EpiSCs, the prior experience of WNT signalling appears to have hard-wired the lineage differentiation potency. The mesendoderm propensity of these EpiSCs is correlated with the expediency and magnitude of activation of Mixl1. Given that Mixl1 activity may be regulated by WNT signalling, this raise the possibility that the activity of WNT-Mixl1 cascade is key to mesendoderm differentiation of the EpiSCs. Modulation of WNT activity and Mixl1 therefore have a convergent function in controlling the differentiation of the progenitor of germ layer tissues.

W2192

GENERATION OF FUNCTIONAL HIPPOCAMPAL NEURONS FROM SELF-ORGANIZING HUMAN EMBRYONIC STEM CELL-DERIVED DORSOMEDIAL TELENCEPHALIC TISSUE

Sakaguchi, Hideya[1], Eiraku, Mototsugu[2], Takahashi, Jun[1] and Sasai, Yoshiki[2], [1]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [2]Center for Developmental Biology RIKEN, Kobe Hyogo, Japan

The developing dorsomedial telencephalon includes choroid plexus and the medial pallium, the latter of which goes on to form the hippocampus. Notably, hippocampal granule and pyramidal neurons are crucial for learning and memory formation, and their dysregulation is associated with several neuropsychiatric disorders, including Alzheimer's disease and schizophrenia. For this reason, generating a reliable source of human dorsomedial telencephalic tissue is an important step for cell-based research into hippocampus-related diseases. Despite previous efforts, however, we currently lack the means to generate human medial pallium and functional hippocampal granule/pyramidal neurons in vitro. Here, we demonstrate the generation of functional hippocampal granule- and pyramidal-like neurons from self-organizing dorsomedial telencephalic tissue using human ES cells (hESCs). First, we developed a hESC culture method that utilizes BMP and Wnt signaling to induce Lmx1a$^+$/Otx2$^+$/TTR$^+$ choroid plexus, the most dorsomedial portion of the telencephalon. We found that titrating BMP and Wnt exposure allowed the self-organization of Foxg1$^+$/Lef1$^+$/Lhx2$^+$ medial pallium tissues. Following long-term dissociation culture, these dorsomedial telencephalic tissues gave rise to Zbtb20$^+$/Prox1$^+$ granule neurons and Zbtb20$^+$/KA1$^+$ pyramidal neurons, both of which were electrically functional and positive for the maturation marker CaMKII. Thus, we have developed an in vitro model that recapitulates human hippocampus development, allowing the generation of functional hippocampal granule- and pyramidal-like neurons.

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W2194

DUSP4 REGULATES NEURONAL DIFFERENTIATION AND CALCIUM HOMEOSTASIS BY MODULATING ERK1/2 PHOSPHORYLATION

Kim, Sun Young[1], Han, Yong-Mahn[2], MiHee, Oh[3], Kim, Won-Kon[1], Oh, Kyoung-Jin[1], Le, Sang Chul[1], Bae, Kwang-Hee[1] and Han, Baek-Soo[1], [1]KRIBB, Daejeon, Korea, [2]KAIST, Daejeon, Korea, [3]KRIBB, Daejeon, Korea, South

Protein tyrosine phosphatases have been recognized as critical components of multiple signaling regulators of fundamental cellular processes, including differentiation, cell death, and migration. In this study, we show that dual specificity phosphatase 4 (DUSP4) is crucial for neuronal differentiation and functions in the neurogenesis of embryonic stem cells (ESCs). The endogenous mRNA and protein expression levels of DUSP4 gradually increased during mouse development from ESCs to postnatal stages. Neurite outgrowth and the expression of neuron-specific markers were markedly reduced by genetic ablation of DUSP4 in differentiated neurons, and these effects were rescued by the reintroduction of DUSP4. In addition, DUSP4 knockdown dramatically enhanced extracellular signal-regulated kinase (ERK) activation during neuronal differentiation. Furthermore, the DUSP4–ERK pathway functioned to balance calcium signaling, not only by regulating Ca^{2+} /calmodulindependent kinase I phosphorylation, but also by facilitating Cav1.2 expression and plasma membrane localization. These data are the first to suggest a molecular link between the MAPK–ERK cascade and calcium signaling, which provides insight into the mechanism by which DUSP4 modulates neuronal differentiation.

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W2196

FUNCTIONAL SCREEN IN EMBRYONIC STEM CELLS REVEALS ESSENTIAL ROLES OF RNA BINDING PROTEINS IN THE EXIT FROM SELF-RENEWAL

Wang, Xue, Ma, Yanni, Yu, Jia and Huang, Yue, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China

Mammalian embryonic stem (ES) cells retain unlimited self-renewal potential in culture and can be induced to differentiate into all somatic cell types under appropriate culture conditions. Now, it has been well defined how ES cells establish and sustain pluripotency during self-renewal, but the process by which ES cells exit from pluripotency is less studied relatively. RNA-binding proteins (RBPs) play crucial roles in various cellular processes mainly through post-transcriptional control of RNAs, including polyadenylation, splicing, stabilization, localization and mRNA translation. However, the functions of RBPs in ES cells differentiation remain largely unknown. Here, by functional screening some pre-selected RBPs in ES cells, we identified a few critical RBPs required for exit from self-renewal and further explored their molecular mechanisms. Firstly, a number of candidate RBPs were picked by bioinformatics analysis based on the mouse ES cell RBPs database and published literatures. Secondly, we disrupted these candidates by using CRISPR/Cas9 system in mouse ES cells and got the mutant cell lines respectively. The differentiation ability of these mutant cell lines were evaluated through embryoid body formation and withdraw LIF from the medium. A few candidate RBP-null cell clones showed significant differentiation defect compared with wide-type ES cells. Thirdly, preliminary mechanism research showed that Srsf4 RBP possibly modulates gene expression through repressing ES cell specific alternative splicing, which is required for the exit from pluripotency. The detailed regulatory pathway and mechanism of these critical RBPs at the onset of differentiation are being investigated.

W2198

THE ROLE OF SUBSTRATE ELASTICITY IN INFLUENCING THE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO NEURONS

Zaltsman, Yefim[1], Musah, Samira[2], Wrighton, Paul J.[1] and Kiessling, Laura L.[1], [1]University of Wisconsin-Madison, Madison, WI, U.S., [2]Harvard Univeristy, Boston, MA, U.S.

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 cell-based regenerative medicine and drug discovery. These applications, however, require efficient and reproducible methods for directing the differentiation of hPS cells to desired cell types. To date, focus has been on soluble factors such as growth factors and small molecules, while 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 substrates—with elasticity similar to human brain tissue—override these signals to induce efficient differentiation of hPS cells to neurons. The underlying molecular mechanism relies on F-actin and the transcriptional coactivator Yes-associated protein (YAP). Inhibiting F-actin polymerization in hPS cells on a stiff substrate phenocopies

the cells on a soft substrate: YAP is excluded from the nucleus and the cells differentiate to neurons. Inhibition of Rho GTPase activity similarly leads to nuclear exclusion of YAP. Notably, this signaling event occurs via a ROCK-independent mechanism. Our findings indicate that by modulating the localization of YAP, mechanical cues can override soluble signals, thereby suggesting that their contributions to early human development and in vitro differentiation are significant. Current hPS cell differentiation protocols are almost exclusively carried out on tissue culture polystyrene, a substrate which is orders of magnitude stiffer than human tissues. Therefore, we envision that utilizing substrates with more physiologically relevant mechanical properties will augment efforts to efficiently direct hPS cell differentiation.

W2200

ANEUPLOIDY IMPAIRED EMBRYONIC STEM CELL DIFFERENTIATION VIA ENDOPLASMIC RETICULUM STRESS

Cheng, Li[1,2], Zhang, Meili[2] and Huang, Yue[1], [1]Institute of Basic Medical Sciences,Peking Union Medical College, Beijing, China,[2]Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing, China

Aneuploidy, an incorrect number of chromosomes, is the leading cause of severe developmental defects and is also a hallmark of cancer. In our previous study, we have found that aneuploidy promoted neoplastic progression in embryonic stem (ES) cells by impairing their differentiation. However, the mechanism of differentiation defects caused by aneuploidy is unknown. Here we analyzed the level of endoplasmic reticulum (ER)-associated protein degradation (ERAD) in aneuploid mouse ES cells and embryoid bodies (EBs). The expression of proteasome subunits was higher in aneuploid ES cells compared with wild-type ES cells, and the proteasome activity was also elevated. The enhanced proteasome activity degraded the excess proteins from the extra chromosomes and maintained the cellular homeostasis. Upon differentiation, the proteasome should be transiently activated to remove the excess and damaged proteins in wild-type ES cells, but in aneuploid ES cells, the elevated proteasome activity cannot be further stimulated to adapt the cell-fate change. The deficiency of ERAD in aneuploid cells resulted in protein overload and subsequently continuous ER stress, which leads to abnormal protein biosynthesis and secretion. By mass spectrometry-based profiling, the secretomes during aneuploid ES cell differentiation were analyzed to discover the abnormal secreted factors. Our results showed that the protein degradation system cannot function properly during the differentiation of aneuploid ES cells and ERAD-associated abnormal secretome might underlie the cell differentiation defect.

EMBRYONIC STEM CELL PLURIPOTENCY

W2202

GLOBAL TRANSCRIPTOME ANALYSIS OF TE03 HESCS REVEALS HIGH SIMILARITY BETWEEN SUSPENSION AND ADHERENT CULTURE CONDITIONS

Dvir, Shlomi[1,2], David-Eden, Hilda[1], Roytblat, Mark[1], Shariki, Kohava[1], Mandel-Gutfreund, Yael[2], Angel, Itzchak[1] and **Amit, Michal**[1,3], [1]Accellta LTD, Haifa, Israel, [2]Faculty of Biology, Technion, Haifa, Israel, [3]The Ephraim Katzir Department of Biotechnology, Braude College, Karmiel, Israel

Clinical applications of stem cell derivatives require large quantities of cells that can only be provided by novel suspension culture technologies. Despite much progress, the effect of carrier-free suspension culturing on global transcription has yet to be elucidated. Here, we set out to investigate the impact of transitioning of TE03 human embryonic stem cells (hESCs) from adherent (2D) to suspension (3D) culture conditions on gene expression profile. To this end, we used RNA-sequencing and the HTA2.0 microarray GeneChip to compare the gene expression pattern between 2D and 3D cultures of TE03. We validated the obtained measurements with quantitative RT-PCR analysis of key markers of pluripotency and differentiation. To assess the generalizability of our findings, we performed a cross-study comparison of gene expression profiles between suspension cultures of TE03 and published studies of adherent hESCs. Overall, we found a high similarity in the pluripotency signature of TE03 under both suspension and adherent conditions. In addition, we found a good correlation in the expression pattern of suspension cultures sampled at two extremely different time points, indicating a high level of reproducibility. As expected, the pluripotent signature of TE03 was consistent with the molecular signature of multiple adherent hES cell lines. Collectively, our study demonstrates that suspension culturing maintains the undifferentiated state of hESC. This finding supports the utility of the 3D suspension model as an effective alternative to static adherent cultures for research as well as clinical applications.

W2204

THE SINGLE CELL EPIGENETIC LANDSCAPE OF HUMAN EMBRYONIC STEM CELL VARIES WITH CELL STATE AND FUNCTION

Baskar, Reema, Stanford University, Palo Alto, CA, U.S.

The diversity of cells in an organism is driven by variation in chromatin structure, which determines gene expression programs for different cell fates and function. These

variations in chromatin structure not only exist between tissue types, but also between individual cells and gives rises to heterogeneity in cell state and function. To date, most studies employ costly sequencing and biochemical methods to understand chromatin structure of bulk samples. They lack the resolution of single cell observations required to delineate heterogeneity in the epigenome of complex and dynamic systems such as the undifferentiated stem cell compartment. To address this, we have developed a single cell, high throughput proteomic method to capture individual, global epigenetic landscapes by measuring abundance of chromatin structure modulators such as histone post-translational modifications, chromatin remodelers and other chromatin-interacting proteins. It has been shown that there are substantial inter-cell differences in the epigenetic state and phenotype of human embryonic stem cells (hESC) and we believe that the differential levels of these chromatin structure modulators play a significant role in giving rise to the continuum of pluripotent and lineage primed states in hESC, ultimately impacting differentiation potential in culture. We have correlated chromatin states to pluripotent and lineage-primed states by analyzing all these states simultaneously in individual hESC. The states were captured using epitope-specific, mono-isotonic elemental reporter-coupled antibodies to regulatory epigenetic marks (i.e. H3K27me3), chromatin remodeling proteins (i.e. Polycomb group members, CTCF), lineage specific markers (i.e. FoxP1, Nestin) and key transcription factors for stemness (i.e. Nanog, Oct4) on a mass cytometry (CyTOF) platform. Here we present our new method for multiplexed, single cell chromatin state measurements (‘chromotyping’) and discuss its application in understanding the heterogeneity in chromatin structure and concomitant variation in cell state of primed and naïve hESC.

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W2206

MULTINUCLEATED MYOTUBE FORMATION FROM HUMAN PLURIPOTENT STEM CELLS

de la Garza, Anabel S.[1], Main, Heather Marie[2], Caron, Leslie[3], Arjomand, Jamshid[4] and Schmidt, Uli[4], [1]Genea Biocells, San Diego, CA, U.S., [2]Genea Biocells, La Jolla, CA, U.S., [3]Genea Biocells, Sydney, Australia, [4]Genea Biocells SD, San Diego, CA, U.S.

Pluripotent stem cells (PSC) have an extensive ability for self-renewal and can differentiate into all cell types of the embryo proper, including Skeletal Muscle (SkM) cells. These features make them a promising source for in vitro myopathic disease modeling and cellular therapies for muscle regeneration. However, while protocols have been developed in mouse PSC for the generation of multinucleated skeletal muscle myotubes, protocols from human PSC (hPSC) tend to generate thinner myocytes at an embryonic developmental stage that, while they express myotube markers, show limited fusion potential with few nuclei per myotube. Establishment of protocols to enhance in-vitro fusion would provide a more accurate model of SkMC maturation, improve myopathic disease modeling, drug screening and potentially clinical applications. This study builds on our proprietary and robust 28 day protocol for generation of SkMCs that we reported previously. Briefly, stem cells are induced to differentiate to myogenic progenitors (Pax3/7+) and intermediate myoblast (MyoD+) stages to form terminally differentiated (MF20+) skeletal muscle (SkM) myotubes in 26 days using 3 defined culture media. We have now further optimized the final step of this differentiation method leading to formation of mature and multinucleated myotubes, with up to 20-30 nuclei per myotube. These myotubes express the muscle specific markers sarcomeric myosin heavy chain and dystrophin. We conclude that upon thaw and culture in specific serum-free medium, Genea Biocells myoblasts are capable of proliferating and fusing to form multinucleated myotubes in a reproducible manner. To our knowledge, this is the first report of consistent and robust multinuclear myotube formation from hPSC. This work provides an in vitro disease model for investigating cellular and molecular mechanisms in the pathogenesis of human skeletal disorders, for drug screening campaigns and possibly for transplantation.

W2208

DETECTION, ISOLATION AND CHARACTERIZATION OF HUMAN PLURIPOTENT STEM CELLS RESISTANT TO DIFFERENTIATION

Keller, Alexander, Dziedzicka, Dominika, Sermon, Karen D. and **Geens, Mieke**, Vrije Universiteit Brussel, Brussels, Belgium

Often during differentiation of human pluripotent stem cells (hPSC) residual undifferentiated stem cells (rSC) that apparently have lost differentiation capacity are observed. The transplantation of differentiated cell populations containing these cells may pose a high risk of tumor formation in patients and therefore represent a major hurdle to exploit the full therapeutic potential of hPSC. As very little research on the mechanisms by which cells remain undifferentiated is currently available, we aimed to detect, isolate and characterize these cells. We developed detection methods utilizing a variety of techniques, including flow cytometry, line-specific standard curve generation by real time RT-PCR, colony formation assay and immunofluorescence. While we readily detected rSCs within differentiated populations, detection ranged from 0.023% to 8.1%. Several possible sources of error in our estimates make the exact quantity difficult to evaluate.

Isolation of rSC was achieved through the use of a novel replating technique, whereby small amounts of a differentiated population were re-introduced into culture conditions that favor undifferentiated hPSC proliferation. This allowed for the clonal growth of rSC colonies that could be easily isolated. Residual SC lines were isolated from two out of three hPSC lines after 21 days of differentiation. Of these two, one line was karyotypically normal prior to the start of differentiation. The rSC line obtained was abnormal however and displayed a chr20p deletion and chr20q amplification, suggesting a possible link between copy number variations and the occurrence of rSC. Although a well-characterized mutation at 20q11.21 has been shown to give hPSC a significant growth advantage, due to the size of the observed mutation, we cannot confirm whether the same driver gene is involved in the change of differentiation potential. As such, further research into the mechanisms which might cause rSC is needed.

W2210

PRMT8 NEGATIVELY REGULATES MESODERMAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS BY ENHANCING THE PI3K/AKT/SOX2 PATHWAY.

Jeong, Hochang[1], Moon, Sunghwan[2] and Cha, Hyukjin[1], [1]Sogang University, Seoul, Korea, South, [2]Konkuk University, Seoul, Korea

Supplement of basic fibroblast growth factor (bFGF) is critical to maintain human embryonic stem cells (hESCs) through activation of PI3K/AKT. Thus, the elaborate molecular mechanisms to preserve PI3K/AKT signaling upon bFGF stimulation may exits in hESCs. Of interest, protein arginine methyltransferases 8 (PRMT8) in hESCs was expressed and gradually decreased during spontaneous differentiation. Through loss or gain of function study with PRMT8, we demonstrated that PRMT8 contributed to longer maintenance of hESC pluripotency, even under bFGF deprivation conditions. Direct interaction of membrane localized PRMT8 with p85, a regulatory unit of PI3K, was associated to longer maintenance of phosphoinositol 3-phosphate (PIP3) and consequent high AKT activity. Furthermore, Sox2 expression level, controlled by PRMT8/PI3K/AKT axis was attributed to the mesodermal lineage differentiation. Thus, we propose that PRMT8 in hESCs plays an important role in not only maintaining bFGF signaling toward PI3K/AKT/Sox2 axis but also controlling mesodermal differentiation.

W2212

COMPUTATIONAL DESIGNED HIGH-AFFINITY INHIBITOR OF EED-EZH2 INTERACTION REVEALS PRC2 REQUIREMENT IN HESC

Xing, Yalan[1], Moody, James D[1], **Levy, Shiri**[1], Mathieu, Julie[1], WANG, Yuliang[2], Sidhu, Sonia[1], Valensisi, Cristina[1], Kim, Woojin[3], Chao, Xu[3], Min, Jinrong[3], Margolin, Adam[3], Orkin, Stuart H.[3], Hawkins, David[1], Baker, David[1] and Ruohola-Baker, Hannele[1], [1]University of Washington, Seattle, WA, U.S., [2]The Chinese University of Hong Kong, Shatin, Hong Kong, [3]Boston Children's Hospital, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, U.S.

Dissecting the molecular mechanism of the earliest human developmental events holds promise for regenerative medicine. Recently, two stable pluripotent states have been derived in humans: pre-implantation naïve and post-implantation primed human Embryonic Stem Cells (hESCs). These stages are distinct in their gene expression, metabolic and epigenetic signatures. Metabolic enzyme NNMT was shown to regulate PRC2 dependent H3K27me3 levels in naïve and primed stages, however, it was not shown whether this modification was essential for the human pluripotent stages. The catalytic subunit responsible for H3K27 trimethylation is the methyltransferase, EZH2. The interaction of EZH2 with PRC2 component, EED (Embryonic Ectoderm Development) is critical for EZH2 catalytic activity, presumably by localizing EZH2 to its specific substrate. We applied computational protein design to engineer a synthetic, novel protein that incorporates the EZH2 N-terminal helical peptide so as to achieve 300-fold tighter binding to EED compared to endogenous EZH2. The synthesized protein (EED Binder) was transfected into the genome of naïve hESCs and serves as a competitive inhibitor of EZH2 through disrupting the EED-EZH2 interface. Additionally, a negative control binder (with two mutated interface amino acids) did not interact with EED. Importantly, we show that the inducible expression from the AAVS1 site of the active, but not the control, EED-binder abolished the stem cell morphology, significantly reduced the level of H3K27me3 marks, EZH2 protein, and stem cell markers (Tra1-60, TG-30, Oct4) measured by Western, ChIP-seq and FACS analysis. Genome wide RNA sequencing experiments revealed upregulation of 25% of all bivalent genes, further suggesting a widely modified epigenetic landscape. EED-binder was also able to impede the transition from naïve to primed hESC stage, a process that mimics human embryonic implantation. When EED-binder was induced at the transition, the affected hESCs failed to transit to primed stage, expressing repressed H3K27me3 marks and pluripotency markers. The PRC2 complex components, EZH2 and JARID2, were rendered unstable due to dissociation of the complex. These data show that in con-

trast to mouse, human PRC2 has an essential function in naïve as well as in the naïve-to-primed hESC transition.

W2214

RNA BINDING PROTEIN TDP-43 SAFEGUARDS PLURIPOTENCY BY REGULATION OF DEVELOPMENTAL ALTERNATIVE POLYADENYLATION PREVENTING PARASPECKLE ASSEMBLY

Modic, Miha[1,2], Rot, Gregor[3,4], Grosch, Markus[5], Shaposhnikov, Dmitry[5], Cacchiarelli, Davide[6], Meissner, Alexander[6], Ule, Jernej[2,7] and Drukker, Micha[5], [1]Institute of Stem Cell Research, Helmholtz Center Munich, Neuherberg, Germany, [2]MRC Laboratory of Molecular Biology Cambridge, Cambridge, U.K., [3]University of Zurich, Zurich, Switzerland, [4]Swiss Institute of Bioinformatics, Zurich, Switzerland, [5]Helmholtz Center Munich, Neuherberg, Germany, [6]Harvard University/Broad Institute, Cambridge, MA, U.S., [7]UCL, London, U.K.

How pluripotent stem cells (PSCs) exit the self-renewing program and become committed to embryonic lineages is a fundamental question. Transcriptional, signaling and epigenetic regulation of PSC fate decisions have been the focus of research, while the understanding of RNA based mechanisms in rapid dissolvent of the self-renewing reprogramming apparatus in PSCs has been lagging behind. Recently, several studies have identified RNA-binding proteins (RBPs) as modifiers of pluripotency. Additionally, alternative polyadenylation (APA) of RNAs is increasingly being recognized as important posttranscriptional mechanism governing gene dosage. Importantly, APA-pattern is dynamically regulated during early PSC differentiation and reprogramming. However, the factors regulating APA are still poorly understood and it is unknown what mechanisms are employed by the relevant RBPs on a transcriptomic level to regulate pluripotency breakdown. We elucidated a novel TDP-43 function in global developmental regulation of APA including core pluripotency circuitry, role of APA in paraspeckle formation and its connection to the differentiation of mouse and human PSCs. We show that unique subnuclear structures termed paraspeckles are formed independent of lineage type during exit from the pluripotency and are important for stabilization of the early commitment state. The long isoform of non-coding RNA NEAT1 serves as the scaffold for paraspeckles assembly produced only upon differentiation. We present evidence that TDP-43 maintains production of nucleoplasmic NEAT1 short isoform by directly regulating APA of nascent NEAT1 transcripts in undifferentiated cells, and that TDP-43 itself is sequestered in paraspeckles upon differentiation leading to formation of NEAT1 long isoform and paraspeckle assembly. This indicates the existence of a feed-forward feedback mechanism underlying paraspeckle assembly during onset of differentiation. Furthermore, we analyzed how paraspeckle assembly regulates exit from pluripotency; we show that cells lacking NEAT1 and paraspeckle proteins exhibit severe developmental delay. Together, this work implicates developmental regulation of APA in RNA-mediated nuclear restructuring leading to stabilization of the early differentiation state and pluripotency breakdown.

W2216

DELETION OF THE POLYCOMB-GROUP PROTEIN EZH2 LEADS TO COMPROMISED SELF-RENEWAL AND DIFFERENTIATION DEFECTS IN HUMAN EMBRYONIC STEM CELLS

Rugg-Gunn, Peter J., Collinson, Adam and Morgan, Natasha, The Babraham Institute, Cambridge, U.K.

The Polycomb-Group (PcG) complex PRC2 mediates trimethylation of histone H3 on lysine 27 (H3K27me3) through the activity of the histone methyltransferase EZH2, and is associated with the epigenetic repression of transcriptional programmes. PRC2 is an essential modulator of developmental and stem cell fate decisions in several model organisms. The well conserved binding profiles of PRC2 components in human embryonic stem cells (ESC) at the promoters of developmental regulators raises the possibility that PRC2 may also have an important role in controlling human ESC pluripotency and differentiation, however no functional studies of PRC2 in human ESC have been reported to date. Here, we describe the generation and characterisation of EZH2-deficient human ESC. Disruption of EZH2 using Crispr-Cas9 resulted in the loss of EZH2 protein and destabilisation of PRC2. Global and promoter-localised H3K27me3 levels were lost upon EZH2 deletion and a large cohort of PcG-target genes encoding developmental regulators were transcriptionally derepressed. Although EZH2-deficient human ESC could be maintained in an undifferentiated state, the cells were severely compromised in their ability to self-renew and proliferate and exhibited increased levels of spontaneous differentiation. Timecourse differentiation studies and teratoma assays showed that EZH2-deficient cells were able to initiate cell differentiation towards early cell lineages, however they were unable to complete differentiation to mature specialised tissues. These findings leads us to conclude that EZH2 has a broadly conserved role in regulating cell fate decisions and the transcriptional programmes of early human developmental cell types.

W2218

REGULATION OF OCT4 EXPRESSION AND STEM CELL POTENCY BY JUN N-TERMINAL KINASE/CJUN SIGNALING IN MURINE EMBRYONIC STEM CELLS

Sprowles, Amy, Hosawi, Manal, Dahl, Lauren, Castillo, Johnny, Petersen, Abigail, Trzeciak, Jacqueline and Roelf, Kelly, Humboldt State University, Arcata, CA, U.S.

The POU family transcription factor OCT4 is a key regulator of pluripotency in embryonic stem cells, and its expression has been reported in some tumors and cancer stem cells. The proto-oncogene c-Jun and its upstream regulator Jun N-terminal kinase (JNK) function in cellular proliferation, apoptosis, and transformation. JNK has recently been implicated in stem cell potency and Oct4 regulation, but a role for cJun has not been described. Through bioinformatic analysis, we identified putative AP-1 binding motifs in the promoter and introns of both the Mus musculus and Homo sapien Oct4 genes. We modulated the c-Jun/JNK pathway in two murine embryonic stem cell lines by 1.) Treatment with the JNK activator anisomycin, 2.) Treatment with the JNK inhibitor SP600125, or 3.) Transient transfection of plasmids expressing GFP-cJun or GFP-cJun L40/42A, a mutant that cannot be bound or phosphorylated by JNK. Immunocytochemistry and Western blot analysis show both overexpression of GFP-cJun and treatment with 10ng/ml or 50 ng/ml anisomycin increases expression of the Oct4A isoform, but not overall levels of Oct4. To see if these conditions effect stem cell potency, mESCs were treated with 10ng/ml anisomycin or transiently transfected with GFP-cJun before directed differentiation to insulin-secreting pancreatic islet-like clusters. Both treatments resulted in significant reduction in cluster number, average size, insulin expression, and density of neuronal projections. Suppression of JNK/c-Jun activity in mESCs had the opposite effect: transient transfection of GFP-c-Jun L40/42A or treatment with 10 mM SP600125 showed an increase in total cluster number, average size and neuronal projections. These results suggest a role for the JNK/cJun pathway in stem cell potency, potentially through changes in the expression level of Oct4A.

W2220

JMJD1C PROMOTES SELF-RENEWAL OF MOUSE EMBRYONIC STEM CELLS AND SOMATIC CELL REPROGRAMMING THROUGH SUPPRESSING MAPK/ERK SIGNALING

Xiao, Feng, Institute of Health Sciences, Shanghai Institutes for Biological Sciences,CAS, Shanghai, China and Jin, Ying, Institute of Health Sciences, Shanghai, China
Shanghai Stem Cell Institute, Shanghai JiaoTong University School of Medicine, Shanghai, China

The ability of embryonic stem cells (ESCs) to self-renew indefinitely and differentiate into all cell types of an organism is sustained through multiple regulatory mechanisms. The role of histone demethylases (HDMs) for establishing and maintaining these properties is not completely defined. Here, we show that the expression of JmjC domain-containing protein 1c (Jmjd1c), a putative histone H3 Lys 9 (H3K9) demethylase, positively correlates with the undifferentiated state and is required for the self-renewal of mouse ESCs. Depletion of Jmjd1c in mouse ESCs leads to activation of the MAPK/ERK pathway and epithelial-to-mesenchymal transition (EMT) to induce differentiation of mouse ESCs. Importantly, inhibition of the MAPK /ERK pathway by PD0325901 largely rescues the differentiation phenotype caused by Jmjd1c depletion. We also demonstrate that Jmjd1c, an "eraser" of H3K9 methylation, interacts with pluripotency transcription factors Oct4 and Nanog, suggesting that they may share gene regulatory functions in mouse ESCs. Additionally, Jmjd1c expression is required for the efficient generation of induced pluripotent stem (iPS) cells. Collectively, we propose an integrated model of transcriptional and epigenetic control mediated by the H3K9 demethylase for ESC self-renewal and somatic cell reprogramming.

W2222

LONG TERM EXPANSION OF HUMAN EMBRYONIC STEM CELLS USING 3-D CULTURE CONDITIONS

McKee, Christina[1,2], Perez-Cruet, Mick[2,3] and **Chaudhry, G. Rasul**[1], [1]Oakland University, Rochester, MI, U.S., [2]OU-WB Institute for Stem Cell and Regenerative Medicine, Rochester, MI, U.S., [3]Beaumont Hospital, Royal Oak, MI, U.S.

The pluripotent nature of embryonic stem cells (ESCs) makes them an ideal source for investigating developmental and disease processes, as well as applications for cell-based therapeutics and regenerative medicine. However, these applications require routine and efficient methods for expansion and controlled differentiation of ESCs to obtain a homogenous population of their derivatives. Current strategies for large-scale propagation of

ESCs are laborious, expensive, and technically challenging. Like other cells, human ESCs are generally expanded using 2-dimentional (2-D) culture conditions in plastic culture dishes. However, propagation of cells to obtain uniform populations is difficult in 2-D culture. Previously, we have developed 3-dimentional (3-D) self-assembling scaffolds using thiol-functionalized dextran and polyethylene glycol (PEG) tetra-acrylate via a Michael addition reaction, which supported long-term 3-D growth while maintaining pluripotency and self-renewal potential of mouse ESCs. Since there are distinct differences between mouse and human ESCs, in this study we investigated various polymers both natural and synthetic which self-assemble and support the growth of human ESCs. We observed that 3-D scaffolds made of PEG-based polymers promoted the growth of human ESCs for an extended period of time. The 3-D scaffold grown cells exhibited ESC morphology, expressed pluripotent markers such as Oct4, Sox2, Nanog, and Klf4 as well as SSEA3, SSEA4, and TRA-1-60 and were capable of differentiating into all three germ layer cells indicating that 3-D grown human ESCs maintained their self-renewal and differentiation potential. These cells were indistinguishable from traditionally grown ESCs, when subcultured on MEF feeder layer under 2-D culture conditions, for morphological and molecular characteristics. Further studies are warranted to determine the molecular mechanism of the maintenance of pluripotency of human ESCs in 3-D culture conditions. A robust and reproducible 3-D culture system would be helpful to advance the use of human ESCs for cell therapy and regenerative medicine.

EMBRYONIC STEM CELL CLINICAL APPLICATION

W2224

CATALOGING THE GENETIC VARIATION ACROSS 121 PUBLICLY AVAILABLE HUMAN EMBRYONIC STEM CELL LINES USING WHOLE GENOME SEQUENCING

Ghosh, Sulagna[1], Merkle, Florian[2], Genovese, Giulio[3], Karczewski, Konrad[3], Handsaker, Robert[3], Kashin, Seva[3], Sapphire, Genevieve[1], Charlton, Maura[1], Pato, Michele[4], Pato, Carlos[5], Palotie, Aarno[3], Schier, Alexander[6], MacArthur, Daniel[3], McCarroll, Steven[3] and Eggan, Kevin Carl[3,6], [1]Harvard Stem Cells and Regenerative Biology Department, Cambridge, MA, U.S., [2]University of Cambridge, Cambridge, U.K., [3]The Broad Institute of Harvard and MIT, Cambridge, MA, U.S., [4]SUNY Downstate Medical Center, New York, NY, U.S., [5]University of Southern California, Keck School of Medicine, Los Angeles, CA, U.S., [6]Harvard University, Cambridge, MA, U.S.

Human embryonic stem cells (hESCs) hold great promise for disease modeling and regenerative medicine because of their unlimited potential for self-renewal and ability to differentiate into multiple somatic lineages. Despite their widespread use and ongoing clinical application, surprisingly little is known about their genetic makeup and variation. Since genetic differences between hESCs can contribute to variability in transcriptional and cellular phenotypes or render cell lines less suitable for transplantation and disease related models, a necessary first step is to systematically characterize the genetic landscape across these cell lines. To this end, we sequenced the whole genomes of 121 cell lines that were readily available for distribution and research. We found that while the genetic landscape of hESCs largely resembled previously characterized somatic cell lines, some had acquired large copy number variants (CNVs). We then surveyed these hESCs for protein-coding genes affected by copy number changes or point mutations that may have arisen in culture as well as known pathogenic structural and sequence variants. We identified both gene knock-out lines that can serve as cellular models for studying gene function and hESC lines that carry disease-causing mutations that might make them less suitable for certain applications. Together, the results described here provide a resource to enable the rational selection of hESC lines for applications ranging from disease modeling to transplantation medicine.

W2226

UNIVERSAL DONOR STEM CELL: TAKING DOWN THE IMMUNE BARRIER TO TRANSPLANTATION USING CRISPR/CAS9

Ferreira, Leonardo[1], Meissner, Torsten Bjoern[1], Strominger, Jack[2] and Cowan, Chad[1], [1]Harvard University, Cambridge, MA, U.S., [2]Harvard Stem Cell Institute, Cambridge, MA, U.S.

Realizing the promise of regenerative medicine depends on our capacity to transplant stem cell-derived cells into patients. One major barrier to transplantation is allograft rejection by the recipient's immune system. Recent advances in induced pluripotent stem cell (iPSC) technology have made it possible to contemplate the use of a patient's own cells as an inexhaustible source for autologous transplantation. However, the generation of iPSCs remains a costly, laborious, and highly variable process with regards to differentiation potential and genomic stability. In addition, alterations arising during reprogramming and prolonged culturing have been found to trigger an adaptive immune response, resulting in immune rejection of even autologous stem cell-derived transplants. To overcome this immune barrier, we are creating a Universal Donor Stem Cell, an off-the-shelf validated source for any transplantable cell type, which will not be rejected, regardless of a patient's genetic make-up. For this purpose, we have successfully employed CRISPR/Cas9 genome editing to delete critical immune genes in human ES and iPS cells, rendering them potentially less prone to immune rejection in an allogeneic setting. In a complementary approach, we are introducing well-established immune tolerance-inducing molecules, such as PD-L1 and HLA-G, into stem cells that can locally suppress the immune system, thus preventing the complications associated with systemic immune suppression. We are currently using humanized mouse models to assess the immunogenicity of our modified pluripotent stem cell lines and their differentiated derivatives *in vivo*.

CANCER CELLS

W2228

CLONAL ANALYSIS OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA IN A MOUSE XENOGRAFT MODEL

Belderbos, Mirjam[1], de Haan, Gerald[2], van der Velden, Vincent[3] and **Bystrykh, Leonid**[1], [1]ERIBA, UMCG, University of Groningen, Groningen, Netherlands, [2]UMCG Stem Cell Biology, Groningen, Netherlands, [3]Erasmus MC, Rotterdam, Netherlands

Relapsed B-precursor acute lymphoblastic leukemia (B-ALL) is the leading cause of pediatric cancer-related mortality. B-ALL in an individual patient consists of multiple genetically diverse subclones. Over 90% of relapsed B-ALL is due to a subclone already present at diagnosis. We aimed to compare the frequency of leukemia-propagating cells and their clonal dynamics of pediatric B-ALL in an *in vivo*xenograft model by three different clonal markers, including immunoglobulin heavy chain (IgH) rearrangements, leukemia-associated genetic mutations and cellular barcoding. Bone marrow cells from pediatric patients with high-risk B-ALL (BCR-ABL or delIKZF1) were barcoded using a lentiviral vector, and transplanted into immune deficient NOD-SCID-IL2R$\gamma^{-/-}$mice. Barcode composition and IgH rearrangements were analyzed by deep sequencing of blood samples at several time points after transplantation, and in bone marrow, liver and spleen at sacrifice. Genetic mutations were analyzed by quantitative real-time PCR. Barcode clonal analysis revealed highly polyclonal leukemia in primary xenografts, with a median of 182 (89-272) in blood and 107 (range 49-199) barcodes in bone marrow. Barcode complexity decreased upon serial transplantation in mice, to a median of 6 (3-25, p<0.001) barcodes in bone marrow of secondary recipients, and 3 (2-4, p<0.001) barcodes in bone marrow of tertiary recipients. In contrast, IgH clonal analysis only identified a limited number of subclones, which stably contributed to leukemogenesis in primary, secondary and tertiary xenografts. qRT-PCR for leukemia-associated genetic mutations demonstrated a deletion of exon 4-8 in the IKAROS gene in secondary xenografts, of which presence increased upon tertiary transplantation. In conclusion, whereas IgH profiling shows stable oligoclonal leukemogenesis, cellular barcoding reveals initial 100 fold higher clonal complexity and strong selection in serial transplantation towards one clone. Apparently, combining different clonal markers is essential to obtain a thorough view on clonal evolution. Detailed characterization of the genetic and other functional factors driving dominance of specific B-ALL subclones may provide further insight into B-ALL clonal evolution and provide strategies to predict and/or prevent relapsed B-ALL.

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W2230

ENABLING ROBUST EXPRESSION PROFILING OF EXOSOMAL microRNA IN CELL MEDIA AND DILUTE BIOLOGICAL SAMPLES.

Gould, Barbara, Exiqon, Woburn, MA, U.S.

MicroRNAs constitute a class of short RNAs which function as post-transcriptional regulators of gene expression and have key roles in pathway regulation, tissue differentiation, and in many diseases. The expression patterns of microRNAs can accurately classify cell differentiation levels, discrete tissue types as well as specific disease states. This has positioned microRNAs as promising new

biomarkers for application in stem cell research and therapy as well as in disease diagnostics. We have developed different technologies enabling the detection of microRNA biomarkers in biological material even when microRNA expression levels are low such as in cell culture media and biofluids. The first of these technologies is a highly sensitive LNA™-based qPCR platform for microRNA detection, which enables profiling in biofluids where microRNA levels are extremely low. At this point we have already applied this platform on thousands of biofluid samples including serum/plasma and urine to establish normal reference ranges for circulating microRNAs as well as to identify biomarkers of disease. The second technology is a simple exosome precipitation system which only requires low-speed centrifugation to harvest exosomes from cell media or biofluids. The poster will describe how the combination of our developed technologies enable discovery and validation of disease biomarkers of prostate cancer in urine where microRNA levels are extremely low. The technologies were applied to profile exosomal microRNA in cell-free urine from two different cohorts of approximately 220 patients each. A number of differentially regulated microRNAs were identified in urine from prostate cancer bearing individuals. Different prostate cancer specific signatures have been developed the simplest being a signature composed of only three microRNAs. This signature allows construction of receiver operating characteristic curves with areas under the curve from 0.95 - 0.90.

W2232

REGULATION OF SOX2 EXPRESSION AND STEMNESS PROPERTIES IN BREAST CANCER CELLS BY A LYSINE DEMETHYLASE

Hung, Wen-Chun, National Institute of Cancer Research, National Health Research Institutes, Tainan, Taiwan

Histone methylation dynamically regulated by histone methyltransferases and demethylases is frequently changed during the carcinogenesis. In this study, we investigated the expression of various lysine demethylases in breast cancer cells and identified JHDM1A as an overexpression target in the cells. Knockdown of JHDM1A in breast cancer cells revealed that inhibition of this demethylase attenuates the NOTCH signaling with concurrent reduction of Jagged1, NOTCH1 and HEY1 in the pathway. Chromatin immunoprecipitation-quantitative polymerase chain reaction demonstrated the binding of JHDM1A to the Jagged1 promoter and the increase of methylation of Lys-36 of histone H3 (H3K36) in the upstream transcription region. Mammosphere formation was significantly reduced in JHDM1A-depleted cells which could be reversed by ectopic expression of Jagged1. A selective JHDM1A inhibitor daminozide also decreased the number of mammosphere and the number of $CD24^{-}/CD44^{hi}$ cells. Pharmacological inhibition or genetic depletion of JHDM1A enhanced the sensitivity of cancer cells to chemotherapeutic drugs. We also identified SOX2 as a direct transcriptional target of JHDM1A to promote cancer stemness. Depletion of JHDM1A in MDA-MB-231 cells inhibited NOTCH activation and SOX2 expression. Moreover, inhibition of JHDM1A significantly decreased tumor growth and SOX2-positive cancer cells in orthotopic animal studyies Collectively, we conclude that JHDM1A functions as an oncogene in breast cancer and promotes cancer stemness by upregulating JAG1 to increase SOX2 expression and chemoresistance.

W2234

HUMAN I-MFA DOMAIN-CONTAINING PROTEIN PROMOTES DRUG RESISTANCE OF EPITHELIAL-TYPE CANCER STEM CELLS IN NON-SMALL-CELL LUNG CANCER

Liu, Yu-Peng[1,2], Chen, Chao-Ju[3], Yang, Chih-Jen[4] and Huang, Ming-Shyang[4], [1]Center for Infectious Disease and Cancer Research, Kaohsiung Medical University, Kaohsiung, Taiwan, [2]Graduate Institute of Clinical Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, [3]Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan, [4]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan

Epithelial-to-mesenchymal transition (EMT) has been demonstrated to induce the cancer stem cell (CSC) phenotypes, including self-renewal and drug resistance. Our previous study showed that the CD133+ cells exhibited CSC properties. However, both epithelial-type and mesenchymal-type CD133+ cells could be found in non-small cell lung cancer cell lines. The drug responses of these treatment naïve CSCs in different EMT status are still unknown. In this study, we isolated the epithelial-type CD133+ (E+/CD133+) and CD133- (E+/CD133-), and the mesenchymal-type CD133+ (E-/CD133+) and CD133- (E-/CD133-) subpopulations from PC14 lung cancer cell line. The E+/CD133+ subpopulation had greater proliferation, sphere formation, tumor initiation and drug resistant abilities compared to other subpopulations. By comparing with the public microarray datasets, a panel of 86 drug resistance-related genes, which were specifically up- or down-regulated in E+/CD133+ subpopulation, was determined. Among of the gene signature, the mRNA levels of 20 genes were significantly correlated with the worse prognosis and higher hazard ratio of lung cancer patients in the public GSE31210 dataset. In addition, we found that the human I-mfa domain-containing protein, HIC, was highly expressed in E+/CD133+ subpopulation. Knockdown of HIC induced EMT and increased the drug sensitivity of E+/CD133+ cells to cisplatin. On the other hand, overexpression of the short form HIC (p32) promoted the cisplatin resistance of E-/CD133+ cells. Mechanistic study showed that HIC increased the protein stability and

transcriptional activity of b-catenin, which contributed to the drug resistance. This study demonstrated the heterogeneity of EMT and drug response in CSC and non-CSC subpopulations of non-small cell lung cancer.

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W2236

ANDROGEN RECEPTOR EXPANDS THE POPULATION OF CANCER STEM CELLS IN UPPER URINARY TRACT UROTHELIAL CELL CARCINOMA CELLS

Shyr, Chih-Rong[1], Chen, Chi-Cheng[2], Hsieh, Teng-Fu[2] and Huang, Chi-Ping[1], [1]China Medical University, Taichung,, Taiwan, [2]Taichung Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taichung, Taiwan

Androgen receptor (AR) affects the development and progression of upper urinary tract urothelial cell carcinoma (UUTUC). However, the regulatory mechanism exerted by AR to affect UUTUC cells remains unclear. Here we investigated whether AR promotes UUTUC development and progression, possibly by expanding the population of cancer stem cells (CSCs), which are a particular population of cells within cancer cells responsible for tumor initiation, drug resistance and metastasis. We compared UUTUC cells with or without the addition of AR on their CSC population with flow cytometry, colony formation and sphere formation assay to determine the effect of AR on CSC activity, and real-time PCR was used to detect the expression stemness genes and miRNAs. In vivo tumor formation was evaluated with the implantation of cancer cells in nude mice. We found that the addition of AR in UUTUC cells, significantly increased the population of CSC, clonogenicity, sphere formation and the expression of stemness genes (Oct4, Bmi1 and Nanog), altered CSC-related miRNA profile, as well as promoted epithelial mesenchymal transition (EMT). And AR inhibitor, enzalutamide was shown to suppress AR's effect on tumorsphere formation. Furthermore, in an immune-deficient mouse model, the addition of AR in UUTUC cells also increased the tumor formation capacity. This study will help us better understand the extent to which AR contributes to UUTUC progression by expanding their CSC population and capacity. Our findings could explain high incidence of UUTUC observed in males. And targeting AR may lead to novel therapeutic approaches for genetically diversified urothelial carcinomas in precision medicine era.

W2238

THE ROLE OF MIR-200c IN ATRA-MEDIATED DIFFERENTIATION OF BREAST CANCER STEM CELLS

Wu, Meng-Ju and Chang, Chun-Ju, Purdue University, West Lafayette, IN, U.S.

The key molecular mechanism governing stemness-differentiation of cancer stem cells (CSCs) remains elusive. Our preliminary data provide the first evidence showing that all-trans retinoic acid (ATRA), which is clinically used for differentiation therapy in various human malignancies, promotes the interaction of a novel retinoid acid receptor (RAR)-epigenetic modulator complex to activate the expression of miR-200c. Genome-wide ChIP-seq analysis results further reveal that RAR complex epigenetically activates a cohort of target genes and miR-200c involved in cell differentiation. We also find that CSC populations isolated from low-grade luminal human breast cancers are suppressed by ATRA at the pharmacological concentration; however, CSCs from aggressive triple-negative breast cancers, which are often deficient in the specific RAR are highly resistant to ATRA. Re- expression of the specific RAR is able to restore nuclear epigenetic modulator and re-sensitize the resistant breast CSCs to ATRA. Together, with the newly-identified ATRA-RAR-miR200c regulatory axis from this study, we expect that re-activation of miR-200c expression may serve as a functional therapeutic marker for ATRA effectiveness on targeting the cancer stemness in multiple epithelial cancers. This study will provide insights into effective therapeutic strategies to eradicate breast cancer.

W2240

OCT4A / MIR-367-MEDIATED AGGRESSIVENESS AND STEM-LIKE TRAITS IN EMBRYONAL BRAIN TUMOR

Kaid, Carolini, Silva, Patricia, Rodini, Carolina Oliveira, **Okamoto, Oswaldo** University of São Paulo, São Paulo, Brazil

Atypical teratoid rhabdoid tumor (ATRT) is a highly malignant embryonal brain tumor and one of the most fatal types of pediatric tumors occurring in young children. Molecular determinants of aggressiveness in ATRT are poorly known and only limited therapeutic options are available. In this study, a patient-derived ATRT cell line has been established and found to abnormally express the pluripotency factor OCT4A and one of its microRNA targets that is also involved in pluripotency regulation, miR-367. Enforced overexpression of OCT4A upregulated miR-367 expression in ATRT cells. Overexpression of either OCT4A or miR-367 enhanced tumor features typically correlated with poor prognosis, namely cell proliferation, 3D tumor spheroid cell invasion, and ability to

generate neurosphere-like structures enriched in CD133 expressing cells. Moreover, inhibition of miR-367 in OCT4A-overexpressing ATRT cells partially abrogated the pro-oncogenic effects of OCT4A. Overall, these findings indicate that aberrant OCT4A expression enhance aggressiveness and stem like traits in ATRT cells through miR-367 upregulation, revealing a mechanism that could be further explored to overcome the poor prognosis associated with this malignant pediatric brain tumor.

TECHNOLOGIES FOR STEM CELL RESEARCH

W3002

DEVELOPMENT OF A FEEDER-FREE PSC CULTURE SYSTEM ENABLING TRANSLATIONAL & CLINICAL RESEARCH

Newman, Rhonda A., Sangenario, Lauren and Kuninger, David, Thermo Fisher Scientific, Frederick, MD, U.S.

Pluripotent stem cell (PSC) culture using the xeno-free Essential 8™ Medium/truncated recombinant human Vitronectin system has been shown to support normal PSC properties and provide a large pool of cells for disease modeling and drug development. As research moves from translational to clinical research, general regulatory guidance from the US Food and Drug Administration (FDA) indicates that, cGMP manufactured, or clinical grade reagents should be used whenever available as ancillary reagents to minimize downstream risk to patients. Thus, we sought to identify regulatory compliant, animal-origin-free alternatives for growth factors contained within the Essential 8™ Medium and incorporate cGMP manufacturing processes for the recombinantly expressed, truncated human Vitronectin, producing a qualified ancillary system for PSC expansion. Here we present data to support a seamless transition from the xeno-free Essential 8™ Medium system to the Cell Therapy Systems (CTS™) animal-origin free system. Compatibility is shown with existing cGMP-manufactured passaging reagents: Versene Solution for clumped cell passaging and CTS™ TrypLE™ Select combined with RevitaCell™ Supplement for single cell passaging. Upon expansion, PSCs are shown to maintain normal PSC properties, including morphology, pluripotency, karyotype, and trilineage differentiation potential. Together this system provides a consistent, feeder-free PSC culture medium for translational and clinical research.

W3004

CONSTRUCTION AND VALIDATION OF PAX7 REPORTER HUMAN PLURIPOTENT STEM CELL LINES

Banuelos Mota, Andrea[1,2], XI, Haibin[2], Hicks, Michael R.[3], Jan, Majib[4], Fujiwara, Wakana[2], Malone, Cindy[1] and Pyle, April[2], [1]California State University, Northridge, Northridge, CA, U.S., [2]University of California, Los Angeles, Los Angeles, CA, U.S., [3]University of California, Los Angeles, Santa Monica, CA, U.S., [4]Bridges to Stem Cell Research, Northridge, CA, U.S.

Duchenne Muscular Dystrophy (DMD) is an X-linked disease characterized by loss of dystrophin and depletion of the endogenous skeletal muscle stem cells (satellite cells or SCs). Loss of SCs is a major driver in DMD progression and therapies to replace SCs could significantly augment muscle regeneration. During development, waves of skeletal muscle progenitor cells (SMPCs) are generated that give rise to PAX7$^+$ SCs. We aim to utilize developmental pathways to derive PAX7$^+$ SMPCs from human pluripotent stem cells (hPSCs) with myogenic potential comparable to SCs. To evaluate endogenous expression of PAX7 in SMPCs during differentiation, we have constructed an hPSC reporter line utilizing CRISPR/Cas9 gene editing technology to insert a Green Fluorescent Protein (GFP) reporter cassette in the 3'UTR region of PAX7. We verified insertion by PCR genotyping as well as sequencing the junction region where the cassette was inserted in multiple clones. We further removed the antibiotic resistant cassette with a recombinant TAT-Cre protein to prevent transcriptional repression of adjacent endogenous promoters of other genes. Additionally for transient experiments, we are utilizing a PAX7 promoter-less GFP lentiviral vector that reports on PAX7 activity. To validate these PAX7 reporters, we used modified directed differentiation protocols that include enriching for NCAM, a SMPC marker, and against HNK1, a marker of neural crest that also expresses PAX7. Transient infection of PAX7 lentivirus revealed a dose dependent increase in GFP expression in NCAM$^+$ SMPCs post-selection. Ongoing experiments are sorting GFP-PAX7$^+$ SMPCs to test their myogenic potential *in vitro* and *in vivo*. Validation and optimization of these reporters to efficiently derive PAX7$^+$ SMPCs, will improve our understanding of skeletal muscle specification from hPSCs and enhance the ability to develop potential cell based therapies for DMD.

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W3006

PERSPECTIVES FOR THE SCALE-UP OF PRODUCTION OF PLURIPOTENT HUMAN EMBRYONIC STEM CELLS THROUGH THE INCORPORATION OF THERMORESPONSIVE POLYMER COMPONENTS

Chen, Xiaoli, Harkness, Linda, Gray, Peter, Monteiro, Michael and Jia, Zhongfan, University of Queensland, St Lucia, Australia

Human embryonic stem cells (hESCs), with their pluripotent and proliferative properties, are viewed as promising candidates for applications in regenerative medicine and therapy. One of the limiting factors impeding use in therapeutic applications is the difficulty in obtaining enough cells from 2D hESC cultures. Hence, a robust and scalable system for larger scale production of clinical-grade cells needs to be developed. This need led to recent developments in scale-up platforms of three-dimensional suspension cultures of hESCs. However, these processes routinely rely on enzymatic dissociation and use of small molecules, such as the Rho Kinase inhibitor, to counteract low cell viability during passaging. These treatments have been reported to cause a detrimental effect on genetic stability of hESC, thus raising concerns regarding the dependence on enzymes and small molecules for hESC maintenance. Our group has previously published a 2-component polymer system, comprising of a polystyrene core coated with thermoresponsive poly(N-isopropylacrylamide) (pBridges) and a block copolymer of PNIPAM conjugated with recombinantly-produced extracellular matrix protein. This system has allowed enzyme-free propagation of hESCs as suspension cultures using a 96-well platform. To demonstrate the feasibility of this technology on scale-up, we have expanded our experiments to larger volumes and a mini-spinner platform. Cell viabilities remained high (> 80% viable at each passage), however, proliferation rates were found to be dependent on hESC line and media composition. Detailed analysis of the distribution of aggregate size, prior to and post passaging, demonstrated that the aggregates were capable of dissociation into relatively uniform smaller aggregates that continued to proliferate between passages. These hESC aggregates demonstrated continued expression of key pluripotency markers, were able to differentiate towards all three germ lineages, and were karyotypically normal. In conclusion, the proof-of-principle study, presented here demonstrates the feasibility of the nanobridge system in suspension cultures of pluripotent hESCs for efficient production of clinical-grade hESCs for stem-cell based therapies.

W3008

PLURTEST: PROVIDING A PLURIPOTENCY ASSAY FOR THE GLOBAL STEM CELL COMMUNITY

Gandre-Babbe, Shilpa[1], George, Kenneth[1], Resch, Alissa[1], Bellafante, Mark[1], Williams, Roy[2], Mason, Dylan[3], Schuldt, Bernhard[4], Mueller, Franz-Josef[5] and Loring, Jeanne F.[6], [1]Coriell Institute for Medical Research, Camden, NJ, U.S., [2]The Scripps Research Institute, La Jolla, CA, U.S., [3]Independent Consultant, Encinitas, CA, U.S., [4]Zentrum fur Integrative Psychiatrie, Schleswig-Holstein, Germany, [5]Zentrum für Integrative Psychiatrie, Kiel, Germany, [6]The Scripps Research Institute, San Diego, CA, U.S.

Induced pluripotent stem cells (iPSCs) are a powerful human model system for studying pathophysiology of genetic diseases and for drug discovery. Generation of pluripotent stem cells from somatic cells is a non-natural process and mandates robust quality control of the newly generated cell line. This quality check does not end upon generation of the cell line. The life cycle of an iPSC line poses a longitudinal challenge and genomic and epigenomic integrity need to be assessed regularly with appropriate assays. PluriTest was developed as an alternative to the teratoma assay to obviate the use of laboratory animal testing for determining pluripotency of human induced pluripotent stem cells. The www.pluritest.org website has 704 unique registered users since its inception in 2011 and over 13,500 gene expression datasets have been uploaded and analyzed by the system so far. Here we will present and discuss extensions to the current PluriTest, report anonymized usage data and data trends, and our ongoing efforts toward improvement of the current version of the PluriTest data model for pluripotency and development of new applications of this bioinformatics-based model.

W3010

KNOCK-IN OF LARGE REPORTER GENES IN HUMAN CELLS VIA CRISPR/Cas9-INDUCED HOMOLOGY-DEPENDENT AND INDEPENDENT DNA REPAIR

He, Xiangjun, the Chinese University of Hong Kong, HongKong, China

CRISPR/Cas9-induced site-specific DNA doublestrand breaks (DSBs) can be repaired by homologydirected repair (HDR) or non-homologous end joining (NHEJ) pathways. Extensive efforts have been made to knock-in exogenous DNA to a selected genomic locus in human cells; which, however, has focused on HDR-based strategies and was proven inefficient. Here, we report that NHEJ pathway mediates efficient rejoining of genome and plasmids following CRISPR/Cas9-induced DNA DSBs, and

promotes high-efficiency DNA integration in various human cell types. With this homology-independent knock-in strategy, integration of a 4.6 kb promoterless ireseGFP fragment into the GAPDH locus yielded up to 20% GFP+ cells in somatic LO2 cells, and 1.70% GFP+ cells in human embryonic stem cells (ESCs). Quantitative comparison further demonstrated that the NHEJ-based knock-in is more efficient than HDRmediated gene targeting in all human cell types examined. These data support that CRISPR/Cas9- induced NHEJ provides a valuable new path for efficient genome editing in human ESCs and somatic cells.

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W3012

THE MINIMUM INFORMATION ABOUT A STEM CELL EXPERIMENT (MISCE) TO CONSISTENTLY REPRESENT STEM CELL RESEARCH DATA

Jain, Sagar B.[1,2], Adams, Mark D.[1], Stuart, Joshua[3] and Scheuermann, Richard H.[1,2], [1]J. Craig Venter Institute, La Jolla, CA, U.S., [2]University of California, San Diego, La Jolla, CA, U.S., [3]University of California, Santa Cruz, Santa Cruz, CA, U.S.

The promise of stem cell therapies has generated an ecosystem of experiment data and hypotheses in regenerative medicine. To make progress in translating the proliferating variety and volume of data, representation standards are required to ensure accurate interpretation of experiment data between independent investigators. Consistent representation is one of the major challenges in ensuring that stem cell experiment data can be translated into clinical value. Thus, minimum data standards for stem cell experiments will pave the way for consistent, unambiguous and insightful interpretation of stem cell experiment information. Here we present the Minimum Information about a Stem Cell Experiment (MISCE) standard, which proposes the minimum information required to report a stem cell experiment. MISCE has been developed based on the Planned Process hierarchy in the Ontology for Biomedical Investigations (OBI). Biomaterial Transformations, Assays, and Data Transformations need to be consistently represented throughout the entire stem cell experiment workflow to provide meaningful information. MISCE-compliant data capture is being implemented using O-Meta, a software tool developed by JCVI to track associated metadata at a project, event, and sample level for the California Institute of Regenerative Medicine (CIRM) Center of Excellence in Stem Cell Genomics (CESCG) specific aims. We have run pilot-tests with CESCG affiliated lab data and are iteratively refining MISCE to effectively represent stem cell data. These initial tests have demonstrated that the proposed metadata in MISCE is interoperable with diverse objectives in stem cells experiments, including induced pluripotent stem cells and differentiation and direct cellular reprogramming. The adoption of MISCE by the research community at large will facilitate consistent stem cell data interpretation and independent data validation.

Funding Source: Funding from the California Institute of Regenerative Medicine (CIRM) Center of Excellence in Stem Cell Genomics (CESCG)

W3014

INCREASED SURVIVAL AND STEMNESS OF STANNIOCALCIN 2 EXPRESSING STEM CELL UNDER OXIDATIVE DAMAGE

Kim, Pyung-Hwan[1], Kim, Keun-Sik[1], Na, Sang-Su[2], Lee, Bom Nae Rin[3], Kim, Joo-Hyun[2] and Cho, Je-Yoel[2], [1]Konyang University, Daejeon, Korea, [2]Seoul National University, Seoul, Korea, [3]Seoul National University, Seoul, Korea, South

Although stem cell-based cell therapy has demonstrated promising application in various diseases, the therapy suffers from some challenges, including cell expansion before treatment and the low survival of the cells treated at the disease site. To overcome these disadvantages, we used stanniocalcin 2 (STC2), a family of secreted glycoprotein hormones that function to inhibit apoptosis and oxidative damage and to induce proliferation. STC2 gene was transfected into two types of stem cells to prolong stem cell survival and protect the cells from chronic environment-induced cellular damage (by exposing to hydrogen peroxide). The stem cells expressing STC2 exhibited increased cell viability and improved cell survival as well as elevated expression of the pluripotency and self-renewal markers (Oct4 and Nanog) under sub-lethal oxidative conditions. Up-regulation of CDK2 and CDK4 and down-regulation of cell cycle inhibitors p16 and p21 were observed after the delivery of STC2. Furthermore, STC2 transduction activated pAKT and pERK 1/2 signal pathways. Taken together, the STC2 can be used to enhance cell survival and maintain long-term stemness in therapeutic use of stem cells.

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W3016

Phf6 GENE KNOCKOUT MICE BY TALEN SHOWED DEFECTS IN LIFESPAN

Liao, Baojian, GIBH, GuangZhou, China

PHF6, located on the X chromosome, mutations in this gene could cause the Börjeson–Forssman–Lehmann syndrome (BFLS), T-cell acute lymphoblastic leukemia (T-ALL), or acute myeloid leukemia (AML). To date, no Phf6 mouse knockout models have been published. Herein, we established the Phf6 knockout mouse model by Transcription Activator-Like Effector Nucleases (TALENs). Briefly, transcribed mRNA of TALENs was microinjected into the mouse fertilized oocytes. 3 out of the 35 born mice carried truncated mutations in a mosaic type. All the mutations of the mice were verified by direct sequencing. Mutant mice exhibited hyperactivity, decreased in body size and weight, and showed a shorten lifespan compared to the control group. More than 1/3 of the mutant mice developed tumors. The thymus of some mutant mice was enlarged. These phenotypes are partly similar to the human PHF6 related diseases. The Phf6 knockout mice could be a useful model for the functional and pathogenic mechanism research of Phf6.

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W3018

‘CUREMOTORNEURON’: NOVEL iPSC APPROACH TO ADVANCE ALS RESEARCH

Lubitz, Sandra[1], Eggan, Kevin Carl[2], Rubin, Lee[3], Kling, Lea[1], Obieglo, Carolin[1], Pelka, Benjamin[1], Fukui, Hirokazu[1], Chilian, Bruno[1], Bohnstengel, Jana[1], Rudhard, York[1], Sternberger, Ina[1], Scheel, Andreas[1], Kuhn, Rainer[1] and Dohrmann, Cord[1], [1]Evotec, Hamburg, Germany, [2]Harvard University, Cambridge, MA, U.S., [3]Harvard University Department of Stem Cell and Regenerative Biology, Cambridge, MA, U.S.

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. 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. The high unmet medical need is compounded by clinical heterogeneity, lack of robustly predictive in vitro/in vivo disease models and limited understanding of the molecular mechanisms of disease pathogenesis. ‘Cure MotorNeuron’, our strategic partnership with the Harvard Stem Cell Institute, aims to use motor neurons derived from a panel of well characterized human induced pluripotent stem cell lines both from familial and sporadic ALS patients as basic models of disease. We have adapted and standardized motor neuron differentiation and phenotypic screening in 384-well format and are progressing towards further disease modeling and systematic screening for new mechanisms, targets and compounds that have therapeutic value for this life-threatening disease.

W3020

ENHANCEMENT OF CELLULAR ADHESION AND PROLIFERATION BY DIRECT INSTILLATION OF TYPE I COLLAGEN BASED RECOMBINANT PROTEIN SOLUTION INTO CULTURE MEDIUM

Muraya, Koji[1], Kawasaki, Tomoyuki[2], Maekawa, Toshihiko[3], Yoshioka, Yasuhiro[3] and Akutsu, Hidenori[2], [1]FUJIFILM Corporation, Kaisei-machi, Japan, [2]National Center for Child Health & Development, Tokyo, Japan, [3]FUJIFILM Corporation, Kanagawa, Japan

Mesenchymal stem cells (MSC) have great potential for wide range of clinical application. It is important to culture and provide large amount of MSC for injured tissue repair. It has been used tissue culture plates pre-coated with substrate like fibronectin, to get enough amount of MSC. But those substrates derived from animal tissues so that they would cause serious problems of contamination of animal components. On the other hand, we newly developed type I collagen based recombinant protein. The characteristic of this protein is xeno-free, increased RGD (Arg-Gly-Asp) sequence and high uniformity of molecular weight. It is the material with biodegradability, biocompatibility, and cell adhesive property. The aim of this study is to make clear the effect of this protein to promote cellular adhesion and proliferation of MSC without coating it to plates, only by direct instillation of this protein solution. By direct instillation of it, we could reduce working time and cost in cell culture. We investigated the cellular adhesion and proliferation of several MSC lines, which were cultured in low serum concentration or serum-free media for MSC. For safety in practical use of MSC, it is desirable to culture cells in serum-free medium or low serum concentration medium as possible. Moreover, changes in gene expression profile during cell culture was monitored by RT-PCR (Human PCR Array). We compared the differences in cell adhesion, proliferation and gene expression profile between those media, or addition methods of this protein during the period of cell culture. It was revealed that this protein would be effective to enhance cellular adhesion and proliferation without coating it to plates, only by direct instillation of this protein solution into culture medium. Comparing the case of this protein pre-coated plate with the direct instillation of same amount of it to pre-coated plate into culture medium, the effect was the same, and both were better than the case of this protein is not contained in culture medium. Regarding the direct

instillation, the result was the same even in one hundredth of this protein concentration to the amount of coating. Our findings indicate that this protein could promote the cellular adhesion and proliferation only by direct instillation into culture medium, and that could reduce the cost in cell culture.

W3022

VEGF LEVEL IN HUMAN ADIPOSE TISSUE DERIVED MESENCHYMAL STEM CELL CULTURE WASTE AFTER FREEZING

Pawitan, Jeanne Adiwinata[1,2], Leviana, Melisa[1], Sukmawati, Dewi[1], Liem, Isabella Kurnia[1,2], Margiana, Ria[1] and Tarcisia, Twidy[1], [1]Universitas Indonesia, Jakarta, Indonesia, [2]RSCM/FKUI, Jakarta, Indonesia

Adipose tissue derived mesenchymal stem cell (AT-MSC) conditioned medium (CM) contained VEGF, which explain the effect of AT-MSC CM on various conditions that needs angiogenesis, such as diabetic ulcers. Moreover, our previous study showed various growth factors were present in umbilical cord derived MSC culture waste, which might be used in therapy of various diseases. The same might apply for AT-MSC culture waste. Before use and for transportation, CM or culture waste can be stored frozen. However, freezing may cause changes in VEGF conformation, which may alter its function and/or epitope location that is important for VEGF detection using ELISA method. The aim of this study was to measure VEGF concentrations in adipose tissue derived mesenchymal stem cell (MSC) culture waste after freezing using ELISA method, to predict conformational change. Adipose tissue derived MSCs were cultured in 10% platelet lysate containing alpha MEM. Culture medium waste at harvests were collected, aliquoted and frozen. Some aliquots were thawed, and frozen for the second time. Some of the twice frozen aliquots were thawed and frozen for the third time. Concentrations of VEGF in the medium waste after freezing once, two and three times were measured using ELISA method. Each GF concentrations were noted. Mean values and standard deviations of VEGF concentrations were calculated. VEGF levels were detected in AT-MSC culture waste, after first, secod and third freeze-thaw cycles. This result showed that VEGF conformation, at least the epitope location was not changed after freezing. However, the efect of freezing on its fuction needs further study. In conclusion, VEGF level in AT-MSC culture waste was still high even after three times of freezing.

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W3024

CHARACTERIZATION OF CELL-NON-AUTONOMOUS SIGNALING OF THE ER-UNFOLDED PROTEIN RESPONSE

Schinzel, Robert Thomas, UC Berkeley, Berkeley, CA, U.S., Moehle, Erica, University of California, Berkeley, Berkeley, CA, U.S. and Dillin, Andrew, Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA, U.S.

Living systems encounter an unpredictable and complex environment. Coping with ever-changing surroundings, they need to spend a considerable amount of their available energy to maintain homeostasis. To meet this challenge, eukaryotic organisms evolved several organelle-specific stress response pathways, including the unfolded protein response (UPRER) of the endoplasmic reticulum (ER). Intriguingly, the activation of UPRER target genes is not restricted to cells directly affected by the stress, but can be communicated to distal tissues. Our work focusses on the identification of these cell-non autonomous signal molecule and its pathway components. For this we use a human pluripotent stem cell (hPSC) *in-vitro* system to complement the research in other model systems. By utilizing hPSCs to study the ER stress response as well as the cell-non autonomous signaling of the stress we hope to provide important insights in understanding disease etiology and guide the development of novel therapeutic strategies. We would like to take this opportunity to provide to novel observations regarding the ability of cells to signal the unfolded stress response of the ER across tissues. Furthermore, we like to discuss our recent efforts to perform a whole genome screen utilizing CRIPR technology in an effort to identify pathway components of the UPR and its associated signaling pathways.

Funding Source: funded by a CIRM research grant

W3026

NUCLEAR MAGNETIC RESONANCE METABOLIC PROFILING OF CELL MATURATION - HUMAN EMBRYONIC STEM CELLS INTO HEPATOCYTE-LIKE CELLS AND ADULT PIGMENTED EPITHELIAL CELLS INTO RETINAL PIGMENTED EPITHELIAL CELLS.

Swann, Carolyn[1], Rudd, Tim R[1], Carr, Amanda-Jayne[2], Hay, David[3], Athersuch, Toby[4], Keun, Hector[4] and Man, Jennifer Sui-Sum[5], [1]NIBSC, Hertfordshire, U.K., [2]Inst of Ophthalmology, University Coll London, London, U.K., [3]University of Edinburgh, Edinburgh, U.K., [4]Imperial College London, London, U.K., [5]UK Stem Cell Bank, National Institute for Biological Standards and Control, Hertfordshire, U.K.

Human embryonic stem cells (hESCs) are valuable models to study disease and drug development, as they can be cultured indefinitely in vitro, and have the potential to form almost any cell types in the body. The ability to measure and assess the cell status and quality is of paramount importance to ensure the production of the desired cell lineage for use in possible regenerative medical applications. The quality and status of cells is generally assessed using techniques such as flow cytometry (utilising fluorescently tagged surface markers), and detection of transcription markers by Quantitative Real Time Polymerase Chain Reaction (qPCR). These methods are destructive and disturb the culturing environment of the cells. With this in mind, there is a need to identify and develop non-invasive monitoring approaches that will accurately predict the cellular and biochemical changes that occur during cell differentiation. Metabolic profiling (metabonomics/metabolomics) of cell cultures captures a snapshot of the multiple phenotypic endpoints to demonstrate cellular processes in hepatocyte-like cells and retinal pigmented epithelial cell (RPE) models. Nuclear magnetic resonance (NMR) spectroscopy is non-destructive and requires minimal sample pre-treatment. The NMR spectral profiles are reproducible, quantitative and the data is rapidly produced. The NMR spectra of these small molecules in spent media can be compared and conclusions drawn to understand the cellular status of cells in culture. This allows large groups of metabolites to be profiled and screened for differences when compared to a control set. In summary ^{1}H NMR Spectroscopy was used to demonstrate the association of specific metabolite signatures that can be directly ascribed to the final stages of differentiation pathways in hepatocytes derived from hESCs and mature retinal pigmented epithelial cells originating from the APRE19 cell line.

W3028

TRANSCRIPTOMIC AND MASS CYTOMETRIC ANALYSIS OF HUMAN IPS CELLS CULTURED IN AN AUTOMATED CELL CULTURE SYSTEM

Watson, Michael[1], Boutet, Stephane[1], Guo, Tingxia[2], Sigal, Natalia[1], Devaraju, Naga Gopi[1], Harris, Greg[1], Lu, Ying-Wei[3], Unger, Marc[1], Mok, Alex[1] and Li, Nianzhen[1], [1]Fluidigm Corporation, South San Francisco, CA, U.S., [2]Fluidigm corporation, South San Francisco, CA, U.S., [3]Fluidigm Corporation, Singapore, Singapore

Cutting-edge microfluidics technologies applied to cell biology represent a significant paradigm shift for tissue culture techniques in terms of increased micro-environment control, reduction of cells used per assay and increased automation, resulting in a significant reduction of experimental variation. We have developed a fully automated cell culture system that requires only small quantities of user interaction to perform highly complex experiments. The system comprises an integrated fluidic circuit (IFC), an electropneumatic controller, experimental designer software and automated run time control software. Each IFC has 32 culture microchambers that are independently addressable and can be dosed with any combinatorial ratio of up to 16 reagents. Here we will present proof of concept data showing that our automated microfluidic cell culture system is compatible with single-cell transcriptome analysis via qPCR or mRNA sequencing and proteome analysis by via mass cytometry. Briefly, knockdown studies on known pluripotent genes such as *POU5F1* and *SOX2*were conducted on human induced pluripotent stem (iPS) cells cultured in IFC chambers. Gene and protein expressions were analyzed in two separate single-cell analysis workflows using 1) the Fluidigm C1™ system for either qPCR or mRNA sequencing to identify upregulated genes upon knockdown of stemness genes and 2) the Fluidigm Helios™ system for mass cytometry analysis of protein changes in the cell population following the knockdown. The results demonstrate that our two analysis methods are generating high-resolution single-cell information of cell populations cultured in the microfluidic environment. Upon gene expression knockdown, the data shows that cell populations are heterogeneous, as siRNA treatment affects each cell differently at both the transcript and protein levels. In conclusion, we show here for the first time the integration of microfluidic cell culture with single-cell transcriptomic and proteomic analysis workflows allowing for determination of cell culture state at high resolution.

W3030

PROTEIN KINASE C AND TANKYRASE INHIBITION STABILIZE TRANSGENE-FREE HUMAN NAIVE PLURIPOTENCY IN CHEMICALLY-DEFINED FEEDER-FREE CONDITIONS

Zimmerlin, Ludovic, Park, Tea Soon and Zambidis, Elias, Johns Hopkins School of Medicine, Baltimore, MD, U.S.

Conventional human pluripotent stem cells (hPSC) adopt developmental, biochemical, and epigenetic phenotypes analogous to “primed” mouse post-implantation epiblast stem cells (mEpiSC), which possess a less primitive and more restricted pluripotency than mouse embryonic stem cells (mESC). Dual inhibition of GSK3β and ERK (2i) is sufficient for homogenizing mESC to a naïve pluripotent ground state akin to the inner cell mass, but cannot similarly stabilize naïve hPSC. Various transgenic and chemical methods have been reported to revert hPSC to a naïve state with mESC-like transcriptome, epigenome, and biochemical signaling. However, reliance on transgenic or dichotomous primed signals in these human cultures may confound biological interpretation of their naïve state. We recently established that small molecule inhibition of WNT, ERK and tankyrase signaling (LIF-3i medium) was sufficient for stable naïve reversion of a repertoire of hPSC lines on feeders, in the absence of exogenous bFGF, TGFβ, or Activin. LIF-3i rewiring reinforced subcellular levels of activated β-catenin in hPSC, permitted acquisition of mESC-like self-renewal signaling (e.g., LIF/JAK/STAT3; BMP4), and augmented expressions of naïve-inducing factors (e.g., NANOG, KLF2, NR5A2). LIF-3i also potentiated naïve-dominant transcript patterns in hPSC (e.g., increased expressions of STELLA, DNMT3L, HERV-H; decreased XIST, HLA), and an epigenome (e.g., 30-50% reduced global CpG DNA methylation, OCT4 distal enhancer predominance) reminiscent of naïve mESC. LIF-3i-reverted hPSC clustered closely with mESC transcriptomes, and supported improved directed multi-lineage differentiation. We now revise and optimize this feeder-dependent naïve reversion system in novel feeder-free (FF) conditions, using defined cGMP-compliant reagents. This FF system propagated hPSC on vitronectin in N2/B27-based LIF-3i media without primed growth factors or transgene induction, but required both tankyrase and supplemental protein kinase C small molecule inhibition (LIF-4i) to sustain long term stability. Stable, FF naïve hPSC system utilizing biochemically-defined conditions will have great impact in mechanistic human developmental biology studies, and also in future GMP-grade therapeutic applications.

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W3032

NOVEL PATHOLOGICAL DETECTION SYSTEM OF INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES USING T-CELL RECEPTOR GENE LOCUS FOR CELL TRANSPLANTATION THERAPY

Kishino, Yoshikazu, Seki, Tomohisa and Fukuda, Keiichi, Keio University School of Medicine, Tokyo, Japan

Recently, patient’s own induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iPSC-CMs) are expected to be cell sources for regenerative therapy. In the assessment of the benefits and the adverse effects of transplantation therapy, pathological assessment of transplanted iPSC-CMs is essential. However, engrafted autologous iPSC-CMs are indistinguishable from the recipient’s tissue. Moreover, additional gene insertion to iPSCs for pathological detection possibly causes tumorigenic transformation and therefore is unsuitable for clinical application. To address this issue, we developed a novel pathological detection method of genomic rearrangement in cardiomyocytes, which were differentiated from T cell-derived iPSCs (TiPSCs). Transgene-free TiPSCs were generated from peripheral blood with temperature-sensitive mutated Sendai virus which expressed OCT4, SOX2, KLF4, and c-MYC. TiPSCs were differentiated into cardiomyocytes (TiPSC-CMs) in vitro and teratomas in vivo. V, D, and J segment usages in T-cell receptor beta (TCRB) gene locus were sequenced and identified by comparison to the database. With DNA fluorescence in situ hybridization (FISH) probes which were designed according to genomic deficit area in TCRB gene locus and chromosome 7 centromere, two color FISH was performed. TiPSCs, TiPSC-CMs and teratomas showed genomic deficits of the rearrangement area in TCRB gene locus. We successfully detected genomic deficits of TCRB rearrangement in TiPSCs, TiPSC-CMs and teratomas with the FISH method. This method enables pathological distinction of autogenous iPSC-CMs from recipient’s tissue without additional gene insertion, and plays a pivotal role in the verification of successful engraftment of donor iPSC-CMs to follow up clinical studies.

TISSUE ENGINEERING

W3034

THREE-DIMENSIONAL SPHEROID FORMATION ENHANCES CARTILAGE REGENERATIVE ABILITY OF HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYMAL STEM CELLS THROUGH INHIBITION OF APOPTOSIS

Lee, Miyoung, Ha, Jueun, Jeon, Hong Bae and Jeong, Sang Young, Biomedical Res Inst, Medipost Co, Ltd, Seongnam, Korea, South

Recent researches have reported that 3D spheroids of MSCs show enhanced tissue regeneration capabilities compared to that of monolayer-cultured cells. However, the mechanisms underlying improved therapeutic efficacy of spheroid of MSCs are poorly understood. Here, we report that 3D spheroids of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) show enhanced tissue regeneration ability for damaged cartilage by inhibiting apoptosis of transplanted MSCs. 3D spheroids of hUCB-MSCs expressed higher level of manganese superoxide dismutase (SOD2) than that of adherent MSCs. As hUCB-MSCs transplanted into cartilage lesion was exposed to pathological condition that induce apoptosis, to represent pathological microenvironment of damaged cartilage lesion, spheroids of hUCB-MSCs were treated with synovial fluid (SF) of osteoarthritis (OA) patients. After SF treatment, spheroids of hUCB-MSCs increased expression of SOD2. On the other hand, activated caspase-3 decreased compared to adherent cell. The pro-survival molecule Bcl-2 was upregulated while the pro-apoptotic marker, Bax, was down-regulated in spheroidal cells. The siRNA-mediated knockdown of SOD2 expression led to increased activation of caspase-3. Improved survival of 3D spheroids exposed to SF contribute to the enhanced therapeutic efficacy of hUCB-MSC spheroids in vivo. Using an osteochondral-defect animal model, we demonstrated that spheroid-derived hUCB-MSCs showed better therapeutic efficacy than their adherent cells in regeneration of damaged cartilage. In conclusion, our data suggest that 3D spheroids of hUCB-MSCs could enhance the regeneration ability through extension residual period of the MSCs in cartilage lesion.

W3036

THE ROLE OF MATRIX METALLOPROTEINASES ON THE PROANGIOGENIC EFFECTS OF CD133+ CACS

Binder, Bernard, Ren, Jun, Liu, Bo and Murphy, William, University of Wisconsin-Madison, Madison, WI, U.S.

Circulating angiogenic cells (CACs) are a hematopoietic population of cells classically identified as “early outgrowth” endothelial progenitor cells. The precise lineage and phenotype of CACs remains poorly defined – however, these cells express CD133, CD34, and VEGFR2 and potentiate in vitro and in vivo angiogenesis without directly participating as endothelial cells in tubule or blood vessel formation. These proangiogenic effects make CACs an attractive candidate for the treatment of ischemic and cardiovascular disease, but a lack of knowledge regarding their specific mechanism of action has hampered clinical progress. We hypothesized that CACs promote angiogenesis not only by secreting canonical trophic factors such as VEGF and bFGF, but also through the production of immunomodulatory cytokines like interleukins and matrix metalloproteinases (MMPs). $CD133^{+}$ cells were isolated from peripheral blood by fluorescence-activated cell sorting and expanded in suspension culture for 2 weeks in StemSpan CC100 media. Expanded cells were allowed to adhere to tissue culture plastic for 48 hours, and non-adherent cells were discarded. Characterization of proangiogenic effects was performed using standard assays including UEA lectin binding, AcLDL uptake, and flow cytometry. We compared growth factors secreted by CACs to mesenchymal stromal cells (MSCs) and smooth muscle cells (SMCs), which have established paracrine effects on endothelial cells. CACs produced significantly lower amounts of canonically pro-angiogenic cytokines such as VEGF, angiopoietin 1, and angiogenin than SMCs or MSCs. However, CACs secreted markedly higher quantities of MCP1, MCP3, uPAR, and MMP9, suggesting that their effects are mediated by inflammatory signaling and matrix remodeling. To further probe the role of matrix metalloproteinases in CAC function, we knocked down MMP9 production via shRNA and assessed the resulting effect on angiogenic potency in vitro and in vivo. Our data suggest that, although CACs produce some angiogenic factors like VEGF and bFGF, their effects on blood vessel formation are also largely driven by regulation of inflammation and tissue remodeling. This information will be valuable for selecting appropriate applications and optimizing manufacturing and delivery strategies for CAC-based treatments.

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W3038

INTERPLAY BETWEEN MACROPHAGES AND HUMAN ENDOMETRIAL STEM/STROMAL CELLS ON MESH PERFORMANCE IN A MOUSE MODEL

Darzi, Saeedeh[1,2], Deane, James[1,2], Edwards, Sharon[3], Gough, Daniel[4], Werkmeister, Jerome[3] and Gargett, Caroline[1,2], [1]Department of Obstetrics and Gynaecology, Monash University, Melbourne, Australia, [2]The Ritchie Centre, Hudson Institute of Medical Research, Melbourne, Australia, [3]CSIRO Manufacturing Flagship, Melbourne, Australia, [4]Centre for Cancer Research, Hudson Institute of Medical Research, Melbourne, Australia

Pelvic Organ Prolapse (POP) is the herniation of pelvic organs into the vagina, a hidden disease burden affecting 25% of all women. It causes sexual, bladder and bowel dysfunction. 19% of women have reconstructive surgical treatment for POP with a third requiring multiple surgeries. Despite broad acceptance of mesh use in POP surgical repair to support the pelvic organs, the complication rate is unacceptable. Human endometrial mesenchymal stem cells (eMSC) are a new, readily available source of MSC, which are purified using SUSD2 antibodies. The anti-inflammatory properties of eMSC are poorly understood. Our aim was track eMSC (100, 000) delivered on novel polyamide/gelatin (PA+G) mesh (1X1 cm^2) to examine their modulatory role on macrophage phenotype in immunocompromised mice. SUSD2$^+$ eMSCs were isolated from endometrial biopsies by magnetic beads sorting. A mCherry lentiviral plasmid was used to permanently label eMSC. mCherry$^+$SUSD2$^+$eMSC were seeded onto PA+G mesh and implanted in a fascial defect made in the abdominal wall. Tissues were harvested at 7, 14, 30 and 90 days. Macrophage markers used for immunofluorescence were F4/80 (Pan), CCR7 (M1) and CD206 (M2). ELISA was used to detect IL-1β, IL-6 and TNF-α. Transduction efficiency of eMSC was >80%. mCherry$^+$ eMSC were identified in mouse tissue after 7 and 14 but not 30 and 90 days, although Alu sequences were detected until 90 days. Immunofluoresence image quantification showed that most macrophages around mesh filaments at 7 days were M1 phenotype but the M1/M2 ratio reduced at later timepoints in both eMSC/PA+G and PA+G control explants. Less IL-1 β was found in eMSC/PA+G explants than PA+G alone after 7 days, but TNF-α and IL-6 were similar for all time-points. This study shows that human eMSC can be transduced with a lentiviral construct for in-vivo tracking and that their immunomodulatory effects on M1-macrophage mediated inflammation was limited by their short lifespan in a xenograft mouse model. Ongoing studies will use higher eMSC seeding densities by delivering cells in a blue-light crosslinked gel onto the PA+G mesh to evaluate the spatial and temporal relationship between eMSCs and macrophages associated with the foreign body response to mesh

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W3040

PHYSIOLOGICALLY MIMETIC DECELLULARIZATION PROTOCOL FOR RAT AORTA GRAFT GENERATION AND STEM CELL REPOPULATION

Goulart, Ernesto[1], Caires, Luiz Carlos[2], Kobayashi, Gerson Shigeru[1], Ishiy, Felipe Augusto André[1], Musso, Camila[1], Torres, Bruno[1], Faria Assoni, Amanda[1], Oliveira, Danyllo[1], Caldini, Elia[2], Fernandes, Thadeu[2], Passos-Bueno, Maria Rita[1], Raia, Silvano[2], Lelkes, Peter[3] and Zatz, Mayana[1], [1]Institute for Bioscience, University of Sao Paulo, Sao Paulo, Brazil, [2]University of Sao Paulo, Sao Paulo, Brazil, [3]Temple University, Philadelphia, PA, U.S.

There is a great demand for bio-mimetic vascular grafts for a variety of clinical applications. However, most of available synthetic and bio-mimetic materials so far fail either in fully bio-integration and/or providing proper tissue physiological properties. In order to be functional in the host, vascular grafts must preserve their mechanical integrity, proper ECM composition and the ability to be fully recellularized and functionalized prior or after implantation with reduced immunogenic potential. Vascular tissue decellularization is predicted to be a good option to fulfil these neeeds but the majority of currently available protocols have failed in functional host integration and immunogenic response. Here we describe a new protocol that aims to create a practical and efficient alternative protocol for aorta decellularization by recreating closely physiological parameters during the process in order to generate a optimal scaffold to be functionally re-seeded with stem cell derivatives. Rat thoracic aortas were harvested from adult male Wistar rats with intercostals branches ligated. Tissue was decellularized using a non-denaturing detergent (Triton X-100 1%, pH=11) for 48 hrs under intraluminal continuous perfusion (variable flow rate) and pressure (120 mmHg) in an adapted bioreactor developed by our group. Decellularization was characterized by histology (HE, picrosirius, alcian blue and verhoeff van gieson stain) immunehistochemistry for major ECM proteins (collagen I/IV, pan-laminin, and fibronectin), residual DNA content and scanning electron microscopy for all aortic tissue layers (adventicia, tunica media and intima). Decellularized aorta grafts present no apparent nuclei and cytoplasm residues with histological resemblance to normal tissue, non-immunogenic residu-

al DNA content (38.2±6.7 ng/mg of dry tissue), preservation of major ECM proteins (collagen I and IV, laminin and fibronectin) and tissue architecture in all layers. Thus, the present results indicate that decellularization of aorta tissue was successfully achieved. Preliminary experiments showed enhanced recellularization cabability and mechanical resemblance to native tissue as well.

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W3042

TISSUE ENGINEERING PERFU.S.BLE VASCULAR NETWORKS FROM HUMAN IPS-DERIVED CELLS

Kurokawa, Yosuke K[1], Tu, Christina H[2], Lock, Leslie F[2], Hughes, Christopher CW[2] and George, Steven C[1], [1]Washington University in St Louis, St Louis, MO, U.S., [2]University of California Irvine, Irvine, CA, U.S.

The development of organ-on-a-chip platforms have led to in vitro models of the human body that can potentially be utilized for drug screening and disease modeling. Many of these organ models lack a vascular supply, despite the fact that viability and normal function of most organ systems requires a vascular network. In addition, the use of induced pluripotent stem (iPS) cell technology provides a compelling source of cells appropriate for personalized medicine strategies and disease modeling. Here, we utilize endothelial cells and stromal cells that are both derived from the same human iPS cell to create a 3D vascular network that can be used to create vascularized organ models. A TGF-β-modulatory protocol was used for endothelial cell (iPS-EC) differentiation, and magnetic-activated cell sorting was used to select for CD31+ cells. To obtain the iPS-derived stromal cells (iPS-SC), embryoid bodies (EBs) were cultured on non-adherent culture plates for 8 days. After plating on gelatin-coated plates, outgrowing cells were passaged and expanded using fibroblast growth medium. In order to form vessel networks, iPS-EC and iPS-SC were co-cultured in a fibrin gel. Human primary endothelial cells derived from cord blood progenitor cells and lung fibroblasts were used as controls. Cells were fixed and stained using a CD31 antibody to visualize the vascular network. Initial experiments showed that that iPS-SC did not support vessel network formation when co-cultured with iPS-EC, whereas primary human lung fibroblasts supported vascular networks (average vessel area of 17.5 and 151.3 μm^2/frame, respectively). However, with the supplementation of vascular endothelial growth factor (VEGF) into the media (50 ng/ml), the co-culture of iPS-EC and iPS-SC successfully formed 3D vascular networks (average vessel area of 133.8 μm^2/frame). The addition of VEGF alone (in the absence of a stromal cell) to iPS-EC resulted in no vessel network formation, suggesting that other paracrine- or contact-mediated signaling from stromal cells is necessary for vessel network formation. In conclusion, we used a completely iPS-derived cell source to create a 3D vascular network, which can provide more advanced and physiologically-relevant organ-on-a-chip models.

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W3044

SPATIOTEMPORAL PATTERNING OF PLURIPOTENT STEM CELLS USING AN INDUCIBLE CRISPR INTERFERENCE PLATFORM

Libby, Ashley[1], Mandegar, Mohammad[1,2], Conklin, Bruce[1] and McDevitt, Todd[3], [1]USCF, Gladstone, San Francisco, CA, U.S., [2]Gladstone Inst of Cardiovascular Disease-Conklin, San Francisco, CA, U.S., [3]Gladstone Institutes, San Francisco, CA, U.S.

The ability to spatiotemporally control and interrogate cell-cell interactions and multicellular organization is critical to advance in vitro morphogenic models of human development. Rho-associated kinase-1 (ROCK1) affects actomyosin contraction and thus cell-cortex tension, which is vital for the separation of germ layer progenitors in embryogenesis. The objective of this study was to interrogate the effects of ROCK1 on multicellular organization within human induced pluripotent stem cell (hiPSC) colonies and aggregates. We have established a doxycycline inducible CRISPR interference (CRISPRi) platform that allows for reversible inhibition of ROCK1 transcription. Based on qPCR analysis, near complete knockdown (99%) of ROCK1 was achieved after doxycycline treatment for 7 days. The ROCK1 inhibited cells exhibited a similar morphology to the wildtype parental hiPSC line (WTC11) treated with Y-27632, a small molecule ROCK inhibitor. Populations of parental hiPSCs and CRISPRi ROCK1 hiPSCs were subsequently mixed at ratios of 1:3, 1:1, or 3:1, aggregated in microwells overnight (100 or 1000 cells total) and immediately re-plated onto Matrigel-coated substrates or transferred to rotary orbital suspension culture. After 4 days of CRISPRi inhibition of ROCK1, the mixed colonies self-organized to form distinct patterns of inhibited and uninhibited cells that were not observed in untreated colonies. In ~50% of the 3:1 mixed colonies, the ROCK1-inhibited cell population appeared at the periphery of the colonies to form a concentric ring pattern. Despite the radial cell patterning effect, the cells throughout the colonies remained pluripotent (Oct4+). Within the 3D aggregates, ROCK1 inhibited cells clustered together and began to bud by 15 days of suspension culture. Similarly to 2D colonies, without ROCK1 inhibition, CRISPRi cells did not sort from the WT cells within 3D aggregates. These results demonstrate that the inducible CRISPRi system enables the study of spatiotemporal dynamics of multicellular self-organization between isogenic cell lines that differ only by the knockdown of a single gene. This system will enable a greater mechanistic understanding

of the specific molecular pathways regulating multicellular dynamics of pluripotent morphogenesis.

W3046

EFFICACY OF FREEZE-DRIED PLATELET-RICH PLASMA ON BONE ENGINEERING

Nakatani, Yuya, Agata,Hideki, Sumita, Yoshinori, Koga, Takamitsu and Asahina, Izumi, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki-shi, Japan

Platelet-rich plasma (PRP) is known to promote wound healing and widely utilized in bone engineering. PRP is freshly isolated and applied on site of the operation in general, but it is making it both a time- and labor-intensive and is sometimes hard to prepare the adequate amount. Then, it is useful if PRP can be stored. We evaluate the efficacy of freeze-dried PRP (FD-PRP) comparing to freshly isolated PRP (f-PRP) on bone engineering. f-PRP was lyophilized to prepare FD-PRP and it was subsequently preserved at -20°C for one month. Then, we produced different concentrations (equal and 3-folds; ×1FD-PRP, ×3FD-PRP, respectively) by resolving FD-PRP to equal or 1/3 amount of water. We assessed the property changes of FD-PRP in gelation ability and also the release of growth factors (PDGF-BB, TGF-β1 and VEGF) in vitro. We also examine the in vivo bone forming ability. PRPs (f-PRP, ×1FD-PRP or ×3FD-PRP) were mixed with β-TCP granules and onlay-grafted on mice calvaria. After 4 and 8 weeks, the specimens were harvested and the new bone-formation is assessed histologically. Each PRP (f-PRP, ×1FD-PRP or ×3FD-PRP) equally formed gel. In terms of growth factors release, ×1FD-PRP released the identical concentrations of PDGF-BB and TGF-β1 to f-PRP, while ×3FD-PRP did approximately 3-fold concentrations of them to f-PRP. In vivo, ×1FD-PRP promoted identical level of the bone formation to f-PRP, and ×3FD-PRP induced more abundant bone formation at both 4 and 8 weeks. In addition, bone formation at 8 weeks was significantly greater than that at 4 weeks in all groups except the β-TCP group. Conclusions; These results suggest that f-PRP can be stored without functional-loss by freeze-drying and the concentration of PRP may promote its validity on bone engineering.

W3048

HUMAN EPITHELIAL STEM/PROGENITOR CELLS RECONSTITUTE A FUNCTIONAL SQUAMOUS STRATIFIED EPITHELIUM WITHIN A TISSUE-ENGINEERED OESOPHAGUS AS A THERAPY FOR OESOPHAGEAL ATRESIA

Phylactopoulos, Demetra Ellie, Onikoyi, Fatai Olansile, Urbani, Luca, Gjinovci, Asllan, De Coppi, Paolo and Bonfanti, Paola, University College London, London, U.K.

Oesophageal atresia (OA) is a severe congenital malformation that causes the oesophagus to end in a blind-ended pouch rather than connecting normally to the stomach. In the case of 'long-gap' OA, primary anastomosis is often not feasible. The therapeutic options for those patients are limited to surgical procedures aiming at oesophageal replacement (e.g. gastric pull-up), but these procedures are associated with a high rate of post-operative complications and poor quality of life. Regenerative medicine and tissue engineering (TE) may provide a therapeutic alternative to these patients by combining natural organ scaffolds with bona fide stem cells. Decellularised scaffolds from xenogeneic source maintain the native extra-cellular matrix (ECM) composition, mechanical properties and favour *in vivo* vascularisation and remodelling. Our study focuses on the reconstruction of the barrier function of a TE oesophagus. We have succeeded in (i) establishing the conditions necessary for human oesophageal epithelial cells (HuOEC) to extensively expand in vitro, while maintaining the capacity to differentiate, and (ii) to reconstitute a fully functional epithelium *ex vivo* on a decellularised scaffold. Both rat and piglet whole oesophagi were decellularised using a combined detergent/enzymatic treatment, which preserves the chemistry, porosity and architecture of the native organ. After effective decellularisation, HuOEC were seeded on the luminal surface of the scaffolds and cultured for up to three weeks *ex vivo,* where cells adhered, migrated, stratified and expressed differentiation markers. Current results indicate that HuOEC are able to give rise to a functional squamous stratified epithelium on a xenogeneic ECM. The newly differentiated tissue maintains a subpopulation of epithelial cells with proliferation capacity and clonogenic potential, thus supporting their stem/progenitor identity. Reconstructing an oesophagus through TE might be beneficial for OA patients, but also for a broader spectrum of oesophageal pathologies, where an organ/tissue replacement is required. The results that will be presented show feasibility of engineering a human oesophageal replacement with mucosal barrier function and suggest the existence of oesophageal epithelial clonogenic and self-renewing stem cells.

W3050

EXCITATION-CONTRACTION COUPLING OF iPSC-DERIVED EMBRYONIC CARDIAC MYOCYTES AND ADIPOSE-DERIVED MSCs ON A 3-D IN SITU DE NOVO VASCULARIZED HUMAN TISSUE-ENGINEERED CONSTRUCT

Thiruvanamalai, Valarmathi, University of Illinois at Urbana-Champaign, Urbana, IL, U.S. and Li, Jiang, University of Illinois at Urbana-Champaign, College of Veterinary Medicine, Urbana, IL, U.S.

Organ tissue engineering, including cardiovascular tissues, has been an area of intense investigation. The major challenge to these approaches has been the inability to vascularize and perfuse the in vitro engineered tissue constructs. Attempts to provide oxygen and nutrients to the cells contained in the biomaterial constructs have had varying degrees of success. The aim of this current study is to develop a three-dimensional (3-D) model of vascularized cardiac tissue to examine the concurrent temporal and spatial regulation of cardiomyogenesis in the context of postnatal de novo vasculogenesis during stem cell cardiac regeneration. In order to achieve the above aim, we have developed an in vitro 3-D functioning vascularized cardiac muscle construct using human induced pluripotent stem cell-derived embryonic cardiac myocytes (hiPSC-ECMs) and human mesenchymal stem cells (hMSCs). First, to generate the prevascularized scaffold, human cardiac microvascular endothelial cells (hMVEC-C) and hMSCs were co-cultured on a 3-D collagen cell carrier (CCC) for 7 days under vasculogenic culture conditions, hMVEC-C/hMSCs underwent maturation, differentiation and morphogenesis characteristic of micro vessels, and formed extensive plexuses of vascular networks. Next, the hiPSC-ECMs and hMSCs were co-cultured onto this generated prevascularized CCCs for further 7 or 14 days in myogenic culture conditions. Finally, the vascular and cardiac phenotypic inductions were analyzed at the morphological, immunological, biochemical, molecular and functional levels. Expression and functional analyses of the differentiated cells revealed neo-angiogenesis and neo-cardiomyogenesis. Thus, our unique 3-D co-culture system provided us the apt in vitro functioning prevascularized 3-D cardiac patch that can be utilized for cellular cardiomyoplasty.

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W3052

MODEL AND RESCUE VENTRICULAR ARRHYTHMIAS OF CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA (CPVT) WITH iPSC AND HEART-ON-A-CHIP TECHNOLOGIES

Zhang, Donghui, Boston Children's Hospital, Boston, MA, U.S.

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a severe inherited arrhythmia that is frequently lethal. Treatment with beta-blockers and implantable defibrillators has improved outcome, but the treatment remains inadequate. Most cases of CPVT are caused by mutation the gene ryanodine receptor 2 (RYR2), the major sarcoplasmic reticulum (SR) calcium release channel of cardiomyocytes (CMs). Mutation in RYR2 causes increases spontaneous channel opening (Ca2+ sparks) and excessive AP-induced SR Ca^{2+}release, which predisposes to membrane depolarization termed delayed early or delayed afterdepolarizations (EADs or DADs). We differentiated patient-specific iPSCs into cardiomyocytes (iPSC-CMs) and enriched the iPSC-CMs by metabolic selection through culturing in lactate-containing media. By characterizing the phenotype of single CPVT iPSC-CMs, we observed that CPVT iPSC-CMs had increased frequency of Ca^{2+}sparks and afterdepolarizations at baseline. These were exacerbated by catecholamine stimulation. We transduced CPVT iPSC-CMs with the optogenetic actuator ChR2 and assembled them into anisotropic engineered two dimensional cell sheets, fabricated on a heart-on-a-chip platform that allows optical mapping of calcium transients and measurement of contractile properties. This platform showed that assembly of CPVT iPSC-CMs into an engineered heart tissue stabilized their activity so that at baseline they were similar to WT iPSC-CMs. Isoproterenol stimulation and increased optical pacing frequency, the in vitro equivalent of an exercise stress test, caused calcium transient abnormalities, consistent with ectopic beats, and spiral wave re-entry and maintenance. Genome editing of potential downstream targets of β-adrenergic signalling identified a key site that is essential for the pro-arrhythmic effect of isoproterenol on CPVT iPSC-CMs. To our knowledge, we have developed the first tissue level model of an inherited human arrhythmia and used it to define a molecular target of catecholamine stimulation that is essential for arrhythmogenesis.

REGENERATION MECHANISMS

W3056

DIRECTLY REPROGRAMMED NEURAL PRECURSOR CELLS – A NOVEL SOURCE FOR CELL REPLACEMENT THERAPY IN SPINAL CORD INJURY

Nagoshi, Narihito[1], **Khazaei, Mohamad**[2], Chio, Jct[1], Ahlfors, Jan-Eric[3] and Fehlings, Michael G.[1], [1]University Health Network, TORONTO, ON, Canada, [2]Toronto Western Hospital, Toronto, ON, Canada, [3]New World Laboratories, Laval, QC, Canada

Spinal cord injury (SCI) is a common cause of disability that is currently without a cure. Cell therapies for treating SCI have shown great promise but are limited due to several translational roadblocks including ethical and practical concerns regarding cell source, safety and the time required to generate cells from pluripotent cells. To circumvent these concerns we have generated a novel population of directly reprogramed neural precursor cells (drNPCs) derived from human somatic cells. The direct reprogramming process has a higher efficiency and shorter duration time than generation of NPCs derived from pluripotent cells. We used a model of traumatic SCI in athymic nude rats with clip compression at the T7 level. Nine days after injury, we transplanted 1) drNPCs, 2) pro-oligodendrocyte precursor cells (pro-OPCs) differentiated from drNPCs, and 3) vehicle only, into the injured spinal cord (n=10 per each group). The cell survival rates at nine weeks after SCI were 28.89% and 24.14% in drNPC and pro-OPC groups, respectively. The grafted pro-OPCs significantly differentiated into APC$^+$ oligodendrocyte compared to drNPCs (50.32% vs. 15.14%). In contrast, drNPCs showed higher differentiation rates into NeuN$^+$ neurons (1.79% vs. 0.56%), and GFAP$^+$ astrocytes (57.58% vs. 40.82%). Histomorphometric analysis showed significantly spared white matter area and a reduction in the size of the lesion area in the pro-OPC group compared to drNPC group. Significant recovery of motor function was observed in the pro-OPC group by BBB score. To evaluate the tumorigenicity, we transplanted the cells into the intact spinal cords of NOD/SCID mice. One-hundred and fifty days after injection, all mice remained healthy and histological analysis revealed no tumorigenicity with less than 1% of Ki67$^+$/HuN$^+$cells in both groups. In summary, pro-OPCs 1) spared white matter area and suppressed the expansion of the lesion, 2) predominantly differentiated into mature oligodendrocytes and myelinated host axons, and 3) contributed to motor neurological recovery. Further, transplanted drNPCs did not form tumors. These findings suggest that oligogenic drNPCs are a safe and promising cell source that preserve tissue and promote functional recovery following traumatic SCI.

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W3058

THERAPEUTIC POTENTIAL OF CO-INJECTION OF ADIPOSE-DERIVED STEM CELLS AND MACROPHAGES FOLLOWING ISCHEMIA

Hsieh, Pei-Ling, Rybalko, Viktoriya, Suggs, Laura and Farrar, Roger, The University of Texas at Austin, Austin, TX, U.S.

It is estimated that more than eight million individuals in the US suffer from peripheral arterial disease (PAD). Progressive ischemia results in muscle damage and reduced strength of the lower extremity. Adipose-derived stem cells (ADSCs) have therapeutic potential in tissue repair and have been shown to modulate regeneration via a variety of mechanisms including paracrine effects on macrophages (MPs). We have shown that co-injection of ADSCs with in vitro-polarized MPs (M2, pro-regenerative) intramuscularly leads to a 20% increase in normalized muscle force and significantly enhanced blood flow in the ligated muscles at day 21 relative to saline controls. However, in vitro cell cytokine-mediated polarization is not a desirable method for developing a cell therapy. Additional data demonstrated that brief in vitro co-culture of ADSC with un-polarized bone marrow-derived MPs induces the upregulation of anti-inflammatory and pro-regenerative markers resulting in M2-like phenotype of MPs, particularly in the presence of a hypoxic culture environment. ADSCs may serve to drive M2 versus M1 (pro-inflammatory) phenotypes under conditions of ischemia. The co-injection of autologous M0 MPs and ADSCs is a clinically attractive strategy involving minimal cell manipulation to treat PAD.

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W3060

EFFECTS OF AGING ON MESENCHYMAL STEM CELL MEDIATED INTERCELLULAR MITOCHONDRIAL TRANSFER AND CELLULAR REPAIR FUNCTION

Mukherjee, Shravani and Agrawal, Dr. Anurag, CSIR Institute of Genomics and Integrative Biology, New Delhi, India

Mesenchymal stem cells (MSC) are potential therapeutic for various diseases and conditions. We have studied the mechanism of intercellular mitochondrial transfer and found a critical role of mitochondrial rho-GTPase 1(Miro-1). The rescue ability of MSC in in-vivo and in-vitro models

of ovalbumin allergen induced lung injury and oxidative stress induced lung epithelial cells (NHBE, MLE) respectively were seen. We also found that suppression of intercellular mitochondrial transfer ability from MSC correlates with a decrease in its rescue and repair potential. However, it is consistently observed that MSC fails to show its typical characteristics and inefficient rescue and repair when derived from aging donor or when in late passage. Thus it was interesting to study the impact of aging conditions and late passage on MSC mitocohondrial donation mediated repair. We studied bone-marrow derived MSC from mouse and human which were assessed for its therapeutic role when either co-cultured in-vitro with oxidative stress-induced lung epithelial cells or administered *in-vivo* into mouse model of allergen induced airway injury. We observed that the MSC derived from aging organism or late passage show diminished repair and rescue efficacy but no change or loss in its mitochondrial transfer ability! We also found that aging MSC transferred functionally 'bad' mitochondria, the cells typically had low ATP levels and mitochondria membrane potential and high reactive oxygen species. The Miro1 protein expression did not change with age. In case of aging MSC, we found that aging MSC have decreased levels of calcium than normal and thus Ca-sensitive Miro1 remains attached to even the functionally 'bad' mitochondria, thereby still facilitating the consistent intercellular mitochondrial transport. Interestingly, increase in the intracellular-calcium in MSC with specific stress inducers, does inhibit the cell mediated rescue and repair along-with considerable inhibition of intercellular mitochondrial transport as well. This study is further into the understanding of why and how aging impairs the therapeutic rescue efficiency of MSC. We conclude that not only the intercellular mitochondrial transport ability of the cells but also the quality and function of MSC mitochondria are the determinants of its rescue and repair ability.

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W3062

ISOLATION AND CHARACTERIZATION OF ADULT STEM/PROGENITOR CELL NICHE LOCATED IN THE PARENCHYMA OF THE RAT PITUITARY GLAND

Yoshida, Saishu[1,2], Nishimura, Naoto[3], Kato, Takako[2,4] and Kato, Yukio[3,5], [1]Division of Life Science, Graduate School of Agriculture, Meiji University, Kawasaki-shi, Japan, [2]Organization for the Strategic Coordination of Research and Intellectual Property, Meiji University, Kawasaki-shi, Japan, [3]Division of Life Science, Graduate School of Agriculture, Meiji University, Kawasaki, Kanagawa, Japan, [4]Institute of Reproduction and Endocrinology, Meiji University, Kawasaki-shi, Japan, [5]Department of Life Science, School of Agriculture, Meiji University, Kawasaki-shi, Japan

The anterior lobe of the pituitary gland is a master endocrine tissue composed of five endocrine-cells and non-endocrine-cells. Among non-endocrine-cells, *Sox2*-expressing stem/progenitor cells exist and play a role in the regeneration of endocrine-cells in the adult pituitary. Recent studies have demonstrated that pituitary stem/progenitor cells form two types of micro-environment (niches): the marginal cell layer (MCL-niche) and the dense cell clusters of SOX2-positive cells in the parenchyma of the adult anterior lobe (parenchymal-niche) via E-cadherin and a homophilic tight-junction protein, coxsackievirus and adenovirus receptor (CAR). However, little is known about the mechanisms and factors for regulating the pituitary stem/progenitor cell niches, as well as the functional differences between the two types of niches. Therefore, this study aimed to isolate and characterize niches of the adult rat pituitary. Cell dispersion by stepwise treatment with collagenase and trypsin allowed us to succeed in isolating a stem/progenitor cell niche, termed a pituicluster. Immunocytochemistry demonstrated that pituiclusters are universally composed of cells positive for SOX2, E-cadherin and CAR. Moreover, most of pituiclusters were positive for PROP1, which is mainly present in the parenchymal-niche of the anterior lobe. Therefore, we concluded that pituiclusters are isolated from the parenchyma-niche, not in the MCL-one. Referring to the contents of S100b-positive cell, these pituiclusters were classified into three subtypes. Cultivation of pituiclusters by the serum-free overlay 3-dimensional culture maintained undifferentiated state. Moreover, bFGF- and EGF-treatment induced proliferation and cyst-formation. Collectively, the present study demonstrated a simple method for isolating the pituitary stem/progenitor cell niches existed in the parenchyma of the anterior lobe, and provided valuable tools to analyze the mechanisms and factors for regulating the pituitary stem/progenitor cell niches.

W3064

TMJ PROGENITOR CELL MARKERS AND FATE SPECIFICATION

Casanovas, Guillem[1], Pylawka, Serhiy[1], Kalajzic, Ivo[2] and Embree, Mildred[1], [1]Columbia University, New York, NY, U.S., [2]UConn Health, Farmington, CT, U.S.

Our lab has identified TMJ progenitor cells (TMJ-PCs) that spontaneously undergo endochondral ossification when transplanted in vivo. However, little is known about the mechanisms underlying TMJ-PC mediated cartilage and bone regeneration. Our goals are to: 1) identify early TMJ-PC markers; and 2) investigate signals critical for determining TMJ-PC cartilage and bone lineage specification. To localize TMJ-PCs, EDU was administered intraperitoneally into pregnant rats to label proliferating cells during TMJ morphogenesis. Label retaining EDU+ cells were detected in newborns and after 8 weeks. Sixteen day-old aSMACreERT2/Ai9 transgenic mice were injected with tamoxifen to trace aSMA expression, a skeletal progenitor cell marker. TMJ-PCs were isolated from rat mandibular condyles. Stem cell markers were analyzed in TMJ-PCs by immunohistochemistry and/or RT-PCR. The effect of Wnt agonist Wnt3a and inhibitor sclerostin was tested on TMJ-PC proliferation and maturation. In newborns, ~60% EDU+ cells were found in the entire condyle. After 8 weeks, ~2% EDU+ cells were specifically localized within the articular surface of the condyle, representing putative progenitor cells. In aSMA-Cre transgenic animals, type II collagen (col2a1) expression was used to distinguish putative TMJ-PCs from chondrocytes. Two days after tamoxifen injection, aSMA expression was restricted to col2a1- cells. By day 15, aSMA+ cells infiltrated the col2a1+ chondrocyte population. Isolated TMJ-PCs were positive for cell surface markers Lgr5, CD90, -44, -29, -105, -146, and negative for leukocyte markers CD-45, CD79a, CD11b. Wnt3a agonist significantly inhibited chondrogenesis (lower sox5, sox 6, and aggrecan) in TMJ-PCs, while sclerostin increased aggrecan gene expression levels over 10 days and significantly decreased TMJ-PC proliferation. These results show that TMJ-PCs are localized in the articular surface layer of the mandibular condyle and express bone marrow stromal/stem cell markers. Wnt agonists and inhibitors regulate TMJ-PCs proliferation and maturation and may be critical for cartilage lineage specification.

ETHICS AND PUBLIC POLICY; HISTORY OF STEM CELL RESEARCH; SOCIETY ISSUES; EDUCATION AND OUTREACH

W3068

HUMANIZED ANIMALS HARBORING HUMAN BRAIN NEURONS AND GERM CELLS: A SURVEY AMONG THE PUBLIC AND RESEARCHERS

Hatta, Taichi, Sawai, Tsutomu and Fujita, Misao, Center for iPS Cell Research and Application (CiRA), Kyoto, Japan

The current situations concerning the regulations of human-animal chimeric embryo (HACE) research differ from country to country. Last September saw the following NIH announcement: “it will not fund research in which human pluripotent cells are introduced into non-human vertebrate animal pregastrulation-stage embryos while the agency considers a possible policy revision in this area.” With sudden turns of the policies at the NIH, ethical concerns for human/non-human chimerism have surfaced as there is a possibility of chimeric animals harboring human neurons and germ cells. Although the current Japanese law allows the development of HACE, it has imposed certain conditions on it: any research using HACE must be aimed at organ transplantation. The Japanese Expert Panel on Bioethics in the Cabinet Office, considering the ethical acceptability, suggested expanding the restrictions of research using HACE. Similar to the US case, such ethical issue also entails the discussion of the possibility of chimeric animals harboring human neurons and germ cells in HACE research in Japan. Before deciding whether to introduce such medical technology to the general public, differences in opinions between the public and researchers have to be ascertained. Now, we are about to conduct a survey concerning the attitudes of the public and researchers toward the humanized animals harboring human neurons and germ cells. In the questionnaire, both background information on the procedure of HACE research and the explanations about current limitations concerning differentiation control are provided to the respondents. Additionally, we investigate the degree to which the respondents accept issues of human pluripotent cells being introduced into non-human embryos, in each harboring scenario for human neurons, germ cells, and other types of cells. The participants for this survey are 500 individuals from the general public and approximately 500 iPS cell researchers. Upon approval from the ethics committee at Kyoto University, the data will be collected using the online survey system. The data will help policy makers better understand the attitudes towards research using HACE between the public and researchers.

In this poster presentation, we will present our research findings.

Funding Source: This research was founded by the Uehiro Foundation on Ethics and Education.

W3070

CHILDREN'S EDUCATION ON THE REGENERATIVE MEDICINE: HOW SHOULD WE NURTURE THE ABILITY OF CHILDREN TO GIVE WELL-CONSIDERED ASSENT/DISSENT IN PARTICIPATING IN CLINICAL TRIALS?

Kusunose, Mayumi[1], Katsui, Keiko[2] and Muto, Kori[1],
[1]The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [2]Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Research on regenerative medicine has been advancing rapidly. At this moment, various types of stem cells are used in the fields of research in regenerative medicine such as embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and somatic stem cells. It is well known that ESCs engender ethical issues since embryos need to be destroyed to obtain ESCs. On the other hand, iPSCs are still carrying a risk of tumorigenicity. Even though some ethical debates have lingered on, stem cell research has already become one of the major research with enormous funding from the government in Japan. Therefore, there is a possibility that the day will come when many minors become research participants in stem-cell clinical trials. In current studies, children are provided with opportunities to express their assent/dissent to participation in research. In this regard, children can be actively involved in the decision-making process if they want. However, the question is whether it is adequate enough for children or adolescents to provide information and obtain assent/dissent to make use of these opportunities. Our study focuses on not only the stage of obtaining informed assent/dissent but also on how to educate children about the process. The purpose of our research is to study elements of educating children to nurture their abilities to give well-though-out assent/dissent. Considering the method, we conduct literature research. We also refer to the Project on Children and Clinical Research, Nuffield Council on Bioethics, at the UK since the goal of the project is to achieve the involvement of children in clinical research and some educational workshops for children were carried out. Then, we proceed to discuss how we should educate children to cultivate their ability and consider their research participation. In conclusion, it is important to make sure that children are educated about their rights while engaging them in the process. Such education will help them decide and make them literate about health and science issues including clinical trials. It is also necessary to establish an educational methodology for pediatric clinical trial participants. Our poster presents a detailed discussion of this significant issue.

Funding Source: This research was funded by the Japan Agency for Medical Research and Development.

LATE BREAKING ABSTRACT

W4002

COMPREHENSIVE ASSESSMENT OF NGS PLATFORMS FOR GENETIC CHARACTERIZATION IN HUMAN PLURIPOTENT STEM CELLS

Park, Mi-Hyun[1], Jo, Hye-Yeong[1,2], Kim, Bo-Young[1,2], Ha, Hye-Yeong[1,2], Uhm, Kyung-Ok[1,2], Han, Minhoon[1,2], Kim, Jung-Hyun[1,2], Kim, Yong-Ou[1,2] and Koo, Soo Kyung[1,2],
[1]Korea National Institute of Health, Chungcheongbuk-do, Korea, [2]National Stem Cell Bank, KNIH, Cheongwon-gun, Korea, South

Human pluripotent stem cells (hPSCs) have a wide range of potential for the clinical application. Since genetic stability in hPSCs is a major issue to keep stem cells pluripotent, we assessed the genetic variations of induced pluripotent cells (iPSCs) in Korea National Stem Cell Bank and constructed the analysis flow of Next Generation Sequencing (NGS) for the genetic characterization. We analyzed copy number variations (CNVs) and single nucleotide variants (SNVs) of human induced pluripotent stem cells (iPSCs) which were reprogrammed by two reprogramming methods sendai virus and modified mRNA. In addition, we analyzed CNV and SNVs in iPSCs of different passage number. Since there are a flood of analysis tools and algorithm, which are technically challenging, we applied hPSCs samples to our established analysis flow including arrayCGH and NGS platforms. Here, we present the comprehensive assessment of genomic platforms and the results of genetic variations to verify if the hPSCs from Korea National Stem Cell Bank is genetically stable. In the near future, we plan to provide genomic information for the quality control of hPSCs via Korea National Stem Cell Bank.

W4004

CULTURE IN NME7AB IS A SIMPLE METHOD FOR INDUCING NAÏVE STATE PLURIPOTENCY IN HUMAN AND NON-HUMAN PRIMATE STEM CELLS

Bamdad, Cynthia, Minerva Biotechnologies, Waltham, MA, U.S.

Most human stem cells cultured in labs today are in the primed state of pluripotency. An earlier state of pluripotency, called naïve, exists in rodent stem cells but has

been elusive in human stem cells. Because rodent stem cells have been much easier to work with than human stem cells and have demonstrated superior ability to differentiate, there is great interest in generating human stem cells in the naïve state. A handful of research groups have now succeeded in generating naive-like human stem cells using a variety of difficult and expensive methods involving cocktails of biochemical inhibitors plus LIF and/or FGF2. These stem cells mimic mouse naïve cells in many ways, but their dependence on the addition of several extrinsic agents, and their propensity to develop abnormal karyotype calls into question their resemblance to a naturally occurring 'naïve' state in humans. We discovered that a truncated form of the human protein NME7 ($NME7_{AB}$) is secreted by human stem cells. A recombinant form of this naturally occurring growth factor alone is sufficient to convert human primed state stem cells to a karyotypically stable naïve state, without the use of inhibitors, transgenes, LIF or FGF2. $NME7_{AB}$, along with the Yamanaka factors, quickly and easily reprograms human or non-human primate fibroblasts or blood cells to iPS cells under serum-free, feeder-free conditions. $NME7_{AB}$ induced naïve cells differentiate down all three germlines as demonstrated by teratoma formation. Directed differentiation to cardiomyocytes, neuronal sub-types and mature hepatocytes was superior to that of FGF-grown cells in terms of yield, expression of markers of the desired cell type.$NME7_{AB}$ has two binding sites for its cognate receptor, MUC1*. Monomeric $NME7_{AB}$ dimerizes MUC1* which stimulates growth and induces pluripotency. At a later stage, $NME7_{AB}$ is down-regulated as NME1 is up-regulated. Dimeric NME1 dimerizes MUC1* stimulating growth and maintaining pluripotency. However, as the population of stem cells increases, the concentration of NME1 increases which shifts the multimerization state from dimer to hexamer. Hexameric NME1 does not bind MUC1* and induces differentiation.

W4008

RAPID AND SCALABLE INTRACELLULAR DELIVERY VIA ULTRASONIC MICROFLUIDIC VORTEX SHEDDING

Pawell, Ryan and Burkhardt, Matthew A., Indee. Inc., San Francisco, CA, U.S.

Cell-derived gene therapies (CDGTs) – cell-based therapies requiring an intracellular delivery step – offer the opportunity to radically impact patient outcomes across several challenging disease areas. A potential limitation in the application and distribution of these therapies is their ability to be manufactured in a scalable and affordable manner. The aim of our work is to address this problem by developing an intracellular delivery platform that scales up and out to meet the demands of the diseased patient populations by delivering functional macromolecules (DNA, RNA, endonucleases, etc.) to therapeutically-relevant cell types (HSCs, iPSCs, T-Cells, etc.). Microfluidic devices offer a solution as they enable the precise processing of cells. Microfluidic devices may be manufactured with existing semiconductor infrastructure: the semiconductor industry developed the ability to manufacture large volumes of parts with micron scale features. Select microfluidic designs can scale to 10 x 10^6 devices per year. In this abstract, we describe a prototype microfluidic device with a 10 mm x 5 mm x 0.7 mm footprint utilizing micro post array geometries to induce ultrasonic microfluidic vortex shedding. These fluid dynamics disrupt the cell membrane in a manner that allows the device to process up to 10 x 10^6 cells s^{-1} with a 7 mm x 1.5 mm x 40 µm flow cell. Here, we demonstrate delivery of fluorescently labeled dextrans (3-, 10- and 70-kDa), purified Cas9 protein, and Cas9 encoding mRNA to Jurkat cells. Dextran delivery resulted in up to 13.4% delivery efficiency with 85.0% - 90.3% viability. These results indicate delivery efficiency is a function of macromolecule size, operating pressure, and array geometry while cell viability is a function of operating pressure and array geometry. Moreover, these fluid dynamics are thought to allow for a cell-type and cell-size independent delivery mechanism. This work demonstrates ultrasonic microfluidic vortex shedding may be used to deliver therapeutically-relevant macromolecules to Jurkat cells with within the context of clinical CDGT development. The high cell viability percentage, cell processing speeds, and mass manufacturing capabilities of our microfluidic chip platform offer a promising path towards large scale clinical manufacturing of *ex vivo* CDGTs.

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W4010

EMT-INHIBITING TRANSCRIPTION FACTOR OVOL2 REGULATES MOUSE SKIN EPITHELIAL REGENERATION AND REPAIR

Haensel, Daniel William, Sun, Peng and Dai, Xing, University of California, Irvine, Irvine, CA, U.S.

Mouse skin epithelia serve as leading systems to study the molecular regulation of adult stem cells. Stem/progenitor cells that reside in the epidermis and hair follicle not only drive normal homeostatic turnover and physiological regeneration, but also contribute to wound repair. Both physiological hair follicle regeneration during hair cycle and formation of a new epidermis during wound healing entail migration and morphological reorganization of stem cell progenies that conceivably must occur in concert with proliferation and differentiation. How these cellular processes are precisely coordinated is not clear. Moreover, the involvement of mechanisms that control

epithelial plasticity remains to be fully investigated. Our previous studies have identified essential, redundant roles for Ovol1/2 transcription factors in epidermal morphogenesis. This function is in part mediated by the ability of Ovol factors to inhibit epithelial-to-mesenchymal transition (EMT), an extreme form of epithelial plasticity. Here we report that loss of Ovol2 alone results in delayed hair follicle anagen progression and wound re-epithelization in adult mice. Additionally, epidermal cells from Ovol2-deficient mice display compromised ability to regenerate hair follicle in vivo, as demonstrated using a patch transplantation assay. Using primary keratinocytes as a model, we have identified *Ovol2* deletion-induced molecular and cellular changes that are indicative of enhanced EMT, defective high-density growth and clonal expansion, reduced intra-clonal cell adhesion, as well as altered keratin gene expression. Furthermore, we will present lineage tracing data that compare and contrast EMT-like events during wound healing in WT and Ovol2-deficient mice. Collectively, these findings highlight an important role of Ovol2 in skin epithelial regeneration and repair, and implicate the coordination of epithelial plasticity and growth as a possible underlying mechanism of its action.

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W4012

MFLISA: A NEW EXPERIMENTAL AND COMPUTATIONAL PLATFORM FOR ANALYSIS OF DYNAMIC SECRETOMES UNCOVERS A SECRETION SIGNATURE PROTECTING CARDIAC CELLS FROM REPERFUSION INDUCED STRESS

Kz, Kshitiz[1], Ellison, David[1], Suhail, Yasir[2], Afzal, Junaid[3], Woo, Laura[2], Spees, Jeffrey[4] and Levchenko, Andre[1], [1]Yale University, West Haven, CT, U.S., [2]Johns Hopkins University, Baltimore, MD, India, [3]Johns Hopkins Medical Institutions, Baltimore, MD, India, [4]University of Vermont, Colchester, VT, U.S.

Secretion of molecular factors is an important and fundamental cellular phenotype. Secretory signature of cells in its composition, dosage, and kinetics can define a cellular response to environmental challenges, or constitute an important means of inter-cellular communication. However, precise measurement of dynamic and context-specific cellular secretion profiles has been challenging. Here we introduce a high throughput platform combining protein microprinting and microfluidics, μFLISA, to directly measure cellular secretion and estimate the kinetics for each of the detected secreted constituents. μFLISA, in combination with a new computational algorithm, can uniquely estimate secretory signature of adherent or non-adherent cells in response to arbitrary biological stimuli. Using μFLISA we provide strong evidence for the hypothesis that secretion by bone marrow derived stem cells (BMSCs) is anti-apoptotic for cardiac cells injured by oxidative stress, and that this secretion is uniquely defined by the biological context. We also precisely determine the cocktail of factors secreted by BMSCs in response to the cardiac injury, and show that this cocktail is sufficient to prevent cardiac cell death in a stem cell-free manner.

Funding Source: American Heart Association Predoctoral Fellowship

W4014

INDUCING INSULIN EXPRESSION IN PDX1-EXPESSING MOUSE ADIPOSE-DERIVED STEM CELLS

Yap, Chui-Sun[1], Sim, Jelvin[1], Lim, Sai Kiang[2], Bee, Yong Mong[1] and Li, Guodong[1], [1]Singapore General Hospital, Singapore, Singapore, [2]Institute of Medical Biology, Singapore, Singapore

Diabetes mellitus is a metabolic disease characterized by a deficiency of insulin relative to metabolic needs, leading to poor blood glucose regulation. Management of the disease involves the administration of insulin with constant glucose monitoring, while transplant of donor or cadaveric pancreas/islets remains the only cure. However, the cure is limited by the scarcity of donors and tissues, hence stem cell-derived insulin-producing cells (IPCs) became regarded as a viable option. With the aim to establish a mouse IPC transplant model, we chose to reprogram adipose-derived stem cells (ADSCs) isolated from Mouse Insulin Promoter (MIP)-GFP mice. To obtain IPCs with high levels of insulin expression, we are screening transcription factors that are involved in the development of beta-cells [e.g. pancreatic and duodenal homeobox 1 (PDX1), neurogenin 3 (NGN3) and forkhead box A2 (FOXA2)], activators/inhibitors of various developmental/signaling pathways (e.g. retinoic acid, activin A and Wnt agonist), reagents known to induce insulin expression (e.g. exendin 4 and nicotinamide), epigenetic modifiers (e.g. trichostatin A and procaine), 3D matrices (e.g. matrigel and fibrin gel), and components of extracellular matrices (e.g. laminins and vitronectin). PDX1 expression proved to be a critical factor in reprogramming ADSCs into IPCs. Co-expressions of FOXA2 and/or NGN3 with PDX1 do not significantly increase insulin expression levels. However, they induce the expression of glucokinase, which is crucial for glucose sensing. Besides exhibiting fluorescence, the IPCs also become cuboidal in morphology; and readily form cell clumps in 3D culture. We identified 13 factors in our successful reprogramming protocols, which are able to induce the expression of pancreatic hormones (insulin and glucagon) in $ADSC^{PDX1}$ cells. The 13 factors were tested in various combinations in a series of fractional factorial design experiments. In 2 rounds of experiments, foetal bovine serum, retinoic acid and nicotinamide were found to be significant contributors to the reprogramming of ADSCs and/or induction of insulin expression, while ac-

tivin A's contribution was marginal. These 4 factors will be included in further screens to obtain IPCs with robust insulin expression and other beta-cell functions, such as glucose response.

W4016

KLF4 RECRUITS CBP/P300 AND MEDIATOR TO ACTIVATE REPROGRAMMING

Zhou, Huajun, Papp, Bernadett, Schmidt, Ryan, Vashisht, Ajay, Mckee, Robin, Wohlschlegel, James, Payne, Gregory and Plath, Kathrin, University of California, Los Angeles, Los Angeles, CA, U.S.

By forced expression of transcription factors Oct4, Sox2, and Klf4, somatic cells undergo conversion to induced pluripotent stem cells (iPSCs), which closely resemble embryonic stem cells (ESCs). To understand the molecular basis of reprogramming, domains important for Klf4 reprogramming activity were mapped. This analysis identified the transcriptional activation domain (TAD) as a domain critical for reprogramming. A single residue mutation (I7A) within the TAD not only disrupted the transcriptional activation activity, but also abolished reprogramming function. To determine binding partners of Klf4's TAD during reprogramming, wild-type and the I7A TADs were used to affinity-purify the proteins from mouse ESC nuclear extracts. The histone acetyltransferase CBP/p300 and Mediator complex were identified as factors that interacted only with the wild-type TAD. We propose that different from Oct4 and Sox2, Klf4 provides a critical link to the basal transcription machinery to facilitate gene activation in reprogramming.

Funding Source: NIH P01 GM099134

W4018

HUMAN HAEMATOPOIETIC STEM CELL DIFFERENTIATION FOLLOWS A CONTINUOUS WADDINGTON-LIKE LANDSCAPE

Velten, Lars[1], Haas, Simon[2,3], Raffel, Simon[2,3], Blaszkiewicz, Sandra[2,3], Huber, Wolfgang[1], Trumpp, Andreas[2,4], Essers, Marieke[2,3] and Steinmetz, Lars[1,5], [1]EMBL, Heidelberg, Germany, [2]DKFZ, Heidelberg, Germany, [3]Heidelberg Institute for Stem Cell Technology and Experimental Medicine, Heidelberg, Germany, [4]HI-STEM gGmbH, Heidelberg, Germany, [5]Stanford University School of Medicine, Stanford, CA, U.S.

Blood formation is believed to occur via the step-wise progression of haematopoietic stem cells (HSCs) through a tree-like hierarchy of discrete progenitor cell types. Although several recent studies have challenged different aspects of this dogma, a comprehensive model of haematopoiesis and entry of HSCs into lineage commitment is currently lacking. Here, we mapped human bone marrow haematopoiesis by quantitatively integrating flow cytometric, transcriptomic and functional lineage fate data at the single-cell level. We found that individual HSCs neither enter lineage commitment at binary branching points nor pass through discrete intermediate progenitor cell stages. In contrast, HSC lineage commitment occurs in a gradual manner best described by a continuous Waddington landscape with initially flat but progressively deepening valleys. Our data determine a detailed model of developmental trajectories within this landscape, as well as their underlying gene expression modules and biological processes. These results establish the concept of a developmental continuum, which can replace the 'differentiation tree' as a comprehensive model of steady state haematopoiesis.

W4020

REGENERATION OF MYOCARDIAL INFRACTED MOUSE HEART WITH FULLY-DEFINED HUMAN CARDIAC PROGENITOR CELLS

Yap, Lynn[1], Wang, Jiong-Wei[2], Sun, Yi[3], Chong, Liyen[1], Chai, Xiaoran[1], Ohman, Miina[1], Bunte, Ralph[1], Cook, Stuart[1], Gosh, Sujoy[1], de Kleijn, Dominique[2] and Tryggvason, Karl[1], [1]Duke-NUS Medical School, Singapore, Singapore, [2]National University of Singapore, Singapore, Singapore, [3]BioLamina AB, Sundbyberg, Sweden

Significant limitations remain concerning the reproducibility, efficiency and effectiveness of new cell therapy approaches for myocardial infarction. We 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 cardiomyocytes (CM) and its progenitors using chemically defined and xeno-free methodology. In this study, we characterize the cells during differentiation using RNA sequencing, immunocytochemistry, flow cytometry, electrophysiology and cytotoxicity. Cardiac progenitors were detected at different days as confirmed by expression of C-KIT, ISL1, NKX2-5 and other distinctive markers. These progenitors continue to differentiate into spontaneously beating CM expressing > 80 % TNNT2. We aim to investigate the potential of these progenitors for myocardial repair in SCID mice. We hypothesize that multipotent progenitors will have higher efficacy than beating CM for myocardial repair and also reduce the risk of arrhythmias. Reperfusion injuries to the ventricles are created and different fluorescently labeled CM progenitors are injected directly into the damaged myocardium. Survival, prolif-

eration and tracking of the injected cells are monitored by bioluminescence in IVIS Spectrum imaging system. The bioluminescence signal continued to be detected at 8 weeks implying long-term survival of these cells. Heart function is assessed by echocardiography at 8 weeks and showed a significant improvement of ejection fraction in treated mice as compared to control mice. Hematoxylin and Eosin staining showed a huge infract and immunofluorescence staining showed the presence of human CM in the infract region as well as integrated into the mouse muscle fibers. This is the first study to show the functional regeneration of damaged heart muscles with well-characterized fully defined, xeno-free human cardiomyoctes. We believe the "stage is now set" for future human clinical trials.

Funding Source: National Medical Research Council Singapore (NMRC)

W4022

CHEMICALLY MODIFIED FRAGMENTED SILICA NANOFIBERS FOR ENHANCED NEURAL STEM CELL ATTACHMENT AND SURVIVAL

Ling-Yu, Guo[1], Wen Shuo, Chen[1], Cheng Kang, Tsai[1], Ting Yu, Chin[1], Mong-Lin, Yang[2], Yui Whei, Chen-Yang[1] and Yi-ting, Fu[1], [1]Chung Yuan Christian University, Taoyuan City, Taiwan, [2]Concordia University, San Paul, MN, U.S.

This study investigates how chemical modification of the fragmented silica nanofibers (fSNF) affects the attachment and survival of neural stem cell. The fSNF with average length of about 25 and 50 μm are obtained by a cryocutting from the electrospun aligned SNF. The fSNF surfaces are coupled with (3-aminopropyl)-trimethoxysilane and (3-mercaptopropyl)-trimethoxysilane, individually, to form the modified fSNF, namely, fSNF-NH_2 and fSNF-SH, respectively. The modified fSNFs are characterized by SEM, FTIR, BET, Si-NMR and TGA measurements. The results show that the modifications did not significantly change the average diameters, while the surface area is drastically decreased. Furthermore, the FE-SEM and florescence microscopic studies indicate that fSNF-NH_2 has significantly higher adhesion ability than fSNF-SH and fSNF. On the other hand, fSNF-SH display much higher viability than both fSNF-NH_2 and fSNF. In addition, all the fragmented nanofibers could be homogeneously dispersed in the PBS solution, and are able to be injected through the 26G syringe needles. This study provides a potential alternative for neural tissue engineering application

Funding Source: the Ministry of Science and Technology, Taiwan, ROC (NSC102-2632-M-033-001-MY3)

W4024

ADULT CANINE SKIN-DERIVED NEURAL PRECURSORS ENGRAFT INTO THE HIPPOCAMPUS AND RESCUE AGE-RELATED MEMORY DYSFUNCTION

Duncan, Thomas[1], Lowe, Aileen[1], Siette, Joyce[1], Sidhu, Kuldip S.[2], Westbrook, R Frederick[3], Sachdev, Perminder[3], Chieng, Billy[1], Lewis, Trevor[3], Lin, Ruby[4], Sytnyk, Valdimir[3] and **Valenzuela, Michael**[5], [1]The University of Sydney, Sydney, Australia, [2]Prince of Wales Hospital, University of New South Wales, Randwick, Australia, [3]University of New South Wales, Sydney, Australia, [4]Concord Hospital, Sydney, Australia, [5]School of Psychiatry, UNSW, Sydney NSW, Australia

The isolation and culture of neural precursors show promise as an autologous stem cell therapy for the regeneration of depleted or dysfunctional neuronal circuits in patients with neurodegenerative disorders. The rapid clinical translation of this research has however been limited by the frequently reported propensity for glial differentiation *in vitro* and *in vivo*, or due to a technical reliance on transgenic modifications. Adapting published methods, we utilize a two-step method to produce high yields of P75-Nestin-CD133-positive neural precursor cells from autologous adult canine skin samples without the use of genetic manipulation. These skin derived neural precursors are highly homogenous in culture, rate-limited by virtue of low number of maximal cell doublings, and differentiate almost exclusively into neurons - demonstrating high expression levels of neuron specific markers such as βIII-tubulin (96%) and MAP2 (74%). Following transplantation into the rodent hippocampus, these cells survive and migrate extensively. This is accompanied by structural and functional maturation, and widespread neuronal engraftment and synaptogenesis within host hippocampal circuitry. Moreover, in aged rats, selective hippocampal-dependent age-related memory deficits are reversed, restoring memory function back to levels equivalent with young rats. Adult skin derived neural precursors are rate-limited, striking a balance between unlimited self-renewal and terminal differentiation. They are patient specific, easily accessible, highly homogenous in culture, and highly restricted to a CNS neuronal fate. Our success in reversing age-related memory impairment in rats has paved the way for the commencement a therapeutic trial in dogs, utilizing these autologous neural precursors for the treatment of canine cognitive dysfunction, a neurodegenerative disorder analogous to Alzheimer's disease.

Funding Source: Rebecca L. Cooper Medical Research Foundation.The ANZ Mason Foundation.National Health and Medical Research Council (NHMRC) of Australia Program Grant (#568969).

W4026

A FEEDBACK AMPLIFICATION LOOP BETWEEN STEM CELLS AND THEIR PROGENY PROMOTES INTESTINAL REGENERATION AND TUMORIGENESIS IN DROSOPHILA

Chen, Jun, Xu, Na, Huang, Huanwei, Cai, Tao and Xi, Rongwen, National Institute of Biological Sciences, Beijing, Beijing, China

Homeostatic renewal of many adult tissues requires balanced self-renewal and differentiation of local stem cells, but the underlying mechanisms are poorly understood. Here we identified a novel feedback mechanism in controlling intestinal regeneration and tumorigenesis in Drosophila. Sox21a, a group B Sox protein, is preferentially expressed in the committed progenitor named enteroblast (EB) to promote enterocyte differentiation. In Sox21a mutants, EBs do not divide, but cannot differentiate properly and have increased expression of mitogens, which then act as paracrine niche signals to promote intestinal stem cell (ISC) proliferation. This leads to a feedback amplification loop for rapid production of differentiation-defective EBs and tumorigenesis. Notably, in normal intestine following damage, Sox21a is temporally downregulated in EBs to allow the activation of the ISC-EB amplification loop for epithelial repair. We propose that executing a feedback amplification loop between stem cells and their progeny could be a common mechanism underlying tissue regeneration and tumorigenesis.

W4028

DYNAMIC REGULATION OF HAIR FOLLICLE STEM CELLS AND THEIR NICHE IN MICE

YI, Rui[1], Wang, Dongmei[1], Wang, Li[1], Siegenthaler, Julie[2] and Dowell, Robin[1], [1]University ofColorado Boulder, Boulder, CO, U.S., [2]University of Colorado Denver, Denver, CO, U.S.

Maintaining a pool of adult stem cells (SCs) is critical for tissue homeostasis and wound repair throughout an organism's life. Self-renewal, a defining property of SCs, is achieved by either symmetrical or asymmetrical cell division, through which new generations of SCs are produced to replenish the SC pool. In many mammalian tissues, notably hair follicles (HFs), blood, and muscle, adult SCs acquire quiescence and infrequently divide for self-renewal. Although mechanisms that govern quiescence and self-renewal in adult stem cells have been investigated extensively, it is unknown for quiescent SCs how they cell-intrinsically respond to the cellular state change induced by self-renewal and how the SC niche also changes their gene expression patterns in coordination with self-renewing and quiescent SCs. We investigate largely synchronized hair follicle SC populations existing during early adulthood in mice to probe this new layer of regulation. Integrating genetic and molecular analyses for transcription factors (TFs), as well as mathematical modelling of HFSC cellular states using mRNA profiling data and global measurement of open chromatin regions, we reveal that activated hair follicle SCs reside in a distinct cellular state from either their quiescent counterparts or the committed hair germ (HG) progenitors. Furthermore, adaptive expression of a Foxc1-Nfatc1-BMP network in the activated hair follicle SCs is required to reinforce the quiescent cellular state and maintain the SC identity. In addition, we find that HFSC niche also dynamically change their gene expression during SC self-renewal. One of the critical roles for the dynamic expression is to enhance HFSC attachment to the niche and protect the integrity of the entire SC compartment. Taken together, these findings illustrate an unexpectedly dynamic and coordinated response by quiescent SCs and their niche to self-renewal and provide new possibilities for examining SC maintenance in both normal and pathological conditions.

Funding Source: NIH/NIAMS

W4030

DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS INTO OVARIAN GRANULOSA CELLS IS PROMOTED BY WNT-4 SUPPLEMENTATION

Zhao, Shi Dou[1,2], Chan, Wai Yee[1], Lu, Gang[1], Tsang, Kam Sze[1] and Ma, Jin Long[1,2], [1]Joint Laboratory of The Chinese University of Hong Kong and Shandong University on Reproductive Genetics, Shatin, Hong Kong, [2]Center for Reproductive Medicine, Jinan, China

Human induced pluripotent stem (iPS) cells which are capable to self-renew and differentiate into different cell types of three germ layers, have a great potential in cellular therapy for diseases and disorders related to cell loss and degeneration. However, it remains a challenge to pursue a high yield of functional somatic cells from iPS cells. In this study, we reported an approach of differentiation of human iPS cells into functional ovarian granulosa cells. The iPS cell-derived cells showed high expressions of granulosa cell markers, FOXL2, AMHR2 and FSHR, in both gene and protein levels as demonstrated by RT-PCR, immunofluorescence staining, Western blotting and flow cytometry. Nonetheless, the low expression of CYP19A1 implied immaturity. Upon further supplement of induction cultures with Wnt-4, high levels of CYP19A1 were noted. More than 50% of the derived cells were positive for both CYP19A1 and FSHR. Estradiol was also detected in the spent medium of cultures of iPS cell-derived cells in the presence of testosterone, indicating that the derived cells are functionally active in steroidogenesis. Data of the study suggest that the pleiotropic Wnt-4 may be crucial

in the induction of human iPS cells into functional ovarian granulosa cells.

W4032

AN INTEGRATED PROTEO-TRANSCRIPTOMIC STUDY IDENTIFIES KEY DEPHOSPHORYLATION EVENTS CRITICAL FOR EPIDERMAL DIFFERENTIATION

Mishra, Ajay, King's College London, London, U.K.

Establishment of an optimal balance between proliferation and differentiation is essential for homeostasis of adult tissue. However, the molecular mechanisms that regulate commitment to differentiation are not well understood. We sought to identify such regulatory mechanisms or 'molecular switches' by executing an integrated genome wide analysis of gene expression at both the protein and transcript levels in combination with phenotypic and functional assays over a time course of human epidermal stem cell differentiation. Significant changes were observed at the protein and transcript level during the phase of transition from proliferation to differentiation i.e. commitment to differentiation. Interestingly, we observed a poor correlation between the expression of transcripts and proteins during the commitment phase of differentiation. This led us to speculate that post-translational modifications such as phosphorylation have an important role in regulating differentiation. To test this, we used an unbiased approach to quantify levels of phosphopeptides using mass spectrometry during the time period of commitment. Integrated analysis of phospho-proteomic, genomic and proteomic data identified candidates which, after being subjected to an siRNA mediated functional screen, were identified as important regulators of epidermal differentiation. Our study suggests that phospho-signaling is crucial in the regulation of epidermal differentiation and demonstrates that integrating genome-wide analytical approaches enables discovery of novel molecular mechanisms regulating stem cell behaviour.

W4034

HARMINE AND HONOKIOL SYNERGISTICALLY PROMOTE OSTEOBLASTIC DIFFERENTIATION IN HUMAN PERIODONTAL LIGAMENT CELLS

Yoshimi Kunishima[1,2], Masako Kobayashi[3], Atsuko Yamashiata[1], Kazunari Akiyoshi[4], Taka Nakahara[2] and Masato Ota[1], [1]Japan Women's University, Tokyo, Japan, [2]The Nippon Dental University School of Life Dentistry, Tokyo, Japan, [3]Tokushima University, Tokushima, Japan, [4]Kyoto University, Kyoto, Japan

BMP2, a famous growth factor to induce osteoblast differentiation of mesenchymal stem cells, has been used for clinical applications. However, the treatment using recombinant human BMP2 protein has some problems such as inflammation, poor cost-efficiency, and individual susceptibilities. In the previous study, it is reported that some small compounds such as Honokiol and Harmine promote osteoblast differentiation in vitro. These osteogenic activities have been shown only in mouse cell lines. In the present study, we first generated a bone-defect model on cranial bone in 4 week-old mice to address functional interaction between BMP2 and Honokiol in vivo. When BMP2 and/or Honokiol were delivered with nanogel to the bone defects, Honokiol improved the efficiency of bone repair in combination with BMP2, evaluated by μCT analysis. We next examined the bioactivity of the 2 compounds to induce osteoblast differentiation in primary human mesenchymal cells. Cells were isolated from human periodontal ligament (hPDL) with enzyme digestion. In presence of ascorbic acid (AA) and β-glycerophosphate (β-GP), Harmine promoted the gene expression of BMP2 in endogenous BMP mRNA-negative hPDL cells, and Honokiol increased the gene expression of ALP by 24 hour-treatment, respectively. Since BMP2 and Honokiol synergistically promoted bone repair in mouse *in vivo*, we then investigated whether Harmine and Honokiol can enhance osteoblast differentiation in hPDL cells. Cells were treated with Harmine and Honokiol at various concentrations with AA and β-GP, and then ALP staining and alizarin red S staining were performed on day 3 and 7. Among more than 10 cell populations isolated from the different individuals, a variety of susceptibilities to Harmine and Honokiol were observed. In most cases, Honokiol dose-dependently promoted ALP activity on day 3, whereas Harmine did not show any significant effects. On day 7, some cell populations showed mineralization of ECM in a dose-dependent manner of Harmine. Interestingly, even at low dose of Harmine, which did not induce mineralization by itself, Honokiol cooperatively improved mineralization of the hPDL cells. These results suggest that Harmine and Honokiol synergistically promote osteogenesis in hPDL cells, and can be utilized for future clinical applications.

W4036

HUMAN FETAL CARTILAGE-DERIVED PROGENITOR CELLS HAVE ANTI-CATABOLIC AND ANTI-INFLAMMATORY EFFECTS ON OSTEOARTHRITIC CHONDROCYTES

Kim, Jiyoung[1], Son, Jeong Hee[1], Min, Byoung-Hyun[2], Park, So Ra[3] and Choi, Byung Hyune[3], [1]Inha University, Incheon, Korea, South, [2]Ajou University, Suwon, Korea, [3]Inha University / GSRAC, Incheon, Korea

We previously reported human fetal cartilage-derived progenitor cells (hFCPCs), as a novel cell source for cell therapy. The surface marker profile of hFCSCs was very similar to that of bone marrow mesenchymal stem cells (BM-MSCs) and they could differentiate well into all 3 mesodermal lineage cells. In particular, they showed im-

mune-privileged and immune-regulatory properties comparable with those of MSCs. We found that hFCPCs could suppress T cell proliferation and expressed the genes of anti-inflammatory cytokines, such as IDO, TSG-6, TGF-b, and LIF. In this study, we have investigated whether hF-CPC can reverse phenotypes of cartilage degeneration using human osteoarthritis (OA) chondrocytes and inflamed chondrocytes by interleukin-1b (IL-1b) in vitro. To enhance the paracrine activity of FCPCs, they were stimulated by treating poly-I:C. In the co-culture experiment, activated hFCPCs decreased expression of a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4), matrix metalloproteinase (MMP)1, monocyte chemotactic protein 1 (MCP1), and interleukin (IL)-6 in inflamed chondrocytes by IL-1b, while that of aggrecan, and collagen type II was increased. Similar results were obtained with ostroarthritic chondrocytes isolated from OA patients. In addition to the above target genes, we also found that the expression of suppressor of cytokine signaling 3 (SOCS 3) was down regulated in osteoarthritic chondrocytes by co-culture of FCPCs. These results suggest that hFCPCs have an ability to modulate inflammatory signals and phenotypes in OA chondrocytes and encourage further studies to apply them in animal models of OA in vivo.

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W4038

CELL SURFACE GLYCOSYLATION ALTERS ELECTROPHYSIOLOGICAL PROPERTIES AND FATE POTENTIAL OF MOUSE NEURAL STEM CELLS

Yale, Andrew Richard[1], Nourse, Jamison[1], Muth, Kayla[1], Ahmed, Syed[2], Arulmoli, Janahan[1], McDonnell, Lisa[1] and Flanagan, Lisa[1], [1]University of California, Irvine, Irvine, CA, U.S., [2]California State University, Fullerton, Fullerton, CA, U.S.

Neural stem cells have great potential as cellular therapeutics to treat a large variety of neurological diseases and injuries due to their ability to form the cell types of the central nervous system. However, since these cells exist as a heterogeneous population of neural stem and progenitor cells (NSPCs) when expanded in vitro as needed for cellular transplantation, understanding the factors regulating cell fate becomes important for predicting or controlling lineage specificity. Whole cell membrane capacitance, an electrophysiological measure of the plasma membrane, reflects fate bias in the neural lineage and is sufficient to identify and enrich neuron and astrocyte progenitor populations in a label-free manner. We hypothesize that molecules such as carbohydrates, which have the potential to alter the electrophysiological and morphological characteristics of the plasma membrane, contribute to whole cell membrane capacitance. We tested this hypothesis by measuring the levels of glycosylation enzymes by qRT-PCR, the levels of cell surface N-glycans by lectin binding and MALDI-TOF, and by modifying the levels of highly branched cell surface N-glycans. We used mouse NSPCs derived from the E12 and E16 developing cortex as populations containing more neurogenic or astrogenic progenitors, respectively. Our analyses indicated that E16 astrogenic mNSPCs tended to have more highly branched complex N-glycans compared to the E12 neurogenic mNSPCs. Moreover, lectin binding to E12 and E16 brain sections indicated that highly branched complex N-glycans on NSPCs in the ventricular zone increase during cerebral cortical development in vivo coinciding with the switch from neurogenesis to astrogenesis. Supplementing E12 NSPCs with N-acetylglucosamine (GlcNAc) enhanced the abundance of highly branched N-glycans on the cell surface and increased whole cell membrane capacitance. Additionally, GlcNAc-treated cells showed a shift in fate bias toward astrocyte generation at the expense of both neuron and oligodendrocyte formation. Taken together, our data provides links between fate-specific membrane capacitance, glycosylation patterns, and cell fate and suggest N-glycosylation as a significant component of fate potential in the neural lineage.

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W4040

COAXIAL PCL-POMA ELECTROSPUN NANOFIBERS PROMOTED CELL PROLIFERATION AND NEURAL DIFFERENTIATION OF MESENCHYMAL STEM CELLS DERIVED FROM RAT BONE MARROW

Li, Wen-Tyng[1], Tsai, Chi-Rong[1], Yeh, Jui-Ming[2] and Chen-Yang, Yui-Whei[2], [1]Chung Yuan Christian University, Taoyuan City, Taiwan, [2]Chung Yuan Christian University, Chung Li, Taiwan

Conductive polymers such as polyaniline and polypyrrole are known for their difficulty for fiber processing. With coaxial electrospinning, core-shell fibers may be prepared by comprising of an electrospinnable core material and a non-electrospinnable shell material. Here, core–shell structured poly(ε-caprolactone) (PCL)- poly(o-methoxyaniline) (POMA) nanofibers were successfully prepared by coaxial electrospinning technique. POMA/PCL fibers were doped with camphorsulfonic acid (CPSA) to form C-POMA/PCL nanofibers. Cell proliferation and neural differentiation of mesenchymal stem cells (MSCs) from rat bone marrow were studied on POMA/PCL and C-POMA/PCL nanofibers. Transmission Electron Microscopy (TEM) images showed that the diameters of POMA/PCL and

C-POMA/PCL nanofibers were 194±37nm (core: 177±6 nm; shell: 14±3 nm) and 199±31 nm (core: 172±8 nm; shell: 16±2 nm), respectively. Fourier Transform Infrared (FTIR) spectroscopy and Thermogravity Analysis (TGA) showed that coaxial electrospun nanofibers had characteristic wave numbers and Decomposition temperature (Td) of POMA and PCL. Contact angle analysis revealed that CPSA doping enhanced surface hydrophilicity of POMA/PCL nanofibers. Cyclic voltammetry (CV) analysis demonstrated electroactivity of POMA/PCL and C-POMA/PCL nanofibers. Four point probe analysis found that C-POMA/PCL nanofibers had higher conductivity in the range of semiconductor. Mechanical analysis at macroscopic and microscopic level found that POMA/PCL nanofibers had better ductility than C-POMA/PCL nanofibers. Cell viability, fluorescence staining and Scanning Electron Microscopy (SEM) analyses exhibited improved attachment and proliferation of MSCs on both types of nanofibers. As demonstrated by immunostaining with neuronal cell markers, C-POMA/PCL nanofibers had higher expression on early neural differentiation marker-β III tubulin, whereas POMA/PCL nanofibers had higher expression on late neural differentiation marker-MAP-2. Therefore, it is feasible to use core–shell nanofibers as a scaffold for neural differentiation of MSCs.

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W4042

A HIGH THROUGHPUT SCREEN IN THE DEVELOPING HUMAN NEOCORTEX INTEGRATES SINGLE-CELL PHYSIOLOGY AND TRANSCRIPTOMICS

Chen, Jiadong[1], Mayer, Simone[1], Pollen, Alex[1], Nowakowski, Tomasz[1], Alvarado, Beatriz[1], Cunha, Carlos[1], Szpankowski, Lukasz[2], Leyrat, Anne[2], West, Jay[2], Diaz, Aaron[3] and Kriegstein, Arnold R.[1], [1]Department of Neurology, University of California, San Francisco, San Francisco, CA, U.S., [2]Fluidigm Corporation, South San Francisco, CA, U.S., [3]University of California, San Francisco, San Francisco, CA, U.S.

We have only just begun to understand the diversity of neural stem and progenitor cells in the germinal zones of the developing human brain. Recent advances in the field of single-cell transcriptomics enable the identification of molecular markers for these progenitor cell types. Several studies have profiled the developing and adult mouse brain, and have identified molecular signatures of cell types in the neuronal lineage. Other studies have combined single-cell transcriptomics with the physiological firing patterns of neurons and thereby identified an even larger diversity of neuronal cell types than previously appreciated. Even though classical synaptic signaling is absent during the early stages of brain development, neurotransmitters modulate critical aspects of neural progenitor cell proliferation and differentiation, as well as the migration and maturation of newborn neurons. Here we combine single-cell transcriptomics with physiological response characteristics of dissociated cells from the germinal zone of the developing human cortex. Cells were loaded with a calcium indicator, captured and maintained in culture using a microfluidic device. Single cells in culture were then dosed sequentially with a series of neurotransmitter receptor agonists while monitoring their intracellular calcium dynamics. This novel high-throughput screen allowed us to measure physiological responses to a range of neurotransmitters in single cells. Subsequently, the same single cells were processed for single-cell sequencing. This platform allowed us to associate a molecular profile with physiological responses on a single-cell level. Our results indicate that the diversity of cell types in the cortical germinal zone is also reflected in the physiological response profiles of cells to different neurotransmitters. The synthesis of the transcriptomic and physiological fingerprints of a cell allows us to start to decipher the signaling pathways that control the cell biology of different cell types in the developing human neocortex. The interrelation of the hence identified cell types is key to understanding the development of a complex tissue, such as the human brain.

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W4044

HIGHLY EFFICIENT SPECIFICATION OF MIDBRAIN DOPAMINE PHENOTYPE FROM HUMAN PLURIPOTENT CELLS UNDER XENO-FREE CONDITIONS

Niclis, Jonathan C[1], Gantner, Carlos William[1], Alsanie, Walaa[1], Mcdougall, Stuart J[1], Bye, Chris R[1], Elefanty, Andrew George[2], Stanley, Ed[3], Haynes, John[4], Pouton, Colin[4], Thompson, Lachlan H[1] and Parish, Clare[1], [1]The Florey Institute of Neuroscience and Mental Health, Melbourne, Australia, [2]University of Melbourne, Melbourne, Australia, [3]Murdoch Children's Research Institute, Melbourne, Australia, [4]Monash University, Melbourne VIC, Australia

Current pharmacotherapies provide only symptomatic benefit for Parkinson's disease patients. In contrast, cellular replacement therapy addresses the loss of ventral midbrain dopaminergic (vmDA) neurons, with clinical trials demonstrating proof-of-principle using fetal tissue. Human pluripotent stem cells (hPSCs) represent an alternative, sustainable tissue source for vmDA neuronal generation. However, existing differentiation protocols rely upon xenogeneic components that hinder clinical translation. Here we describe the first fully defined feed-

er- and xeno-free protocol for the generation of vmDA neurons from hPSCs and utilise two novel reporter lines (LMX1A-eGFP and PITX3-eGFP) for in depth in vitro and in vivo tracking. Across multiple embryonic and induced hPSC lines this 'next generation' protocol increases both the yield and proportion of vmDA neural progenitors (OTX2/FOXA2/LMX1A) and neurons (FOXA2/TH/PITX3) that display classical vmDA functional properties. We identify the mechanism underlying these improvements and demonstrate the clinical applicability of this protocol for high-throughput scalability and cryopreservation of progenitors at a time amenable to transplantation. Finally, transplantation of xeno-free vmDA progenitors from LMX1A- and PITX3-eGFP reporter lines into rodents demonstrates improved engraftment outcomes. This highly efficient and standardized xeno-free system will prove useful for future purposes of in vitro disease modelling, drug screening and cell replacement therapy in Parkinson's disease.

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W4046

PRIMARY CILIA REGULATES POLARIZATION AND MATURATION OF HUMAN IPSC-DERIVED RETINAL PIGMENT EPITHELIUM

Wan, Qin[1], Jha, Balendu[2], Sharma, Ruchi[2], Hartford, Juliet[2], Khristov, Vladimir[2], Miyagishima, Kiyoharu[2], Hua, Fang[2], Memon, Omar[2], Miller, Sheldon S[2] and Bharti, Kapil[2], [1]NEI/NIH, Bethesda, MD, U.S., [2]National Eye Institute, National Institutes of Health, Bethesda, MD, U.S.

Induced pluripotent stem cell (iPSC) derivatives often do not fully-mature in vitro, limiting their use in cell therapy and disease modeling. Retinal pigment epithelium (RPE), a ciliated monolayer critical for maintaining the health and integrity of adjacent photoreceptors, is an attractive candidate for stem cell therapies to treat blinding eye diseases including Age-related macular degeneration. The primary cilium serves as a signaling and sensory hub and controls developmental cellular processes in some cell types, but the role of primary cilium in human RPE development remains largely unknown. The goal of this study is to explore the function and underlying mechanism of primary cilium in RPE development and maturation. Human iPSC derived RPE (iPSC-RPE) were grown on semi-permeable transwells to generate a confluent monolayer. Primary cilia in iPSC-RPE were manipulated using cilium inducers or blockers, or specific canonical WNT agonist or antagonist. Electrophysiology, immunocytochemistry, electron microscopy, gene expression, and phagocytosis assays were used to determine the polarization and maturity of iPSC-RPE monolayer. Our results show that the progressive development of primary cilia in human iPSC-RPE coincides with the formation of RPE tight junctions and epithelial cell morphology. Experimentally enhanced activation of primary cilia led to extensive apical processes, improved pigmentation, increased expression of adult-specific RPE genes, increased phagocytic capability, and significantly enhanced electrical responses that mimic native human RPE, demonstrating improved RPE maturation and functionality. Electrophysiological recordings in combination with immunostaining and phagocytosis using WNT activator or inhibitors revealed that primary cilia-induced iPSC-RPE maturation was regulated through the suppression of canonical WNT signaling. Our results demonstrate a developmental role for primary cilia in human iPSC-RPE maturation. It provides a mechanistic tool to mature RPE or other epithelial cell types derived from human iPSCs, and also provides insight into retinal degeneration caused by ciliopathies.

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W4048

GENERATION AND PROSPECTIVE ISOLATION OF HUMAN PANCREATIC EXOCRINE CELLS FOR IN VITRO MODELING OF CHRONIC PANCREATITIS

Amin, Sadaf and Chen, Shuibing, Weill Cornell Medicine, New York, NY, U.S.

Chronic Pancreatitis (CP) is a progressive inflammatory disease in which the exocrine compartment of the pancreas is destroyed and replaced by fibrous tissue. This condition causes debilitating pain and eventually leads to diabetes and malnutrition. Despite decades of research, treatment of CP remains mostly empirical and patients are admitted to hospitals repeatedly for interventional procedures. This lack of progress in treatment is a sign of uncertainty about how identified causative factors lead to the disease. Mechanistic in vitro studies on human cells for drug discovery has always been limited by the scarcity of primary exocrine cells and their instability in culture.

Human embryonic stem cells (hESCs), represent an inexhaustible source to potentially generate different cell types of the body. Here, we present a stepwise protocol to efficiently induce the differentiation of pancreatic exocrine cells from hESCs. The hESC derived exocrine cells express markers of the exocrine cells and organize themselves to form acini- the functional unit of the pancreatic exocrine compartment. Using high throughput surface marker screening we were able to prospectively isolate the hESC-derived exocrine cells. This approach enables us to model environmental and genetic aspects of CP and establish a platform for high throughput drug screening.

W4050

MOLECULAR AND CELLULAR MECHANISMS OF SPINAL CORD REGENERATION IN AMPHIBIANS

Larrain, Juan[1], Edwards, Gabriela[1], lee-Liu, Dasfne[1], Mendez, Emilio[1], Munoz, Rosana[1], Peñailillo, Johanny[1], Tapia, Victor[1], cebrian-Silla, Arantxa[2], Dedomenico, Elena[3], Sun, Liangliang[4], Dovichi, Norman[4], Gilchrist, Mike[3] and García-Verdugo, José Manuel[2], [1]P. Universidad Catolica, Santiago, Chile, [2]Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Universidad de Valencia, Valencia, Spain, [3]The Francis Crick Institute, London, U.K., [4]University of Notre Dame, notredame, IN, U.S.

The African clawed frog *Xenopus laevis* at tadpole stages (stage 50-54, R-stages) regenerate in response to spinal cord injury (SCI) a capability that is lost at the metamorphic climax (stage 56-66, NR-stages), providing a unique model system to study spinal cord regeneration. Here we will discuss results from three experimental approaches aiming to understand the molecular and cellular mechanisms of spinal cord regeneration in *Xenopus laevis*. First we will show studies on the function of neural progenitor cells in spinal cord regeneration. We have found that in R-stages Sox2$^+$ cells have a rapid and transient activation in response to injury 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. We have also found that Sox2$^+$ cells are necessary for proper regeneration. Electron microscope characterization showed a very different cellular response between the two stages, with active proliferation and formation of cellular bridges and cellular rosettes of neural progenitors in R-stages compared to extensive cell death and the formation of a glial scar and extracellular matrix deposition in NR-stages. In addition, we will present results of cell transplantation using cells isolated from spinal cord at stage 50 and transplanted into non-regenerative animals. The third approach to be presented is the global analysis of the transcriptome and the proteome deployed in response to SCI in R- and NR-stages. Extensive and different changes in the transcriptome and proteome of regenerative tadpoles were already detected at 1 day after injury in R and NR-stages. In general terms we have found differential regulation between the two stages of: 1) genes related to neurogenesis and the axonal growth cone; 2) genes from biological processes including cell cycle, response to stress, metabolism, development and immune response and inflammation and 3) we have also identified previously uncharacterized transcripts regulated differentially after SCI. Currently we are testing by gain and loss-of-function studies the role in spinal cord regeneration of a subset of genes and signaling pathways identified by these global analyses.

Funding Source: Millenium Nucleus for Regenerative Biology

W4052

WNT3A IS ESSENTIAL FOR DERIVATION AND MAINTENANCEOF BOVINE TROPHOBLAST STEM-LIKE CELLS FROM IN VITRO-PRODUCED BLASTOCYSTS IN THE 2 INHIBITOR SYSYTEM

Li, Xueling, Inner Mongolia University, Hohhot, China

Trophoblast stem cells (TSCs) are important tools to study the trophoblast formation during the implantation. The effects of different culture systems for establishment and proliferation of bovine trophoblast stem-like (bTS) cells derived from in vitro-produced blastocysts were investigated in this study. The culture systems included adding 2i, bFGF and Wnt3a into the basic media combined using L-Wnt-3A/MEF mixed cells or BFFs as feeder cells. The results showed that the cells derived from two culture systems of 2i with L-Wnt-3A and mouse embryonic fibroblasts (MEF) as feeder cells and 2i with Wnt3a and bovine fetal fibroblasts (BFF) as feeders had a typical morphology of mouse trophoblast stem cells. The bTS cells showed flat outgrowths and had no signs of altered morphology or differentiation during passaging and positive for OCT4 and CDX2 immunofluorescence staining. However, the cells derived from the culture systems with PD0325901 removed from the 2i media and basic fibroblast growth factor (bFGF) added into the 2i media proliferated slowly, regardless of using L-Wnt-3A/MEF mixed cells or BFFs as feeder, and the cells are negative for OCT4 and CDX2 immunofluorescence staining. These cells resembled three-dimensional morphology of mouse ESCs and could not been cultured beyond 18 passages. The cells derived from bovine in vitro-produced blastocysts in all culture systems are positive for alkaline phosphatase staining.

W4054

PATHWAYS DYSREGULATED IN ALS IDENTIFIED BY GENETIC CORRECTION OF SOD1 AND FUS MUTATIONS

Bhinge, Akshay, Genome Institute of Singapore, Singapore, Singapore

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder usually affecting the aged population with an incidence of 1 in 100,000. ALS is characterized by progressive loss of both the spinal and cortico-spinal motor neurons, leading to muscle paralysis and subsequent death due to respiratory failure. Mutations in various genes across diverse physiological functions, including SOD1 and FUS, have been implicated in ALS with almost 20% mutations proved to be familial. However, in spite of

extensive research over the last two decades, the molecular networks driving specific motor neuron loss in ALS are poorly understood. Patient derived induced pluripotent stem cells have emerged as powerful tools to study disease in a dish. Using the CRISPR-Cas9 system, we genetically corrected patient-derived induced pluripotent stem cells bearing SOD1 and FUS mutations to model ALS in vitro. Genome-wide transcriptome and enhancer analysis using H3K27Ac ChIP-Sequencing on spinal motor neurons derived from disease and corrected iPSC revealed dysregulated pathways common to SOD1 and FUS mutations. We are currently modulating these pathways to affect increased motor neuron survival in the diseased genetic backgrounds.

Funding Source: A*STAR

W4056

MODELING CARDIOMYOGENESIS IMPAIRMENT IN DOWN SYNDROME WITH TRISOMY 21 INDUCED PLURIPOTENT STEM CELLS

LU, Huai-EN[1], Syu, Shih-Han[1], Wen, Cheng-Hao[1], Ko, Hui-Wen[1], Huang, Hsien-Da[2] and Hwang, Shiaw-Min[1], [1]Food Industry Research and Development Institute, Hsinchu, Taiwan, [2]National Chiao Tung University, Hsinchu, Taiwan

Down syndrome (DS), one of the most common chromosomal abnormalities, is caused by an extra duplication of chromosome 21 (Trisomy 21, T21). Clinical statistics showed that about 50% children with DS have congenital heart disease. MicroRNAs (miRNAs) are post-transcriptional modulators of gene expression that play an important role in heart developmental processes. Previous reports demonstrated that human chromosome 21 (Hsa21) harbors five miRNAs; let-7c, miR-99a, miR-125b-2, miR-155 and miR-802 (Hsa21-miRNAs). However, how these miRNAs evolve in cardiomyogenesis in DS remain unclear. In order to ascertain perturbations in cardiomyogenesis, we use T21 induced pluripotent stem cells-derived cardiomyocytes (T21-iPSC-CM) to model cardiomyogenesis impairment in vitro. In this study, we show that the expression of mesodermal associated gene, Mesp1 is significant difference in T21-iPSC-CM. Furthermore, the expression levels of Hsa21-miRNAs in T21-iPSC-CM are highly elevated with the presence of low expression of Ezh2 and Smarca5 which are direct targets of let-7c and miR-99a respectively. These results suggest that the abnormal expression levels of these Hsa21-miRNAs cause the perturbations of heart development in T21-iPSC-CM.

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W4058

THE ALLEN INSTITUTE FOR CELL SCIENCE: AN OPEN AND LARGE-SCALE TEAM APPROACH TO STEM CELL BIOLOGY

Gunawardane, Ruwanthi, Allen Institute for Cell Science, Seattle, WA, U.S.

The new Allen Institute for Cell Science aims to produce novel, dynamic, image-derived data to develop predictive models of human inducible pluripotent stem (hiPS) cells in the contexts of homeostasis, disease and regeneration. Towards this aim, we are using CRISPR/Cas9 technology to create a large collection of isogenic fluorescently tagged hiPS cell lines spanning several hundred gene targets, comprising the major signaling complexes, organelles and molecular machines. In doing this, we are engaging the community to identify the targets, optimize the efficiency of the gene editing, and develop rigorous quality control standards that include molecular and image-based analysis and deep sequencing. Initially, the Institute will study undifferentiated hiPSCs and cardiomyocytes derived from them. The structural and functional organization and dynamics of these cells is being studied using a live cell imaging pipeline capable of generating information from 10,000s of cells in many contexts and in response to perturbations that include disease mutations and drugs. The data are analyzed using a variety of computational and modeling approaches including machine learning. Data-driven animated cells are the visual output of the image data and models. The breadth and scale of this project will allow us to both understand and hopefully predict cellular behavior in both the normal and pathological contexts. Our goal is to integrate diverse technologies, approaches, models and data into a common standardized framework that is open to the community and serves as a single, unified data resource for biomedical research around the world. We have initiated this work by tagging a set of markers that represent several of the major cellular organelles in an established hiPS cell line. The resulting images clearly show the epithelial properties of these iPSCs and highlight the value of endogenous gene tagging for image-based studies.

THURSDAY

POSTER SESSION II ODD

PRE-CLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL CELLS

T1001

BNIP3-MEDIATED MITOPHAGY IS ESSENTIAL FOR CONTROL OF FREE FATTY ACID SYNTHESIS AND FUNCTIONS BY HYPOXIA IN UMBILICAL CORD BLOOD-DERIVED HUMAN MESENCHYMAL STEM CELLS

Han, Ho Jae[1], Lee, Hyun Jik[1], Ko, So Hee[1], Choi, Gee Eun[1], Lee, Ki Hoon[1] and Ryu, Jung Min[2], [1]Seoul National University, Seoul, Korea, South, [2]Chonnam National University, Gwang-ju, Korea, South

Stem cell regulation by hypoxia is a potent and promising therapeutic strategy in regenerative medicine. Recently, there have been several reports that hypoxia-induced lipid metabolic alteration is a critical factor for stem cell regulation by hypoxia. However, physiological process which is important for hypoxia-induced stem cell lipid metabolic and functional regulation still remains poorly understood. In this study, we investigated the role of mitophagy in hypoxia-induced lipid metabolism and functions, such as apoptosis and migration, and its related signaling pathway in umbilical cord blood-derived human mesenchymal stem cells (UCB-hMSCs). In our results, we presented that hypoxia reduced mitochondria marker expression in a time-dependent manner and increased mRNA and protein expression levels of mitophagy regulating proteins including BNIP3 and BNIP3L. We found that hypoxia-induced BNIP3 mainly regulates hypoxia-induced reactive oxygen species generation, ER stress marker CHOP expression and apoptosis. Silencing of BNIP3 expression by siRNA transfection inhibited hypoxia-induced de novo free fatty acid (FFA) synthesis, lipid metabolic enzyme expression and mTOR activation in UCB-hMSCs. In addition, we observed that BNIP3-silenced UCB-hMSC lost hypoxia preconditioning-induced cytoskeletal regulating protein expression and migration. Hypoxia-induced bnip3 mRNA expression is inhibited by hif-1α siRNA transfection. Furthermore, we confirmed that inhibition of FASN by cerulenin under hypoxia induced UCB-hMSC apoptosis, and blocked hypoxia preconditioning-stimulated migration. In mouse skin wound healing model, transplantation of hypoxia-preconditioned UCB-hMSC stimulated wound healing. Conversely, transplantation of BNIP3-silenced UCB-hMSC delayed wound healing. In conclusion, BNIP3-mediated mitophagy under hypoxia is an essential factor for FASN-induced FFA synthesis, which leads to anti-apoptosis and migration on UCB-hMSCs.

T1003

EFFECT OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS ON INTRINSIC NEUROGENESIS AFTER TRANSIENT GLOBAL CEREBRAL ISCHEMIA

Chung, Tae Nyoung, CHA Bundang Medical Center, CHA University School of Medicine, Seongnam-si, Gyeonggi-Do, Korea, South

Global cerebral ischemia due to ischemia/reperfusion injury is an important cause of poor neurologic outcome after cardiac arrest. Various attempts were tried to overcome serious global cerebral ischemia and to yield a better neurologic outcome, but mostly in vain. Stem cell is considered as one of the most desirable therapeutics for treating many incurable diseases. Effect of adipose-derived mesenchymal stem cells (MSC) on global cerebral ischemia was shown in recent research, which focused on neuroprotection effect of MSC in acute stage of injury (Stem Cells Transl Med 2015). We aimed to assess the effect of MSC on relatively delayed stage of global cerebral ischemia in terms of the effect on intrinsic neuro-regeneration. 7 min of electroencephalography-confirmed transient global cerebral ischemia was performed on four groups (2 groups of 4 and 2 groups of 3) of Sprague-Dawley rats using 2-vessel occlusion method with controlled exsanguination. For first two groups, human adipose derived MSC (1×10^6 cells) and placebo agent were injected intravenously for each group at immediate, 1, 2 and 3 weeks after the insult. Thymidine analog BrdU was intraperitoneally injected twice daily for four consecutive days starting 3 weeks and 3 days after the insult. The number of BrdU (+) cells was counted after sacrifice of the animals at 4 weeks passing the insult to assess intrinsic neurogenesis. For second two groups, human adipose derived MSC (1×10^6 cells) and placebo agent were injected intravenously for each group at immediate after the insult, and the count of live neurons on hippocampus CA1 was measured using immunostaining with monoclonal anti-NeuN antibody 6 weeks after the insult. Mann-Whitney U test was used to compare the count of BrdU (+) cells and live cells between two groups. Values were described as median (interquartile range). There was a significant difference in the count of BrdU (+) cells between MSC and placebo injected groups [p=0.043, 8.3 (5.3-13.1) vs 18.1 (11.5-34.5)]. However, there was no significant difference in the count of NeuN (+) cells between MSC and placebo injected groups (p=0.825). Administration of adipose-derived mesenchymal stem cells after transient global cerebral ischemia stimulates intrinsic neurogenesis.

Funding Source: This research was supported by the Basic Science Research Program through the National Research

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T1005

INTRAVITREAL ADMINISTRATION OF ADIPOSE-DERIVED MULTIPOTENT MESENCHYMAL STROMAL CELLS TRIGGERS A CYTOPROTECTIVE MICROENVIRONMENT IN THE RETINA OF DIABETIC MICE

Ezquer, Marcelo[1], Urzúa, Cristhian[2], Montecino, Scarleth[3], Leal, Karla[3], Conget, Paulette Andrea[3] and **Ezquer, Fernando**[3], [1]Instituto de Ciencias, Facultad de Medicina, Clínica Alemana-Universidad Del Des, Sanitago, Chile, [2]Departamento de Oftalmología, Facultad de Medicina, Universidad de Chile, Sanitago, Chile, [3]Center for Regenerative Medicine, Facultad de Medicina, Clinica Alemana-Universidad Del Desarrollo, Sanitago, Chile

Diabetic retinopathy (DR) is a common complication of diabetes and the leading cause of irreversible vision loss in the Western world. The reduction in color/contrast sensitivity due to the death of neural cells in the ganglion cell layer of the retina is an early event in the onset of DR. Multipotent mesenchymal stromal cells (MSCs) are an attractive tool for the treatment of neurodegenerative diseases, since they could differentiate into neuronal cells, produce high levels of neurotrophic factors and reduce oxidative stress. Our aim was to determine whether the intravitreal administration of adipose-derived MSCs was able to prevent the loss of retinal ganglion cells in diabetic mice. For this, diabetes was induced in C57BL6 mice by the administration of streptozotocin. When retinal pro-damage mechanisms were present, animals received a single intravitreal dose of $2x10^5$adipose-derived MSCs or the vehicle. Four and 12 weeks later we evaluated (a) retinal ganglion cell number (histology); (b) neurotrophic factor levels (RT-qPCR and ELISA); (c) retinal apoptotic rate (TUNEL); (d) retinal levels of reactive oxygen species and oxidative damage (ELISA); (e) electrical response of the retina (electroretinography); (f) pro-angiogenic and anti-angiogenic factor levels (RT-qPCR and ELISA) and (g) retinal blood vessels (angiography). Furthermore, 1, 4, 8 and 12 weeks post-MSC administration, the presence of donor cells in the retina and their putative differentiation into neural and perivascular-like cells were assessed (immunofluorescence and flow cytometry). We observed that MSC administration completely prevented retinal ganglion cells loss. Donor cells remained in the vitreous cavity and did not differentiate into neural or perivascular-like cells. Nevertheless, they increased the intraocular levels of several potent neurotrophic factors (NGF, bFGF and GDNF) and reduced the oxidative damage in the retina. Additionally, MSC administration has neutral effect on the electrical response of the retina and did not result in a pathological neovascularization. We demonstrated that the intravitreal administration of adipose-derived MSCs triggers an effective cytoprotective microenvironment in the retina of diabetic mice. Thus, MSCs represent an interesting tool in order to prevent DR.

Funding Source: Supported by grant FONDECYT 1130470 to Fernando Ezquer

T1007

REPARATIVE EFFECTS OF INTRATHECAL MSC-NP CELLS IN PATIENTS WITH MULTIPLE SCLEROSIS MEDIATED BY TROPHIC AND IMMUNOMODULATORY FACTORS

Tuddenham, John[1], **Harris, Violaine**[1], Vyshkina, Tamara[1] and Sadiq, Saud[2], [1]Tisch MS Research Ctr of New York, New York, NY, U.S., [2]Tisch Multiple Sclerosis Research Center of New York, New York, NY, U.S.

Multiple sclerosis (MS) is an autoimmune-mediated demyelinating disease of the CNS. Patients with progressive MS experience a steady worsening of neurologic function attributed to chronic demyelination and axonal loss. There are limited therapeutic options available to treat the neurologic disability caused by progressive MS. To address this unmet need, we are currently conducting a Phase I FDA-approved trial to evaluate safety, tolerability, and preliminary efficacy of a novel cellular therapy utilizing autologous bone marrow MSC-derived neural progenitors (MSCNP) administered intrathecally into patients with progressive MS. Preliminary efficacy data from the clinical trial suggests that treatment with MSCNPs results in improved motor strength and bladder function in a subset of patients, despite their established disability prior to the treatment. Pre-clinical studies have described the trophic and immunomodulatory effects of MSCNPs both in vitro and in the EAE animal model of MS. The purpose of the current study is to elucidate the therapeutic mechanism of action of MSCNPs through the identification of biological factors that correlate with clinical response. MSCNPs were isolated and expanded from bone marrow of MS patients. Analysis of gene expression of MSC-NPs demonstrated upregulation of a panel of candidate trophic and immunomodulatory factors, including HGF, IGF, LIF, VEGF, IL-11, and TGF-beta. Secreted protein levels in MSCNP conditioned media were quantitated by ELISA and by luminex assays and correlated with promotion of neurogenesis/oligodendrogenesis in an in vitro co-culture assay with neural stem cell lines. To explore whether the candidate MSCNP factors could serve as biomarkers of therapeutic response, we collected and banked cerebrospinal fluid from clinical trial patients at baseline and after each intrathecal treatment with autologous MSCNPs. Multiple factors, including HGF, were detectable in cerebrospinal fluid and correlated with clinical responses. These

results elucidate the trophic mechanisms of action of MSCNPs and identify novel biomarkers of stem cell-mediated neural repair.

T1009

B CELLS - A NEW PLAYER IN SOLID ORGAN TRANSPLANT PATIENTS INFUSED WITH BONE MARROW DERIVED MESENCHYMAL STEM CELLS

Kaundal, Urvashi[1,2], Ramachandran, Raja[1], Kanwar, Deepesh[1], Sharma, Ratti Ram[1], Bagai, Upma[2], Minz, Mukut[1], Jha, Vivekanand[1] and Rakha, Aruna[1], [1]PGIMER, Chandigarh, India, [2]Panjab University, Chandigarh, India

Studies directed towards identification of signature cell populations associated with graft rejection have revealed the role of humoral immune responses involving B cells as an imperative moderator, responsible for graft dysfunction. Immune responses triggered by B cells against transplanted solid organ can cause an Antibody-mediated rejection or a T cell-mediated rejection. However, certain subsets of B cells known as regulatory and transitional B cells have been explored and are linked with allograft tolerance. Therefore, for establishing long term allograft acceptance, there is a need of a therapy that can either minimise effector B cell population responsible for causing organ rejection or can expand the regulatory B cell population which would help in prolonging the graft acceptance. According to the ongoing research, Mesenchymal Stem Cells (MSC) therapy may prove as one such therapy which can enhance the graft acceptance without having any side effects which are associated with immunosuppressive (IS) drug therapy. Most of the literature, which suggests, MSCs as a potential therapy, is focussed on their immunomodulatory effect on the T cells which play a crucial role in allograft rejection. However, our understanding of the effect of MSCs on B cells is limited. In lieu of this, we conducted an in vivo study, which employed the infusion of BM-MSCs along with the standard IS drug therapy for Kidney Transplant (KTx) patients. Post sterility tests and phenotypinng, MSCs were infused into the patients in two doses. The harvested mononuclear cells at various time intervals were used in immunophenotyping for various B cell subsets. Flow Cytometric data revealed a decrease in the percent population of B cells in the patients who underwent MSC infusion as compared to the control group. Moreover, there was an increase in the Virgin Naïve B cells at 1 year time point for patients infused with MSCs. A decrease in early memory B cell subset was observed along with an increase in the un-switched memory B cell subset. Data from our experiments, is indicative of an alteration in B cell phenotype into a tolerogenic phenotype. This study will further be conducted for more KTx patients so as to have a better understanding of the mechansims involved in immunomodulation by MSCs.

Funding Source: Department of Science and Technology (DST), India

T1011

HUMAN PLACENTA-DERIVED MESENCHYMAL STEM CELLS RESTORE HEPATIC LIPID METABOLISM IN THE RAT BILE DUCT LIGATION MODEL

Lee, Yun Bin[1], Choi, Jong Ho[2], Kim, Eun Nam[1], Lee, Hyun-Jung[2], Hwang, Seong Gyu[1] and Kim, Gi Jin[2], [1]CHA Bundang Medical Center, CHA University, Seongnam-si, Korea, South, [2]CHA University, Seongnam, Korea

Mesenchymal stem cell (MSC) transplantation was determined to promote hepatic regeneration and reduce liver fibrosis. However, the influence of MSCs on hepatic lipid metabolism is not yet elucidated. We transplanted human placenta-derived mesenchymal stem cells (PD-MSCs) in bile duct-ligated rats to investigate whether alterations in lipid metabolism are restored. Serum biochemical analysis and liver histology were performed. The expression levels of enzymes involved in hepatic lipid metabolism were examined by quantitative real-time polymerase chain reaction analysis, immunoblot analysis, or quantitative enzyme-linked immunosorbant assay (ELISA) analysis. Bile duct proliferation and periductal inflammation were reduced by PD-MSCs transplantation. Elevation of serum levels of total bilirubin and alkaline phosphatase was attenuated in the transplanted rats. Moreover, serum lipid levels decreased, mainly in the fraction of low-density lipoprotein cholesterol and triglyceride. Fatty acyl-CoA synthetase concentrations measured by ELISA in liver tissues increased following PD-MSC transplantation, and the mRNA levels of long-chain acyl-CoA synthetase 1 and fatty acid transport protein 2 were also elevated. Whereas mitochondrial carnitine palmitoyltransferase 1a (CPT1a), a rate-limiting enzyme in the mitochondrial β-oxidation, expression at the mRNA level was augmented in the transplanted rats, its protein expression was suppressed. The expression levels of microRNA-33 (miR-33), which has been shown to posttranscriptionally regulate genes involved in fatty acid oxidation, were markedly higher in the transplanted rats, indicating that CPT1a expression is repressed by miR-33. These results suggest that the transplantation of PD-MSCs restores altered hepatic lipid metabolism in bile duct-ligated rats.

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T1013

IMPACT OF hMSCs ON SOLID TUMOR GROWTH IN NOVEL TUMOR/IMMUNE SYSTEM HUMANIZED MICE

Moquin-Beaudry, Gaël[1], Benabdallah, Basma[2], Desaulniers-Langevin, Cynthia[1,3], Palacio, Lina[1,3] and Beausejour, Christian[3], [1]CHU Sainte-Justine, Montreal, QC, Canada, [2]Chu Ste Justine, Laval, QC, Canada, [3]University of Montreal, Montreal, QC, Canada

Clinical trial approbation for novel therapies have been steadily decreasing since the 1990s. Nowadays, only about 10% of therapies entering phase I clinical trials will receive approval from regulation agencies, with most failing at the phase II trials. The picture is even worse for solid tumor oncology trials where barely 7% of therapies will result in final approval. This presents considerable health and financial concerns that need to be addressed. One of the potential reasons explaining such worrying numbers is the inability of current cancer pre-clinical models to properly simulate key cellular mechanisms of cancer biology. Among these, the impact of the patient's own immune system on tumor emergence, development and elimination is probably the most overlooked. To address this issue, we developed a unique, novel and flexible humanized tumor/immune system mouse model to study the implication of functional human immune cells in anti-cancer therapies. First, fibroblasts from a donor are harvested from a skin biopsy and transformed into tumor cells by modification with defined proto-oncogenes (hTERT, Ras^{V12}, SV40ER). Alternatively, fibroblast-derived iPSCs could be generated and differentiated into various cell types to form various tumor cell lines from a single donor. Peripheral blood mononucleated cells (PBMCs) allogenic or autologous to the donor are then obtained and injected along with the tumor cells in NOD/SCID/γc (NSG)/MHC-knockout mice. These mice allow for the study of tumor growth in presence of human mature immune cells while avoiding graft versus host disease (GVHD). With this model, we have studied the impact of human mesenchymal stromal cell (MSC) injection of on tumor growth in humanized mice reconstituted in an autologous and/or allogeneic setting. Our preliminary results revealed that allogenic tumors are efficiently rejected and that tumors show massive infiltration by CD8 T cells. Surprisingly, despite their well-known immune-modulatory properties in vitro, the injection of 10 million MSC per mouse did not interfere significantly with tumor growth and had little impact on the tumor immune infiltrate. Overall, we have generated a unique and versatile tumor/immune system mouse model and our results provide essential pre-clinical insight for the safe use of MSC cell therapies in humans.

T1015

CHARACTERIZATION OF A NOVEL PROGENITOR CELL WITH THERAPEUTIC POTENTIAL DERIVED FROM ADULT HUMAN INTERVERTEBRAL DISC TISSUE

Silverman, Lara Ionescu[1,2], Dulatova, Gala[1], Tandeski, Terry[1] and Foley, Kevin[1,2], [1]DiscGenics, Inc, Salt Lake City, UT, U.S., [2]University of Tennessee, Memphis, TN, U.S.

We have developed an in vitro process to generate progenitor cells from differentiated nucleus pulposus cells derived from the intervertebral disc, which we call discogenic cells. These cells have potential therapeutic utility in treating disc disease, as demonstrated by multiple preclinical studies in rabbits and pigs where new extracellular matrix was formed *in vivo*. The matrix-forming potential of the cells have been explored previously through histology and PCR. In this study, the stem properties of these plastic-adherent derived novel cells are characterized in order to better understand potential therapeutic mechanisms of action *in vivo*. For this study, discogenic cells were cultured in a pro-osteogenic, pro-chondrogenic and pro-adipogenic environment to assess multipotentiality. Next, expression of surface antigens classically associated with stemness (positive for CD105, CD73, CD90; negative for CD34, HLA-DR) were assessed on the discogenic cells. Additional surface markers Stro-1 and CD271 were assessed. Finally, RT-PCR was used to assess expression of Sox9 and NANOG. Results: Discogenic cells were successfully differentiated into the three lineage cell types, as evidenced by positive staining for hydroxyapatite, lipids and proteoglycan in each condition. Flow cytometry demonstrated positive (>85%) expression for CD105, CD73, CD90 and negative (<3%) expression for CD34 and HLA-DR. Also, the discogenic cells were negative (<10%) for Stro-1 and CD271. PCR showed an upregulation of Sox9 and NANOG expression during the culture process. In conclusion, discogenic cells satisfy key criteria for stemness, including plastic adherence, expression of certain surface markers, and multipotentiality. Further, PCR shows expression of genes that are associated with stem cells. Given that the discogenic cells also function as committed cells by producing extracellular matrix native to the nucleus pulposus, we define these novel cells as progenitors. We now plan to examine additional *in vivo* mechanisms of action related to stemness, such as anti-inflammatory properties and paracrine effects. Human trials are anticipated.

T1017

REGENERATIVE MEDICINE IS "GOING TO THE DOGS": TREATING EARLY ONSET PARKINSON'S DISEASE (EOPD) WITH ALLOGENEIC MESENCHYMAL STEM CELLS IN CANINE PATIENTS

Vulliet, Richard[1,2], Rosman, Pamela[1], Halloran, Mitch[2] and Tallon, Kelli[2], [1]ReGena-Vet Labs, Davis, CA, U.S., [2]University of California Davis, Davis, CA, U.S.

Lysosomal storage diseases (LSDs) are terminal neurodegenerative conditions characterized by the pathological accumulation of metabolic breakdown products and precipitated proteins. Most LSDs result from rare mutations found in both humans and dogs. Known breed-specific LSDs common to canine and human patients include: American Bulldogs (Cathepsin D), Border Collies (CLN5), English Setters (CLN8), Miniature Longhaired Dachshunds (CLN2, TPP1 or tri-peptidyl peptidase) and Tibetan Terriers (ATP13A2). The ATP13A2 mutation is also known as early onset Parkinson's Disease (eoPD) or the Park9 mutation. Signs of eoPD in dogs have been well described by Coates, Katz and co-workers at the University of Missouri. Affected dogs are normal at birth with the severity of clinical signs increasing with age. In the early stages, starting around 4 to 6 years of age, affected dogs become anxious, withdrawn and avoid novel situations. They lose vision and become dyskinetic. In advanced stages, they will seizure and exhibit other CNS deficits. All affected dogs demonstrate severe behavioral pathology in the advanced stages of the disease. Late stage eoPD is readily confirmed on necropsy with characteristic gross morphological and histological lesions. Affected Tibetan Terriers were recruited by following known pedigrees through dog clubs. All treated dogs were verified to have the ATP13A2 mutation by DNA testing. MSCs from normal dog bone marrow were confirmed to express normal ATP13A2 mRNA by PCR and were the cells were administered intravenously. We have treated nine TTs with confirmed eoPD. Clinical efficacy and behavioral improvement of the treated dogs was evaluated by attending veterinarians and owner observation. All improved in motor ability and sensory perception, but none returned to normal status. Affected dogs with naturally occurring single copy homozygous mutations represent excellent models for evaluating therapies of human genetic diseases.

MESENCHYMAL STEM CELL DIFFERENTIATION

T1021

DIFFEREN TIATION OF HUMAN DENTAL PULP STEM CELLS INTO DOPAMINERGIC NEURON-LIKE CELLS IN VITRO

Jung Yeon, Kim[1], Hyo-Jung, Lee[1], Na Hee, Yu[1], So Young, Chun[1] and Tae Gyun, Kwon[2], [1]Kyungpook National University, DAEGU, Korea, South, [2]Kyungpook National university Hospital, DAEGU, Korea

We investigated the potential of human dental pulp stem cells (hDPSCs) to differentiate into dopaminergic neurons in vitro as an autologous stem cell source for Parkinson's disease treatment. The hDPSCs were expanded in knockout-embryonic stem cell (KO-ES) medium containing leukemia inhibitory factor (LIF) on gelatin-coated plates for 3–4 days. Then, the medium was replaced with KO-ES medium without LIF to allow the formation of the neurosphere for 4 days. The neurosphere was transferred into ITS medium, containing ITS (human insulin-transferrin-sodium) and fibronectin, to select for Nestin-positive cells for 6–8 days. The cells were then cultured in N-2 medium containing basic fibroblast growth factor (FGF), FGF-8b, sonic hedgehog-N, and ascorbic acid on poly-l-ornithine/fibronectin-coated plates to expand the Nestin-positive cells for up to 2 weeks. Finally, the cells were transferred into N-2/ascorbic acid medium to allow for their differentiation into dopaminergic neurons for 10–15 days. The differentiation stages were confirmed by morphological, immunocytochemical, flow cytometric, real-time PCR, and ELISA analyses. The expressions of mesenchymal stem cell markers were observed at the early stages. The expressions of early neuronal markers were maintained throughout the differentiation stages. The mature neural markers showed increased expression from stage 3 onwards. The percentage of cells positive for tyrosine hydroxylase was 14.49%, and the amount was 0.526 ± 0.033 ng/mL at the last stage. hDPSCs can differentiate into dopaminergic neural cells under experimental cell differentiation conditions, showing potential as an autologous cell source for the treatment of Parkinson's disease.

Funding Source: This work was supported by a Biomedical Research Institute grant from Kyungpook National University Hospital.

T1023

INVESTIGATING THE EFFECT OF INJURY ON DERMAL STEM CELLS BEHAVIOR

Abbasi, Sepideh, Hagner, Andrew and Biernaskie, Jeff, University of Calgary, Calgary, AB, Canada

The dermal papilla (DP) is one of the two main mesenchymal compartments of the hair follicle (HF). We previously showed that a population of dermal stem cells (DSCs) contribute cells into the adult DP to maintain the normal cycling of HFs, and support the production of larger hair fibers. However, the influence of wounding at different hair-cycle stages on the behaviour of DSCs and their progeny has not been previously addressed. Here, we asked whether injury alters the behaviour of DSCs and if this is further influenced by hair-cycle stage. Male α-SMA-CreER^{T2}-ROSA^{YFP} mice received tamoxifen at postnatal day 3 and 4 to label DSCs and their progeny. Full-thickness excisions (8mm) were made on the dorsal skin during the 1st telogen, 2nd anagen, 2nd catagen and 2nd telogen. Skin was harvested at the next anagen, 15-34 day post-wounding. Our results show that anagen HFs around the wound bed with ≥1 YFP^{+} DP in the injured skin are not significantly different than the unwounded anagen HFs (P=0.417), and also when injured at different stages of hair cycle (P=0.374). However, there is a significant increase in recruitment of DSC progeny into the DP after injury (P<0.0001) compared to follicles in uninjured skin. This bias toward a DP fate only occurred after wounding at autonomous anagen, propagating anagen and competent 2nd telogen but not at catagen or refractory 2nd telogen (p<0.0001). In summary, injury modifies the fate of DSC progeny, biasing them toward recruitment into the DP in a hair-cycle dependent manner.

Funding Source: Faculty of Veterinary Medicine, University of Calgary

T1025

SIRTUIN-3 FAVORS ADIPOCYTE DIFFERENTIATION TO REGULATE SKELETAL HOMEOSTASIS IN AGING MALE MICE

Ho, Linh[1], Pan, Yong[2], Roth, Theresa[3], Tsu, Chia-Lin[4], Ng, ChePing[4], Hsiao, Edward[1], Verdin, Eric[4] and Nissenson, Robert[5], [1]University of California, San Francisco, San Francisco, CA, U.S., [2]Edison Pharmaceuticals, Mountain View, CA, U.S., [3]Novartis, Emeryville, CA, U.S., [4]Gladstone Institutes, San Francisco, CA, U.S., [5]SFVAMC-UCSF, San Francisco, CA, U.S.

The mechanisms by which post-translational modifications of Sirtuins and cellular metabolism regulate cellular potency and differentiation remain elusive. A possible role for Sirt3 in regulating the fate of bone marrow mesenchymal stem cells has not been investigated. Using Sirt3 overexpression (Sirt3Tg) and Sirt3 knockout (Sirt3KO) mice in a C57BL/6 background, we show that Sirt3 is a positive regulator of adipogenesis and a negative regulator of skeletal homeostasis. Sirt3Tg mice exhibited an increased number of adipocytes in the tibia when compared to WT mice. Bone marrow stromal cells (BMSCs) from Sirt3Tg mice displayed an enhanced ability to differentiate into Oil Red O positive adipocytes in contrast with BMSCs from Sirt3KO mice compared to BMSCs from control mice. Interestingly, adipocytes differentiated from Sirt3KO mesenchymal stem cells displayed reduced expression of macrophage colony-stimulating factor 1 (CSF1) and monocyte-chemoattractant protein-1 that are important for osteoclastogenesis. This data suggests lacking of Sirt3 reduced adipogenesis leads reduced mCSF1 and MCP-1 and thus reduced osteoclastogenesis. In fact, there was almost 2 fold increase in osteoclastogenesis (P<0.01) from Sirt3Tg and 2 fold decrease in osteoclastogenesis (P<0.05) from Sirt3KO bone marrow cells grown in the presence of CSF1 and receptor activator of nuclear factor kappa-B ligand (RANKL). Accordingly, there was a 2.5 fold increase in the number of tartrate resistant acid phosphatase (TRAP) positive cells/bone surface in Sirt3Tg mice compared with control (P< 0.03), indicating increased osteoclastogenesis. The increased adipogenesis and marrow adipose tissue in Sirt3Tg have an adverse effect on bone mass. Sirt3Tg male mice exhibited a significant reduction in cortical thickness at the tibio-fibular junction (TFJ) (0.201 ± 0.003 vs. 0.220 ± 0.004 mm in Sirt3Tg and control mice, respectively, P <0.05). In summary, the positive energetic effects of Sirt3 in bone marrow cells are associated with increased adipogenesis and increased osteoclastogenesis resulting in a negative effect on bone mass. These findings demonstrate that Sirt3 is an important regulator of adipogenesis and skeletal homeostasis in vivo, and identify Sirt3 as a potential target for the treatment osteoporosis.

T1027

AUTOPHAGY IS ESSENTIAL IN ODONTOGENIC DIFFERENTIATION DURING REPARATIVE DENTIN FORMATION

Kim, Won-Jae, Jung, Ji-Yeon, Park, Sam-Young, Woo, Su-Mi, Cho, Heui-Seung and Seong, Kyung-Ju, Chonnam National University, Gwangju, Korea, South

Autophagy plays an important role in cell survival or death pathway. However, there is little information about the role of autophagy in odontogenic differentiation of dental pulp cells (DPC). The present study is aimed to establish whether autophagy is involved in odontogenic differentiation during reparative dentin formation of DPC. For in vivo experiment, the tooth cavity preparation models were set on Sprague-Dawley rats. After tooth cavity preparation, odontogenic differentiation and reparative

dentin formation were induced. Also, immunofluorescence intensity of dentin matrix protein 1 (DMP1) and dentin sialoprotein (DSP), which are odontogenic differentiation markers, were increased. The intensity of autophagy markers LC3B and p62 were enhanced also. By intra-peritoneal injection of autophagy inhibitor 3-methyladenine (3MA), despite tooth cavity preparation, expressions of LC3B, p62, DMP1 and DSPP were not increased and induction of reparative dentin was not shown. After that, to investigate detail regulation of autophagy-related genes in odontogenic differentiation of DPC, qPCR array was performed. In the tooth 8 days after tooth cavity preparation, up- and down-regulation of various autophagy-related genes was detected. Especially, some of ATG8 family gene levels were shown much higher fold changes than others. To investigate expression pattern of ATG8 family genes in odontogenic differentiation in vitro, human dental pulp cells (HDPC) were isolated from human dental pulp tissues and cultured in differentiation inductive medium (DM). MAP1LC3C level selectively increased more than others in DM. MAP1LC3C and GABARAPL2 levels were decreased by 3MA.

Taken together, these findings suggest that autophagy plays crucial role in odontogenic differentiation during reparative dentin formation of DPC.

Funding Source: Medical Research Center in Republic of Korea

T1029

A NEW SERUM-FREE MEDIUM FOR MAINTAINING THE STEM CELL CHARACTERISTICS OF HUMAN PERIODONTAL LIGAMENT STEM CELLS

Murabayashi, Dai[1], Tamaki, Yuichi[1], Sato, Kazutoshi[2] and Nakahara, Taka[1], [1]School of Life Dentistry at Tokyo, The Nippon Dental University, Chiyoda-ku, Japan, [2]Department of Regenerative Medicine and Stem Cell Biology, University of Tsukuba, Tukuba-shi, Japan

Introduction: We previously isolated and characterized periodontal ligament stem cells (PDLSCs) derived from human wisdom teeth using a culture medium containing fetal bovine serum (FBS). However, the allergenic potential and the viral contamination caused by FBS pose a barrier to transplantation, and consequently lead to the introduction of cell therapy methods in clinical applications. In the present study, we developed a FBS-free medium (FFM), designated MSC-T4, and examined whether the new media maintains or stimulates the proliferative potentials and the multidifferentiation capacity of PDLSCs. Materials and methods: The PDL tissues were gently separated from the extracted human wisdom tooth of healthy volunteers aged 27–32 years, and they were digested by collagenase/dispase-based enzymes. Isolated cells were cultured in either FFM or DMEM/F12 with 15% FBS (SCM) up to passage 3. Cells were subjected to proliferative evaluation, RT-PCR, flow cytometry, and multi-differentiation induction experiments into osteogenic, adipogenic, and chondrogenic lineages. Results: FFM cells exhibited significantly greater growth than that of SCM cells ($P < 0.01$) based on growth curve examination. Population doubling time was 22.5 and 30.6 hours in FFM cells and SCM cells, respectively. Cell cycle analysis revealed that FFM cells contained a significantly higher incidence of cells in the G2/M phase and a significantly lower incidence of cells in the G0/G1 phase compared to those of SCM cells ($P < 0.01$). Both PDLSCs cultures were positive for CD44, CD90, and CD105 by flow cytometry analysis, and expressed genes of osteogenic (Vimentin, Runx2, and Type I collagen), neurogenic (Nestin), and stem cell markers (Nanog, Oct3/4, and Sox2)—as determined by RT-PCR. FFM cells showed multidifferentiation capabilities equivalent to those of SCM cells. Conclusions: These results suggest that the MSC-T4 medium can maintain the stem cell phenotype and multidifferentiation potentials, and promotes stem cell proliferation compared to the SCM. This serum-free medium will contribute to the development of cell-based therapies using PDLSCs.

T1031

TET-MEDIATED REGULATION OF CHONDROGENIC DIFFERENTIATION VIA 5hmC DEPOSITION

Smeriglio, Piera, Grandi, Fiorella Carla, Taylor, Sarah and Bhutani, Nidhi, Stanford University, Stanford, CA, U.S.

Cartilage development is a tightly regulated process that entails proper proliferation and stepwise differentiation of mesenchymal stem cells into chondrocyte progenitors and differentiated chondrocytes. Deregulation of this process is linked to pathological cartilage and bone structure, leading to dwarfism, chondrodysplasia and skeletal deformities in fetuses and children. While the key regulators for the cartilage development have been defined, there is limited understanding of how these factors are regulated by epigenetic modifications namely DNA methylation. Recent reports demonstrated that methylated cytosines (5mC) can be further oxidized to hydroxymethylcytocines (5hmC) by the Ten-eleven-translocation enzymes (TET1, 2 and 3) leading to DNA demethylation via DNA repair. We therefore sought to explore the role of the TET enzymes and its derivative in cartilage development. Our global mapping of 5hmC distribution showed that dynamic changes of 5hmC levels are essential to cartilage differentiation in vitro and in development in vivo. In particular, stable accumulation in 5hmC was observed in specific subsets of genes required in embryonic development including cartilage lineage-specific genes. We demonstrated that stable 5hmC enrichment was strong-

ly associated with activated genes indicating a potential regulatory role for DNA hydroxymethylation in chondrogenic gene expression. Moreover, loss of TET1 in chondrocyte progenitors, in vitro and in vivo, decreased 5hmC levels and impaired differentiation suggesting a functional role for TET1-mediated 5hmC dynamics in chondrogenic differentiation. Through genome-wide gene expression analyses, we identified novel TET1 targets that regulate chondroprogenitor differentiation and are known to contribute to several skeletal diseases. Our findings indicate a novel role for TET1, 5hmC deposition and the underlying molecular mechanism critical to chondrogenic differentiation during skeletal development. Furthermore, we may be able to exploit epigenetic reprogramming through TET1 function as means to enhance chondrogenic gene expression, leading to proper cartilage development.

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MESENCHYMAL CELL LINEAGE ANALYSIS

T1035

ASSOCIATION OF LINE-1S EXPRESSION WITH APOBEC3B GENOTYPES IN HUMAN MESENCHYMAL STEM CELLS

Kono, Ken, Sawada, Rumi and Sato, Yoji, National Institute of Health Sciences, Tokyo, Japan

Long interspersed nuclear element 1s (LINE-1s) are retrotransposons that comprise approximately 17% of the human genome. Although most of LINE-1s have been rendered inactive by mutations, the average human genome is estimated to contain 80-100 retrotransposition (RTP)-competent LINE-1s. LINE-1s are considered to retrotranspose in the germ line or during early development, contributing to the genetic diversity. On the other hand, uncontrolled RTP induces genome instability and may cause genetic disorders and cell transformation. LINE-1s mRNA is known to be expressed in embryonic stem cells and induced pluripotent stem cells, and we previously demonstrated that LINE-1s mRNA was also expressed in human mesenchymeal stem cells (hMSCs). 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 and the *A3B* deletion is more common in East Asians and Amerindians (frequency of 36.9% and 57.7%) compared with Africans and Europeans (0.9% and 6%). In the present study, we examined the association of LINE-1s expression with *A3B* genotypes in Japanese MSCs. First, we analyzed *A3B* genotypes in 25 Japanese MSC 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 (deletion frequency is 26%). Next, we examined the relation between *A3B* genotypes and LINE-1s mRNA expression, but no relation was found. However, RNA-seq analysis indicated that LINE-1s that have high homology to RTP-competent LINE-1s were highly expressed in hMSCs from *A3B* deletion homozygotes compared with WT homozygotes. These results indicated that *A3B* genotypes affect LINE-1s RTP and the genome integrity was thought to be threatened in *A3B* deletion homozygotes.

T1037

PLATELET-DERIVED GROWTH FACTOR RECEPTOR BETA IDENTIFY MESENCHYMAL STEM CELLS WITH ENHANCED PRO-ANGIOGENIC PROPERTY

Wang, Shan and Wu, Yaojiong, Tsinghua University, Shenzhen, China

Mesenchymal stem cells (MSCs) are heterogeneous with respect to phenotype and function in current isolation and cultivation regimes, which often lead to incomparable experimental results. Flow cytometry analysis showed that approximately 70% of MSCs derived from human bone marrow and 25% of MSCs derived from human placenta in early adherent culture expressed platelet-derived growth factor receptor beta (PDGFR-β) on the surface. In consideration of the potent role of PDGF signaling, we compared the biological behavior and therapeutic effect in wound healing of MSCs with and without surface expression of PDGFR-β. Culturing expanded MSCs derived from human placenta were separated into PDGFR-β^+ and PDGFR-β^- subpopulations by flow cytometry or immunomagnetic beads (MACS). PDGFR-β^+ MSCs exhibited greater proliferating rates and generated more colony-forming unit-fibroblast (CFU-F), compared to PDGFR-β^- MSCs. Notably, PDGFR-β^+ MSCs expressed higher levels of pro-angiogenic factors such as VEGF, Ang1, Ang2 and bFGF. To examine their effect in wound healing, one million MSCs, which were pre-labeled with green fluorescence protein (GFP) gene, were topically applied into excisional wounds in Balb/C mice. PDGFR-β^+ MSCs actively incorporated into the wound tissue, resulting in more MSCs sustained in the wound as determined by flow cytometry at day 7 and accelerated wound closer; meanwhile, PDGFR-β^- MSCs tended to remain on the top of the wound bed with significantly fewer cells in the wound, suggesting enhanced chemotactic migration and engraftment of PDGFR-β^+ MSCs. Real-Time PCR and immunostain analyses revealed that the expression of PDGF-B was up-regulated after wounding; transwell migration analysis showed 8-fold more PDGFR-β^+ MSCs responded to PDGF-B. Intriguingly, PDGFR-β^+ MSCs-treated wounds showed significantly enhanced angiogenesis in the wound compared to PDGFR-β^- MSCs- or vehicle- treated wounds. Thus our results

indicate that PDGFR-β identify a specific subnet of MSCs with superior effect in promoting angiogenesis and tissue repair, implying enhanced therapeutic potential for certain diseases.

Funding Source: Mesenchymal stem cells (MSCs) are heterogeneous with respect to phenotype and function in current isolation and cultivation regimes, which often lead to incomparable experimental results. Flow cytometry analysis showed that approximately 70% of MSCs deriv

HEMATOPOIETIC CELLS

T1039

IN VIVO SCREEN NOMINATES VALPROIC ACID AS PHARMACOLOGIC ENHANCER OF HEMATOPOIETIC STEM CELL ACTIVITY AND INDUCES HSC-MSC INTERACTION DURING EX VIVO EXPANSION

Arulmozhivarman, Guruchandar[1], Kräter, Martin[2], Wobus, Manja[2], Stöter, Martin[3], Friedrichs, Jens[4], Mueller, Katrin[2], Bickle, Marc[3], Shayegi, Nona[5], Stölzel, Friedrich[2], Bonin, Malte[2], Ehninger, Gerhard[2], Brand, Michael[6] and Bornhäuser, Martin[2], [1]University Hospital Carl Gustav Carus, Dresden, Dresden, Germany, [2]Medical Clinic and Policlinic I, University Hospital Carl Gustav Carus, Dresden, Germany, [3]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany, [4]Leibniz Institute of Polymer Research, Max Bergmann Center of Biomaterials Dresden, Dresden, Germany, [5]Universitätsklinikum, Essen, Germany, [6]BIOTEC/CRTD, Technische Universität Dresden, Dresden, Germany

The identification of compounds which either increase the number and/or enhance the activity of hematopoietic stem and progenitor cells (HSPCs) could improve the clinical outcome after autologous and allogeneic hematopoietic stem cell transplantation (HSCT). The zebrafish represents a versatile vertebrate model allowing to study the regulation of HSPC development during embryogenesis and adulthood. From our semi-automated chemical screen we identified few modulators of HSPC activity by using transgenic (cmyb:EGFP) zebrafish embryos. One identified hit was valproic acid (VPA) which significantly increased the number of hematopoietic precursors. To investigate the potential of VPA as an ex vivo modulator of human HSPCs, we isolated cells from mobilized peripheral blood (PB) of healthy donors using CD34+ microbeads. The cells were treated with VPA in either single-or co-culture with human mesenchymal stromal cells (MSC) for 5 days and were subsequently analyzed phenotypically and functionally. VPA increased the percentage of CD34+CD90+ cells up to 60% compared to controls which showed only 2% of double positive cells and generated 3 fold higher absolute cell number of CD34+ and CD34+CD90+ cells. In a static adhesion assay, 3 fold more VPA treated CD34+ cells attached to the MSC layer compared to control cells. FACS and real-time PCR for various adhesion molecules revealed a 30% induction of CD146 protein expression as well as significantly higher mRNA levels in VPA treated cells compared to controls. This finding led us to quantify the adhesive property of cells using AFM-SCFS. Interestingly, detachment forces of VPA treated cells were 3 fold higher on MSCs compared to controls. Finally, an equivalent number of re-isolated CD34+ cells of VPA treated and control samples were transplanted by retro-orbital injection into NSG mice. In all recipient mice the same amount of PB chimerism was detected until 12 weeks suggesting that they retain their repopulating capacity in vivo after ex vivo expansion. Our data suggest that VPA and potentially other histone-deacetylase inhibitors may be used ex vivo to improve the expansion efficacy of mobilized PB HSPCs. Whether the induction of CD146 expression on HSC and its interaction with MCAM on MSC is functionally involved has to be addressed in loss-of function experiments.

T1041

IN HUMANS AS WELL, SELECTIVE EXPRESSION OF KAI1 ON HUMAN PRIMITIVE HEMATOPOIETIC STEM CELLS REGULATES THEIR QUIESCENCE BY INTERACTION WITH DARC

Chae, Cheong-Whan[1,2], Hur, Jin[3], Choi, Jae-Il[1], Kim, Tae-Won[1], Nham, Pniel[1], Jun, Jong Kwan[1], Baek, Sung Hee[4] and Kim, Hyo-Soo[4,5], [1]Seoul National University Hospital, Seoul, Korea, South, [2]Seoul National University Hospital, Seoul, Korea, South, [3]Seoul Natl University Hospital Division of Cardiology Department of Internal Medicine, Seoul, Korea, South, [4]Seoul National University, Seoul, Korea, South, [5]Seoul National University Hospital, Seoul, Korea, South

KAI1/CD82 marks murine long-term hematopoietic stem cells (LT-HSCs) and the molecule governs LT-HSC quiescence through direct interaction with DARC (Duffy antigen receptor for chemokines, CD234) expressed by macrophages in the bone marrow. Importantly, two different populations of human primitive hematopoietic stem cells ($Lin^-CD34^+CD38^-$ and $Lin^-CD34^-CD38^-CD93^+CD45RA^-$) derived from umbilical cord blood exhibited significant surface KAI1 levels. Particularly, KAI1 was predominantly expressed on dormant HSCs while much less was expressed on actively cycling HSCs and precursors. As in mouse, DARC was mainly expressed by human monocytes/macrophages and maintained KAI1 expression on human HSCs, thereby downregulating cell cycle progression of HSCs.Recombinant human DARC protein also preserved surface CD82 expression and dormancy of HSCs. To sum up, KAI1 is not only a marker for human primitive

HSCs but also a suppressor of HSC cell cycle progression. The KAI1/DARC axis represents a new strategy to make HSC therapies available for broader spectrum of patients (e.g. *ex vivo* expansion of umbilical cord blood).

T1043

BIOMECHANICS OF THE MURINE BONE MARROW NICHE IN HEMATOPOIETIC STEM AND PROGENITOR CELL FATE DECISIONS

Edwards, Nathan C.[1], Weaver, Valerie[2] and Passegué, Emmanuelle[1], [1]University of California, San Francisco, San Francisco, CA, U.S., [2]Department of Anatomy and Department of Bioengineering and Therapeutic Sciences, Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research and Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, U.S.

Many stem cells, including embryonic, mesenchymal and muscle stem cells, are influenced by the biomechanical properties of their surrounding microenvironment such as the extracellular matrix (ECM) and environmental elasticity. Recent studies have shown that biomechanical forces may also affect the activity of hematopoietic stem cells (HSCs). However, little is known about how the biomechanical properties of the bone marrow (BM) niche regulate HSC function. We are interested in comparing the long-lived self-renewing HSCs with the short-lived, myeloid-lineage committed granulocyte macrophage progenitor (GMP) to determine how these two distinct hematopoietic populations with different fate potential and self-renewal activity integrate biophysical cues. At steady state, HSCs preferentially localize to two distinct niches, bone endosteum and perivasculature, while GMPs are found scattered throughout the BM cavity including the central marrow. In vitro adhesion analyses indicate that HSCs and GMPs rely essentially on fibronectin (Fn) for adhesion, the most abundant ECM protein found everywhere in the BM cavity. GMPs, but not HSCs, also adhere to Collagen I, an ECM found solely at the endosteum where GMPs preferentially expand during myeloid regeneration. Preliminary scanning electron microscopy analyses reveal that after 3hr incubation on Fn, HSCs form surface structures similar in appearance to lymphocyte microvilli, while GMPs do not, directly suggesting that HSCs and GMPs sense and respond differently to the same biophysical cues. We are now exploring the significance of this observation, and using atomic force microscopy as well as various immunofluorescence staining approaches to create a biomechanical map of the elastic modulus and ECM composition of the endosteum, perivascular space and central marrow. We are also employing biomimetic 2D polyacrylamide gels (PAGs) to model the elastic properties of these BM environments. Our initial testing indicates that increasing stiffness stimulates HSC proliferation and differentiation while, conversely, decreasing GMP proliferation and differentiation. Our results will provide the first characterization of the physical features of distinct BM niche microenvironments, and their role in controlling HSC and progenitor fate decisions.

T1045

MiR-221 AND MIR-222 REGULATE HEMATOPOIETIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS BY TARGETING C-KIT

Heo, Hye-Ryeon[1], An, Borim[1], Kim, Eunbi[1], Yang, Se-Ran[2] and Hong, Seok-Ho[1], [1]Kangwon National University, Chuncheon, Korea, South, [2]Kangwon National University, Chuncheon, Korea

Hematopoietic development of human pluripotent stem cells (hPSCs) is governed by multidimensional regulatory mechanisms that include environmental factors as well as transcriptional and translational regulation. In recent years, a growing body of evidence suggests that microRNAs (miRNAs) are involved in regulating the self-renewal and differentiation of hPSCs. Meanwhile, stem cell factor (SCF) exerts its biological functions by binding to the receptor tyrosine kinase, c-KIT and plays a critical role in the hematopoiesis during embryonic development. In this study, we sought to investigate whether miRNAs can regulate hematopoietic development of hPSCs by targeting c-KIT as well as SCF can enhance hPSC-derived hematopoiesis. Flow cytometry analysis revealed that about 30% of undifferentiated hPSC cultures expressed c-KIT and were highly enriched with OCT4(+) and SSEA-3(+) fractions as compared with their negative fractions. Supplementation of SCF with other hematopoietic growth factors during hPSC hematopoietic development significantly augmented output of hematopoietic progenitors (CD34+CD45+) and mature blood (CD34-CD45+) cells. Interestingly, single treatment of SCF showed an increased output of hematopoietic progenitor cells, which was suppressed by treatment of Glivec (c-KIT inhibitor). In addition, we found that knockdown of miRNA-221 and miR-222 enhanced hematopoietic induction efficiency. These results suggest that SCF/c-KIT signaling play a key role in the generation of hematopoietic progenitors from hPSCs, which are regulated by modulating of specific miRNAs targeting c-KIT transcripts at the translational level.

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T1047

DUAL ROLES OF MUCIN-TYPE O-GLYCAN IN MAINTENANCE OF HEMATOPOIETIC STEM CELLS IN DROSOPHILA

Itoh, Kazuyoshi[1], Fuwa, Takashi[2], Kinoshita, Takaaki[2], Nishida, Hiroshi[2] and Nishihara, Shoko[2], [1]Graduate School of Engineering, Soka University, Hachioji-shi, Japan, [2]Soka University, Graduate School of Engineering, Hachioji, Japan

Hematopoietic stem cells (HSCs), which exist in an undifferentiated state, possess the ability to differentiate into mature blood cells. Disrupted activity of the core 1 ß1,3-galactosyltransferase 1 (C1GalT1), which synthesizes T antigen, one of major mucin-type *O*-glycans, causes thrombocytopenia and hemolytic anemia in Tn syndrome. It has been suggested that these phenotypes are partially attributed to defective HSCs. We previously reported that C1GalT1 mutants display excessive differentiation of HSCs in the lymph gland (the Drosophila larval hematopoietic organ). However, the precise roles of T antigen in hematopoiesis are unknown. Here, we show dual roles of T antigen in the lymph gland. The lymph gland contains three domains: (1) the medullary zone (MZ), which consists of hematopoietic stem cells (the HSCs); (2) the cortical zone (CZ), which contains differentiated blood cells; and (3) the posterior signaling center (PSC), which are responsible for transmitting signaling molecules to maintain the HSCs as the stem cell niche. T antigen was expressed in both the MZ and CZ. We found that HSC-specific knockdown of C1GalT1 expression promotes HSCs differentiation, demonstrating that T antigen, which is expressed in HSCs, is required for HSCs maintenance. Next, we found that C1GalT1 mutants display decreased length of filopodial extensions, which is required for HSCs maintenance, from the PSC cells. In addition, a shell-like structure was formed around the lymph gland of the C1GalT1 mutants owing to disruption of the hemolymph environment, which is attributed to hypoglycosylation of hemolectin, an orthologue of the human von Willebrand factor (VWF). This shell-like structure could physically inhibit the filopodial extensions from the PSC cells, leading to HSC differentiation. Finally, the differentiated blood cell-specific overexpression of C1GalT1 in the C1GalT1 mutant background restored the lymph gland morphology, filopodial extensions, and HSC population. These data demonstrate that T antigen, which is supplied from the differentiated blood cells, controls the HSC population through maintaining the hemolymph environment that supports filopodial extensions from the PSC cells. Thus, T antigen, which is expressed in both HSCs and differentiated blood cells, is essential for HSC maintenance.

T1049

INVESTIGATING THE MECHANISMS INVOLVED IN LONG DISTANCE REPRESSION BY SILENCERS IN HEMATOPOIESIS

Krueger, Shawn[1], Repele, Andrea[1], Bertolino, Eric[2], Reinitz, John[2] and Manu, Manu[1], [1]University of North Dakota, Grand Forks, ND, U.S., [2]University of Chicago, Chicago, IL, U.S.

During hematopoiesis, cell type is specified by gene regulatory networks (GRNs) whose structure and composition remain to be fully elucidated. One main organizing principle, termed cross-lineage antagonism, is that transcription factors (TFs) expressed in alternative lineages repress each other's expression. We investigated whether distal silencers mediate cross-lineage antagonism by binding TFs expressed in alternative lineages. Identifying and determining the function of *cis*-regulatory modules (CRMs) such as silencers is a challenging task because they can be regulated by multiple interacting TFs. To overcome the challenge posed by complex regulation, we developed a computational model that simulates the joint regulation of CRMs by multiple TFs. Our approach integrates multiple datasets, such as DNA sequences, estimates of TF concentrations, TF binding properties, and CRM activity to infer the TFs regulating CRMs at the resolution of individual binding sites. We investigated the cis-regulation of Cebpa, which encodes a TF necessary for myeloid development in PU.1-inducible estrogen receptor (PUER) cell lines. Using this methodology, we show that the *Cebpa* locus contains 9 silencers, which, in fact, outnumber enhancers. One putative silencer, lying 15kb downstream of the *Cebpa* transcription start site is predicted to be repressed by GATA2 and EBF1, TFs expressed in the erythroid-megakaryocytic and lymphoid lineages respectively. Further experiments testing the repression of this silencer by GATA2 and EBF1 are ongoing. We used the computational model to design mutations impairing GATA2- or EBF1- driven repression while avoiding off-target effects. We are utilizing a synthetic biology approach, Gibson Assembly, to make reporter constructs carrying the mutated CRMs. The activity of these mutant constructs will be assayed in PUER cell lines and G1ME cell lines, representing the myeloid lineage and the erythroid-megakaryocytic lineages respectively. These sequence-level tests will be complemented with knockdown experiments that test model predictions in trans. Establishing the silencer-driven mechanisms of cross-lineage antagonism will help us understand the structure of GRNs involved in hematopoiesis and pave the way for predictively modeling this important developmental phenomenon.

T1051

THE EFFECT OF MESENCHYMAL STEM CELLS ON MITOCHONDRIAL DNA COPY NUMBER OF EXPANDED UMBILICAL CORD BLOOD CD34+ CELLS

Mansouri, Fatemeh[1], Atashi, Amir[1] and Soleimani, Masoud[2], [1]Tarbiat Modares University, Tehran, Iran, [2]Tarbiat Modares University, Hematology Department, Tehran, Iran

Umbilical cord blood is a limited but rich source of hematopoietic and mesenchymal stem cells (UC-MSC). It has been used in medical treatments as it does not have limitations related to bone marrow stem cells such as invasive access. However, umbilical cord blood has limited stem cells; hence in vitro cell expansion is conducted in this regard. CD34+ stem cell culture in the vicinity of mesenchymal stem cells increases and improves the transplanted cells. It is demonstrated that long-term and short-term hematopoietic stem cells are different in term of mitochondrial content and metabolism. Long-term hematopoietic stem cells have fewer mitochondria. The number of mitochondrial DNA (mtDNA) copies of CD34+ cells in the vicinity of UC-MSC was examined in this study. Isolated CD34+ cells from umbilical cord blood were expanded in Stemline II serum-free medium containing TPO, SCF, Flt-3 ligand (routine expansion method) and also co-cultured on UC-MSC (MSC co-culture method). The total extracted DNA from CD34+ cells at day 7 was subjected to TaqMan Real-Time PCR analysis to assess mtDNA copy number. mtDNA copy number of CD34+ cells significantly increased in the routine expansion method versus MSC co-culture method (CD34+ cell prior to expansion: 214 copy per cell, expanded CD34+ cell : 517 copy per cell, expanded CD34+ cell on MSC : 388 copy pre cell) (p-value <0.001). Lower mtDNA copy number of expanded CD34+ cells co-cultured on MSC indicates that the cells are mainly long-term rather than short-term stem cells.

T1053

DARC+ MACROPHAGES MAINTAIN THE DORMANCY OF CD82+ LONG-TERM HEMATOPOIETIC STEM CELLS IN THE BONE MARROW

Nham, Pniel[1], Hur, Jin[2], Kim, Tae-Won[1], Choi, Jae-Il[1], Chae, Cheong-Whan[1], Jang, Hyunduk[1], Baek, Sung Hee[3] and Kim, Hyo-Soo[1], [1]Seoul National University Hospital, Seoul, Korea, South, [2]Seoul Natl University Hospital Division of Cardiology Department of Internal Medicine, SEOUL, Korea, South, [3]Seoul National University, Seoul, Korea, South

Among many types of mouse hematopoietic stem-progenitor cells, CD82/KAI1 is expressed exclusively by long-term hematopoietic stem cells (LT-HSCs), which is at the top of the hematopoietic hierarchy. In this study, we searched for binding partner of CD82 and investigated its influence on regulating HSC quiescence. Duffy antigen receptor for chemokines (DARC/CD234), the counter molecule of CD82, was found to be expressed by bone marrow (BM) macrophages located in the endosteal and arteriolar niches. Upon BM macrophage ablation, LT-HSCs lost direct contact with DARC, resulting in ubiquitination and endocytosis of surface CD82 molecules. Decreased surface CD82 level of LT-HSCs led to cell cycle entry and proliferation, which could be reversed by macrophage co-culture. Taken together, our findings highlight the importance of BM niche components – DARC^{+} macrophages – in maintenance of LT-HSC dormancy.

T1055

DIRECT CONVERSION OF FIBROBLASTS TO FUNCTIONAL MEGAKARYOCYTE PROGENITORS

Pulecio, Julian[1], Alejo, Oriol[1], Capellera Garcia, Sandra[2], Vitaloni, Marianna[1], Rio, Paula[3], Mejia, Eva[1], Caserta, Ilaria[1], Bueren, Juan A.[3], Flygare, Johan[2] and Raya, Angel[1], [1]CMRB, Barcelona, Spain, [2]Lund University, Lund, Sweden, [3]Ciemat, Madrid, Spain

Transfusion of platelets collected from blood donors is a potentially life-saving treatment for thrombocytopenia. Current sources of platelets for transfusion are frequently insufficient and are associated with risks of alloimmunization and blood-borne infection. These limitations could be addressed by the generation of autologous megakaryocytes (mK) derived in vitro from somatic cells. Here we demonstrate that the overexpression of a set of core transcription factors important for mK/erythroid lineage specification during development allows the direct fate conversion of human and murine fibroblasts to mK-like progenitors. We identified a core of 4 transcription factors, including Gata1 and c-Myc that directly and rapid-

ly converted fibroblasts into mK/erythroid progenitors. Addition of Gata2 and Runx1 to this core set of factors skewed the balance of transdifferentiation toward the megakaryocytic lineage, as judged by the appearance of CD41+ cells. These cells were isolated and cultured in conditions favoring mK maturation, giving rise after 7 days to mK-like cells with polylobulated nuclei, ploidies higher than 4N and colony-forming ability. We also found proplatelet-like structures with the capacity to form maturing platelets in vitro. Furthermore, injection of in vitro generated mK-like progenitor cells into NSG mice demonstrated successful engraftment and further maturation in vivo. The application of the same conversion protocol to human fibroblasts resulted in their transdifferentiation to CD41+/CD42+ mK-like cells showing hallmarks of bona fide megakaryocytes such as polyploidization, formation of proplatelet-like structures, and generation of CD41+ platelets-like-particles. Finally, we applied this approach in the context of hematological diseases, successfully obtaining mK-like cells from gene corrected fibroblasts from Fanconi anemia patients. Our results combined demonstrate that autologous functional mK can be obtained in vitro, addressing the current limitations of transfusing allogeneic blood cell progenitors and circumventing the use of pluripotent stem cells.

T1057

USE OF SMALL MOLECULES TO ENHANCE THE IMMUNOREGULATORY PROPERTIES OF HUMAN HSPCS FOR THE TREATMENT OF INFLAMMATORY AND AUTOIMMUNE DISEASES

Robbins, David[1], Tacke, Robert[1], Lai, Kevin[1], Rezner, Betsy[1], Ben Nasr, Moufida[2], Le, Thuy[1], Foster, Heather[1], Truong, Christopher[1], Raynel, Sarah[1], Sahaf, Newsha[1], Driver, Emily[1], Rosen, Jonathan[1], Sabouri-Ghomi, Mohsen[1], Lee, Tom[1], Guerrettaz, Lisa[1], Fiorina, Paolo[2], Parone, Philippe[1] and Shoemaker, Daniel[1], [1]Fate Therapeutics, Inc., San Diego, CA, U.S., [2]Boston's Children's Hospital, Harvard Medical School, Boston, MA, U.S.

Hematopoietic stem and progenitor cells (HSPCs) possess the ability to self-renew, proliferate, and differentiate into all types of mature blood cells. HSPCs have significant therapeutic potential both in the setting of hematopoietic stem cell transplantation as well as for the treatment of autoimmune or inflammatory diseases. Post infusion, HSPCs traffic to sites of inflammation via the CXCR4/SDF-1 axis where they can suppress cytotoxic T cells. Our genome-wide characterization studies of HSPCs in the presence of activated T cells shows significant upregulation of key immunoregulatory genes (e.g., PD-L1 and IDO1). Our goal is to upregulate key trafficking (e.g., CXCR4) and immunoregulatory pathways using pharmacological programming of HSPCs to enhance the therapeutic potential of these cells. We have previously demonstrated that ex vivo programming of HSPCs with 16,16-dimethyl prostaglandin E2 (dmPGE$_2$) enhances the expression levels of CXCR4, which results in improved migration to gradients of SDF-1. A combinatorial screening approach was used to identify additional small molecules, which synergize with dmPGE$_2$ to further increase the expression levels of CXCR4 and other immunoregulatory genes. Of the top 52 screening hits, 26 were glucocorticoids while no other class of molecules was significantly enriched. Treatment of HSPCs with the combination of dmPGE$_2$ and a glucocorticoid synergized to increase the levels of CXCR4 mRNA by 60-fold relative to vehicle controls. While treatment with a glucocorticoid or dmPGE$_2$ alone resulted in only a 3- and 20-fold increase in mRNA, respectively. Ex vivo programmed HSPCs also had increased expression levels of key immunoregulatory genes (e.g., PD-L1) as well as enhanced performance in an in vitro assay, which simultaneously measures migration and suppression. Finally, in vivo models demonstrated that HSPCs programmed with the combination of modulators had improved trafficking to sites of inflammation and suppressed T cell proliferation and effector function. We are currently evaluating the therapeutic potential of programmed HSPCs in a number of inflammatory and autoimmune diseases.

T1059

HEMATOPOIETIC STEM CELLS ARE PROTECTED BY NF-κB PRO-SURVIVAL SIGNALS FROM TNFα-DRIVEN NECROPTOSIS

Yamashita, Masayuki[1], Kohli, Latika[2], Warr, Matthew[2] and Passegué, Emmanuelle[2], [1]University of California, San Francisco, San Francisco, CA, U.S., [2]The Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, Department of Medicine, Division of Hematology/Oncology, University of California San Francisco, San Francisco, CA, U.S.

Hematopoiesis relies on the ability of hematopoietic stem cells (HSCs) to self-renew and differentiate to give rise to a spectrum of progenitor cells, including granulocyte-macrophage progenitors (GMPs), that, in turn, generate all lineages of mature blood cells. Blood production is also controlled by signals from the bone marrow (BM) microenvironment, especially the cytokine milieu, which can rapidly change in composition upon various insults and inflammatory stresses hence impacting on the survival and functionality of different blood cells. The prototypical death ligand TNFα is a prominent cytokine that is detected at abnormally high levels in the BM of patients with chronic inflammation and blood malignancies. However, how TNFα affects HSC and progenitor function remains controversial. Our *ex vivo* exposure experiments reveal that HSCs are resistant and GMPs sensitive to the killing effect by TNFα. Consistent with these findings, we

find that *in vivo* treatment with TNFα preserves the HSC pool, while significantly reducing the number of progenitors and mature myeloid cells. Furthermore, in the absence of TNFα, we observe a major loss of HSCs upon serial treatment with pIpC or 5-FU, indicating a protective role of TNFα in HSCs during inflammation and hematopoietic regeneration. Using both genetically engineered mutant mice and pharmacological inhibitors disrupting the various signaling pathways downstream of TNFα receptor, we demonstrate that HSCs resist the killing effect of TNFα due to a strong induction of the NF-κB pro-survival pathway, and die from necroptosis rather than apoptosis upon NF-κB blockade or removal of TNFα in inflammatory contexts. In contrast, we find that TNFα only weakly induces the NF-κB pro-survival pathway in GMPs, which essentially die from apoptosis rather than necroptosis. Our results demonstrate that a differential wiring of the TNFα pathway machinery supports blood homeostasis by providing pro-survival cues to HSCs, while eliminating more committed progenitors and mature blood cells. They also indicate that HSCs are protected by NF-κB pro-survival signals from TNFα-driven necroptosis, which has significant implications for the treatment of patients with chronic inflammation and blood malignancies.

T1061

SMALL MOLECULE INHIBITION OF RECEPTOR TYROSINE PHOSPHATASE SIGMA PROMOTES HEMATOPOIETIC STEM CELL REGENERATION

Zhang, Yurun[1], Quarmyne, Mamle[1], Himburg, Heather[2], Yan, Xiao[1], Jung, Michael[1], McBride, William[1] and Chute, John[1], [1]University of California Los Angeles, Los Angeles, CA, U.S., [2]UCLA, Los Angeles, CA, U.S.

The proliferation, differentiation and self-renewal of hematopoietic stem cells (HSCs) are regulated by receptor tyrosine kinases (RTK) such as c-kit, Flt-3 and Tie2. Receptor protein tyrosine phosphatase sigma (PTPσ), which counterbalances RTK signals in HSCs, has been shown to inhibit HSC regenerations in vivo. However, the clinical application of targeting PTPσ in HSCs to treat radiation injury has been impeded by the lack of PTPσ specific inhibitors. To search for modulators that can inhibit PTPσ signals, we performed a small molecule screening and identified 5483071 as a potential PTPσ inhibitor. In silico simulation also showed that small molecule 5483071 is docked into the binding site of PTPσ with rigid docking through hydrogen bonding and electrostatic interactions. Subcutaneous administration of 5483071 to sub-lethally irradiated mice significantly improved the survival rate compared to mice in control groups ($p=0.0007$). Treatment of irradiated BM HSCs with 5483071 in vitro displayed a 11-fold increase of myeloid progenitor cells (GEMM) colony formation ($p=0.002$). PTPσ$^{-/-}$ BM cells previously displayed higher expression levels of the activated Rac1 protein, which is a RhoGTPase that regulates HSC engraftment capacity. In vitro culture of 5483071 significantly increased the expression levels of activated Rac1 in PTPσ$^{+/+}$ Lin^{-} BM cells but not in PTPσ$^{-/-}$ Lin^{-} BM cells, which suggested the specificity of inhibitor 5483071 to PTPσ in vitro. Furthermore, treatment of BM HSCs with 5483071 in vitro significantly promoted cell cycle at steady state ($p=0.01$). Taken together, our results suggest that small molecule 5483071 functions as a specific inhibitor to PTPσ, and its modulations to PTPσ promote HSC regeneration via Rac1 activation and cell cycle regulation.

CARDIAC CELLS

T1065

NEONATES SERVE AS A BIOREACTOR TO GENERATE ADULT CARDIOMYOCYTES FROM PLURIPOTENT STEM CELLS

Cho, Gunsik and Kwon, Chulan, Johns Hopkins University, Baltimore, MD, U.S.

Pluripotent stem cells (PSCs) provide unprecedented opportunities for disease modeling and personalized medicine. However, PSC-derived cells exhibit fetal-like characteristics, and the immaturity remains a key challenge for their application. This is particularly important for diseases that manifest in the adult such as cardiomyopathies, Parkinson's disease, and Alzheimer's disease. Here, we show that PSC-derived cardiomyocytes (PSC-CMs) undergo full maturation when introduced into early postnatal hearts. The in vivo-incubated PSC-CMs were indistinguishable from adult CMs in morphology and formed the transverse tubular system, a structural hallmark of CM maturation. Functionally, they displayed sarcomere shortening and calcium transients of adult CMs. Moreover, the in vivo-incubated PSC-CMs recapitulated the phenotype of cardiomyopathies, allowing us to directly model adult heart disease. This study lays a foundation for understanding the maturation and pathogenesis of human CMs and opens a new avenue for PSC-based modeling and treatment of adult diseases.

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T1067

GENETIC REGULATION OF HUMAN CARDIOMYOGENESIS USING PLLURIPOTENT STEM CELL MODELS

Anderson, David[1,2], Kaplan, David[3], Bell, Katrina[1], Koutsis, Katerina[1], Haynes, John[4], Ng, Elizabeth S[1], Elefanty, Andrew George[1], Stanley, Ed[1], Mummery, Christine L.[5], Petrou, Steven[3,6] and **Elliott, David**[1], [1]Murdoch Childrens Research Institute, Melbourne, VIC, Australia, [2]University of Cambridge, Cambridge, U.K., [3]Florey Institute, Melbourne, Australia, [4]MIPS, Monash University, Parkville, Australia, [5]Leiden University Medical Center, Leiden, Netherlands, [6]University of Melbourne, Parkville, Australia

Congenital heart disease is the most common form of birth defect, with a prevalence approaching 1 in 100 children. Similarly, cardiovascular disease is a major cause of illness and death in the Western world. Although the etiologies underlying congenital heart disease and cardiovascular disease differ, the development of new treatments for either condition will depend on a detailed understanding of how the human heart is formed and how it functions at the cellular and molecular level. Human pluripotent stem cell (hPSC) derived cardiomyocytes are the only tractable platform for illuminating the fine detail of the genetic networks that control human cardiomyocyte cell biology. We have developed a cellular framework to investigate the genetic regulation of human cardiac cell lineage specification. A major focus has been to use genetically modified hPSCs to expressing fluorescent proteins from key lineage restricted transcription factor loci with cell surface markers to enrich for cardiovascular cell types ranging from emerging progenitors to committed cardiomyocytes. We are now utilizing these reagents to study heart development using differentiating hPSCs. In particular, we are examining the role of the cardiac transcription factor NKX2-5 during in vitro cardiac differentiation of hPSCs. Cardiomyocytes derived from *NKX2-5* null hPSCs fail to up-regulate VCAM1 and maintain expression of PDGFRA indicating that cardiomyogenesis is blocked. Furthermore, *NKX2-5* null cardiac monolayers display asynchronous contraction and individual mutant cardiomyocytes have altered action potentials. Molecular profiling (RNA-seq and ChIP-seq) identified a number of myogenic transcription factors in the NKX2-5 dependent transcriptional network. We are now dissecting the contribution of these transcription factors to cardiomyogenesis. Understanding the molecular details of *NKX2-5* function in a human model will assist in dissecting the pathogenesis of *NKX2-5* mutations that underlie congenital heart defects.

T1069

MULTI-PARAMETRIC ASSESSMENT OF DRUG EFFECTS ON CARDIOMYOCYTE PHYSIOLOGY USING HUMAN iPSC-DERIVED CARDIAC SPHEROIDS

Sirenko, Oksana[1], Carlson, Coby B[2], Mann, David[2], Hancock, Michael[2], Luke, Steve[1], Seshadri, Anish[1], **Hesley, Jayne**[1], Cromwell, Evan[3] and Gentry, Jason[1], [1]Molecular Devices, Sunnyvale, CA, U.S., [2]Cellular Dynamics, Madison, WI, U.S., [3]Protein Fluidics, Hayward, CA, U.S.

Cell models are becoming more complex in order to better mimic the in vivo environment and provide greater predictivity for compound efficacy and toxicity. There is an increasing interest in exploring the use of three-dimensional (3D) spheroids for modelling developmental and tissue biology with the goal of accelerating translational research in these areas. Such 3D models seek to span the gap between two-dimensional (2D) cell cultures and whole-animal systems, and can provide unique perspectives on the behavior of stem cells and developing tissues *in vitro*. Accordingly, the development of quantitative assays in higher throughput using 3D cultures is an active area of investigation. In this study, we have developed methods for the formation of 3D spheroids of cardiomyocytes organoids derived from human iPS cells. Using both high content and fast kinetic fluorescence imaging, we have measured the impact of various compounds on the beating rate and pattern of cardiac spheroids as monitored by changes in intracellular Ca^{2+} levels with calcium-sensitive dyes. The assay was optimized for HTS in 384-well plates and allows for the characterization of beating profiles by using multi-parametric analysis outputs such as beating rate, peak frequency and width, or waveform irregularities. Additionally, the impact of drug treatment on cell viability and mitochondrial integrity was evaluated by high content imaging. Here, we tested known cardioactive and cardiotoxic compounds, including a- and b-blockers, hERG inhibitors, ion channel blockers, as well as selected environmental toxins (e.g. pesticides and flame retardants). The assay was further characterized using a commercially available cardiotoxicity library representing even other classes of compounds, including anti-cancer drugs and kinase inhibitors. We compared the IC_{50} values for the 3D versus 2D models and demonstrated significant differences in the assay sensitivity to compound-induced effects. In conclusion, we have demonstrated that both assays reported here are suitable for HTS and show utility for evaluation of potential cardiotoxicity using in vitro 3D model systems formed with human iPSC-derived cardiomyocytes.

T1071

EFFECT OF BMP10 AND CROSSVEINLESS 2 ON CARDIOMYOCYTE DIFFERENTIATION IN DFAT CELLS

Jumabay, Medet[1], Niklasson, Katharine[1], Penton, Ashley[1], Saparov, Arman[2] and Bostrom, Kristina[1], [1]Division of Cardiology The David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, CA, U.S., [2]School of Science and Technology, Nazarbayev University, Astana, Kazakhstan

The formation of the heart, cardiomyogenesis, is highly regulated to ensure proper formation and function. The objective of this study was to examine aspect of bone morphogenetic protein (BMP) signaling in cardiomyogenesis. We used an adipocyte-derived multipotent cell model termed dedifferentiated (DFAT) fat cells which readily differentiate into cardiomyocytes. We treated DFAT cells with BMP10 and crossveinless-2 (CV2), a BMP inhibitor. BMP10 is expressed exclusively in the heart and activates the activin receptor-like kinase 1 (ALK1) which is a BMP receptor that induces expression of cardiogenic factors such as Nkx2.5 through the SMAD signaling pathway. We demonstrated that CV2 binds directly to BMP10 and inhibits BMP10 from initiating SMAD signaling using co-immunoprecipitation and a luciferase reporter gene assay. We showed that BMP10 treatment induced proliferation of DFAT cells using a ^{3}H-thymidine proliferation assay, whereas CV2 enhanced differentiation of DFAT cells into cardiomyocytes, as demonstrated by the induction of expression of cardiomyocyte markers by real-time PCR and immunostaining. The results indicated that BMP10 increased the number of early cardiomyocyte progenitor cells, whereas CV2 induced differentiation of DFAT cells into cardiomyocyte-like cells that were electrically active and beating. Taken together, these results supported a model where cardiomyogenesis occurs via a two-step mechanism where BMP10 increases the pool of cardiac progenitor cells, and CV2 inhibits BMP10 and promotes cardiomyocyte differentiation.

T1073

ENGINEERED FUNCTIONAL ANISOTROPIC AND 3-DEMITIONAL hiPS-DERIVED CARDIAC TISSUE-LIKE CONSTRUCT FOR DRUG ASSESSMENT AND CARDIAC DISEASE REPAIRING

Li, Junjun[1], Minami, Itsunari[1], Yu, Leqian[1,2], Shiba, Yuji[3], Nakatsuji, Norio[1], Liu, Li[1] and Chen, Yong[1,4], [1]Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto, Japan, [2]Department of Micro Engineering, Kyoto University, Kyoto, Japan, [3]Department of cardiovascular Medicine, Shishu University, 3-1-1 Asahi, Matsumoto, Japan, [4]Ecole Normale Supérieure, CNRS-ENS-UPMC UMR 8640, 24 Rue Lhomond, Paris, France

The development of human pluripotent stem cells derived cardiomyocytes (hPSCs-CMs) has opened up novel paths for the cardiac drug development and the myocardial regeneration. Despite the exciting progress, the immature and poorly organized CMs in two dimensional culture widely used in the present drug screening may not reflect the translational results of the *i*n vivo conditions. On the other hand, the state-of-art myocardial regeneration technologies, e.g., cell injection, cell sheet, still utilized hPSC-CMs in random formation and low organization, fundamentally different from those under in vivo condition, which may hamper the cardiac regenerative effect. To these regards, we aimed to develop a highly functional, matured and well-organized cardiac tissue-like constructs (CTLCs) as a better candidate for both drug screening and cardiac regeneration therapy. The CTLCs were obtained by cultivating high purity hPSC-CMs on aligned nanofibers made of poly (lactic-co-glycolic acid) (PLGA), a FDA approved biodegradable polymer. The CTLCs demonstrated up-regulated cardiac-related gene expression, enhanced electrophysiology signal and less arrhythmia over control, which proved the suitability of CTLCs for cardiac drug screening. The CTLCs were also used for engraftment modelling in vitro, showing rapidly coupling of isolated cardiac tissues and efficiently eliminating of re-entrant arrhythmias within scarred tissue. We thus proved CTLCs as a good candidate for cardiac drug screening and repairing of infarcted heart tissues.

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T1075

DEVELOPMENT OF A LARGE FORMAT ENGINEERED CARDIAC TISSUE FROM HUMAN IPS CELL-DERIVED CARDIAC CELLS

Nakane, Takeichiro[1,2], Masumoto, Hidetoshi[1], Tinney, Joseph[1], Yuan, Fangping[1], Kowalski, William[1], Ye, Fei[1], Sakata, Ryuzo[2], Yamashita, Jun[3] and Keller, Bradley[1,4], [1]Cardiovascular Innovation Institute, University of Louisville, Louisville, KY, U.S., [2]Kyoto University Graduate School of Medicine, Kyoto, Japan, [3]Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan, [4]University of Lousiville, Louisiville, KY, U.S.

We have been investigating linear-shaped engineered cardiac tissues (ECTs) using rat or chick embryonic cardiomyocytes, and recently adapted this approach to incorporate human iPS cell (hiPSC)-derived cardiovascular cells. We have now developed a large format ECT from hiPSC-derived cardiovascular cells relevant to large animal preclinical studies. We fabricated 20mm square tissue culture molds using Polydimethyl siloxane. Six million (6M) cells containing hiPSC-derived cardiomyocytes, endothelial cells, and vascular mural cells were mixed with collagen I and Matrigel. The cell/matrix mixture was poured onto the tissue molds and cultured for 14days to generate ECTs with various shapes. A mold with rectangular 7mm posts at staggered position brought a mesh (ME) ECT, on the other hand, molds without loading posts or with 16mm long parallel posts brought a patch sheet (PS) ECT or a multiple linear-bundled (ML) ECT, respectively. PS ECTs held the largest tissue area among the 3 ECT types (167.0±35.9mm2) indicating the least gel compaction process. Tissue area and bundle width were similar between ME and ML ECTs (89.3±10.4mm2 / 0.49±0.09mm vs 76.2±4.0mm2 / 0.49±0.04mm). Active stress was similar between ME and ML ECTs (0.31±0.12 vs 0.32±0.14mN/mm2). However, ME ECTs formed more uniform bundles indicating more homogenous distribution of cells compared to ML ECTs. We assessed the cell survival by staining with Ethidium homodimer III, and ME ECTs contained the lowest percentage of dead cells compared to ML or PS ECTs (ME vs ML vs PS: 3.5±2.2 vs 10.1±6.2 vs 45.8±16.8%, $p<0.001$) indicating that mesh structure facilitates cell survival and maturation. Next, we determined the effect of initial cell seeding number on construct maturation and function by comparing 6M, 9M, and 12M cells as well as 12M at a reduced total volume (i.e. higher cell density, 12MH). ECTs with increased cell number showed more dead cells (12M: 15.6±9.7, 12MH: 16.9±10.9%, $p<0.05$ vs 6M) and lower active stress (9M: 0.13±0.04, 12M: 0.098±0.031, 12MH: 0.15±0.08 mN/mm2, $p<0.01$ vs 6M) indicating that 6M would be favorable for viable tissue formation. Finally, we generated a larger (30x30mm) ME ECT and confirmed its scalability. This hiPSC-based ECT construction method may hold promises for further hiPSC-based preclinical and clinical cardiac regeneration.

T1077

USING DIFFERENTIAL INTERACTION PROTEOMICS ON IPSC-DERIVED CARDIOMYOCYTES TO UNRAVEL THE MOLECULAR BASIS OF BAG3-RELATED DILATED CARDIOMYOPATHY

Perez-Bermejo, Juan[1,2], Judge, Luke M.[2], Truong, Annie[2], Yoo, Jennie[2], Johnson, Jeffrey[1,2], So, Po-Lin[2], Krogan, Nevan[1,2] and Conklin, Bruce[1], [1]University of California, San Francisco, San Francisco, CA, U.S., [2]Gladstone Institutes, San Francisco, CA, U.S.

Genetic association studies are generating a wealth of information about genetic variants associated with disease, but the molecular mechanism underlying most of those mutations is still not understood. Protein interactions are a crucial determinant of gene function. We propose that the use of interaction proteomics on protein variants associated to disease may help us understand the functional impact of disease-related mutation (what processes is the protein associated with; which interactions are affected in disease), and also shed light on the observed genetics (e.g. how genes associated to a disease phenotype are connected; why only a subset of genes is pointed out by genetic studies). Due to the tissue-specific nature of protein expression and interactions, the interactome of disease-related proteins need be studied in a relevant cell type. We are using a combination of affinity purification/mass spectrometry (AP-MS), genome editing in human induced pluripotent stem cells, and directed differentiation into cardiomyocytes (iPS-CMs) to study the functional consequences of mutations in the BAG3 gene on human cardiac muscle. Multiple variants of BAG3 have been associated to DCM development with diverse manifestations. In addition, its muscle-specific localization and multi-domain structure suggest its involvement in multiple processes, with a special role in muscle cells. In order to test the mechanism of BAG3 function in iPS-CMs, we used genome editing technology to generate multiple isogenic disease lines. We are using 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. Functional studies of this allelic series will help 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.

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T1079

CRISPR/CAS9-MEDIATED GLA GENE KNOCKOUT CELL LINES AS THE FABRY DISEASE MODEL: THE CO-ADMINISTRATION EFFECTS OF MG132 ON THE INTRACELLULAR PHARMACOKINETICS OF THE RECOMBINANT HUMAN Α-GALACTOSIDASE A

Song, Hui-yung, Yang-Ming University, Taipei, Taiwan

Fabry disease (FD) is the second most common of lysosomal storage disorders in the world that results from mutational deficiency of α-galactosidase A (GLA) and characterized by progressive accumulation of globotriaosylceramide (GL-3), in various organs. Up-to-date, the only effective therapeutics for Fabry disease is the enzyme replacement therapy (ERT) which regularly administrates the recombinant human GLA (rhGLA) via intravenous injection to supplement the GLA enzyme activity of FD patients. However, the efficacy of ERT is limited by the low stability of rhGLA at physiological condition as well as the quick clearance rate of rhGLA in cell. In addition, lack of the appropriate in vitro cell model slow down the pharmaceutical studies for improving the ERT treatment. Therefore, it is worth to establish a FD cell model as the platform to screen the potential candidates for enhancing the rhGLA stability and prolonging its potency. In the present study, by using the CRISPR/Cas9-mediated gene editing technique, we have knocked out GLA gene in HEK-293T cells. The GLA-null HEK-293 cells are completely deficient of detectable GLA protein expression and enzyme activity that provided a clear background for pharmacokinetic study of the exogenous rhGLA administration. Following rhGLA treatment, the GLA protein expression and enzyme activity was remarkably elevated in the GLA-null cells. Subsequently, the rhGLA was decreased with time and had the half-life of 24 hr. To improve the rhGLA stability and potency, the MG132 was co-treatment with rhGLA for 24 hr in the GLA-null cells. Co-administration of 0.1 and 1 μM MG132 with rhGLA in the GLA-null cells significantly restored the GLA enzyme activity by 100 % and 150 %, respectively, compared to rhGLA alone. The residue rhGLA protein levels in the MG132-treated cells were two-folds higher than that in the vehicle control cells. Furthermore, in comparison with the treatment of rhGLA alone, co-treatment of MG132 and rhGLA resulted in more effective clearance of the lysosomal accumulated GL-3 in the FD patient derived iPS-cardiomyocytes. Collectively, the CRISPR/Cas9-mediated GLA-null HEK-293T cells could be the appropriate in vitro FD cell model for evaluating the intracellular pharmacokinetics of the rhGLA as well as for screening the candidates to prolong the rhGLA potency.

T1081

LARGE-SCALE MANUFACTURE OF HPS CELL-DERIVED CARDIOMYOCYTES WITH cGMP COMPLIANT SUSPENSION CULTURE SYSTEM

Ye, Jingjing[1], Hua, Giau[1], Liu, Jian-Chang[1], Chen, Danlin[1], Lin, Ziguang[1], Chai, Jing[1], Krishnan, Aparna[1], Patel, Jennil[1], Dang, Wei[1], Huang, Patricia[1], Shukla, Praveen[2], Gold, Joseph[2], Wu, Joseph[2], Chen, Vincent C.[1], Hsu, David[1] and Couture, Larry A.[1], [1]Beckman Research Institute of City of Hope, Duarte, CA, U.S., [2]Stanford University, Stanford, CA, U.S.

Human pluripotent stem cell (hPSC)-derived cardiomyocytes (CMs) are promising cell source for cell replacement therapy to treat cardiac diseases. The amount of cells required for transplantation has been estimated at a scale of billion cells per dose. To meet the need of a large quantity of hPSC-derived CMss for pre-clinical and clinical studies, a robust, scalable, and GMP-compliant manufacturing process to produce hPSC-CMs is essential. Previously we have established a robust, scalable and GMP-compliant hPSC suspension culture system to expand undifferentiated hPSCs in the form of aggregates in defined media. Using undifferentiated hPSC aggregates generated in this suspension culture, we have developed a process to directly differentiate the hPSC in aggregate form into CMs with small molecules in suspension. We have identified the critical parameters, including shear stress, cell aggregate size, concentrations and induction timing of small molecules, for cardiac differentiation in suspension. By optimizing these parameters, we were able to consistently differentiate hPSCs to >90% CM purity with cell yields of 1-2x10^6 CMs/mL using 125, 500, 1000, and 3000 mL spinner flasks. Our gene expression analysis showed that small molecules induced differentiation of hPSC in suspension into cardiac lineages in a fashion recapitulating sequential development of cardiac specification. CMs generated in suspension culture displayed typical striated structures and action potentials of ventricular-, atrial-, and nodal-like profiles, confirming the cardiac phenotypes. In summary, we have developed a robust and scalable process for large-scale manufacturing of hPSC-CMs in suspension culture. This suspension culture system enables seamlessly transition from hPSC expansion to CM differentiation in a continuous suspension culture under cGMP conditions. It not only provides a cost and labor effective process for large scale hPSC-CM production, but also serves as a foundation for CM manufacturing in large industrial-scale bioreactor, which will accelerate the advance of hPSCs research towards therapeutic applications.

MUSCLE CELLS

T1085

MATURATION AND SARCOMARE FORMATION OF SKELETAL MYOCYTES DIRECTLY PREPARED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS USING A SPHERE-BASED CULTURE

Jiwlawat, Saowanee, Van Dyke, Jonathan M, Smit-Oistad, Ivy and Suzuki, Masatoshi, University of Wisconsin-Madison, Madison, WI, U.S.

Skeletal muscle progenitor cells (also known as myogenic progenitors) have received much attention because of their potential for use in in vitro modeling and cell-based therapy for neuromuscular diseases. Human induced pluripotent stem cells (iPSCs) are a promising resource for propagation of human myogenic progenitors. Our group recently reported a unique protocol for the derivation of myogenic progenitors directly (without genetic modification) from human pluripotent cells using free-floating spherical culture. In this study, we determined how differentiation duration, culture surface coatings, and nutrient supplements in the medium could influence the formation of mature striated myofibrils with contractile sarcomeric units. We prepared myogenic progenitors from iPSCs (IMR-90) using our sphere-based culture method and terminally differentiated them into myotubes. We found that a terminal differentiation period of ≥6 weeks significantly enhanced maturity and number of multinucleated myotubes. Electron microscopy revealed that these myofibrils contained well-defined sarcomeric structures consisting of organized myosin and actin. Spontaneous contractions in myotubes were also observed and recorded by video capture. Further, culturing myogenic progenitors as 3-D muscle constructs facilitated terminal differentiation and formation of elongated mature myotubes. Lastly, we tested different supplements (B27 serum-free supplement vs. horse serum) and culture surface coating matrices (laminin vs. Matrigel) for terminal differentiation. While both Matrigel and laminin coatings showed comparable effects on muscle differentiation, B27 serum-free supplement in the differentiation medium significantly enhanced differentiation of iPSC-derived myogenic progenitors into myotubes compared to horse serum. iPSC-derived myotubes containing matured myofibrils are an attractive tool for muscle biology, allowing analyses of myofibril formation, sarcomeric assembly and functional contraction. Importantly, as patient-specific iPSC lines are currently available, our findings support the ability to create mature striated myofibrils *in vitro* from those cell lines for use in drug screening, and disease modeling for neuromuscular diseases.

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T1087

HIGH-RESOLUTION LINEAGE MAPPING OF MYOGENESIS IN VIVO BY SINGLE-CELL MASS CYTOMETRY

Porpiglia, Ermelinda[1], Bendall, Sean C[1], Samusik, Nikolay[1], Davis, Kara[1], Ho, Andrew[1], Cosgrove, Benjamin D.[2], Fantl, Wendy[1], Nolan, Garry[1] and Blau, Helen M.[1], [1]Stanford University School of Medicine, Stanford, CA, U.S., [2]Cornell University, Ithaca, NY, U.S.

Muscle regeneration is a dynamic process during which the state and identity of the cells involved changes over time. Adult muscle stem cells are the driving force in skeletal muscle repair and regeneration. Most are quiescent in healthy individuals and become activated in response to muscle injury. While the phenotypic identity of muscle stem cells has been previously established, muscle progenitor cells, which are at a stage in between the stem cell and the mature functional myogenic cell, have not yet been identified in vivo. Progenitor cells hold enormous potential as a platform to study the cellular behavior and molecular control of stem cell fate, to identify novel therapeutic targets for muscle diseases, and to develop cell therapy applications for regenerative medicine. However, a major roadblock in their identification has been a lack of tools to resolve cellular heterogeneity in skeletal muscle, underscoring the importance of single cell studies. We have capitalized on single-cell mass cytometry (CyTOF), a transformative technology that allows the discovery of novel subsets within a complex cell population, to capture stem cell fate decisions *in vivo*. We discovered novel cell surface markers that define a myogenic progression in vivo, by combining a high-throughput flow cytometry screen with CyTOF analysis of skeletal muscle in adult mice. Importantly, using new marker sets we identified previously unrecognized stem and progenitor cell subsets. We showed by lineage tracing experiments that these novel subsets originate from Pax7^{+} cells and exhibit distinct regenerative potential in vivo. Moreover, high dimensional CyTOF analysis in response to acute injury revealed the cellular and molecular dynamics of muscle regeneration at a level of resolution not previously possible.

T1089

REGULATION OF mRNA BINDING PROTEIN AUF1 EXPRESSION BY CTCF CONTROLS MUSCLE STEM CELL DIFFERENTIATION

Yang, Ming[1], Chenette, Devon[2] and Schneider, Robert[2], [1]NYU Medical Center, New York, NY, U.S., [2]New York University Medical Center, New York, NY, U.S.

Muscle stem cells, also known as satellite cells, are vital for muscle regeneration following wounding and for preservation of the regenerative potential of muscle with age. Understanding the key molecular mechanisms by which satellite cell fate is regulated and how satellite cells contribute to muscle maintenance with increasing age can advance our knowledge of the nature of myopathic diseases. Post-transcriptional regulation of mRNAs plays a crucial role in the control of the expression of genes that determine stem cell fate. The mRNAs encoded by some of the key genes in muscle stem cell determination are regulated by an AU-rich element (ARE), located in the 3' untranslated region (3'UTR), which controls their targeted rapid degradation. Our lab found that the ARE binding protein AUF1 (hnRNP-D) plays crucial role in regulating stem cell fate. In our auf1 knock out mouse model, we observed that auf1$^{-/-}$ mice undergo accelerated loss of skeletal muscle mass and develop severely increased muscle weakness with age. Our genome-wide mRNA analysis also showed that auf1 mRNA is strongly (>70 fold) and specifically increased in expression only with activation of satellite cells, suggesting that the regulation of AUF1 expression is important for satellite cell fate determination. Using a transcription factor-wide siRNA screen, we found that transcription factor CTCF is essential for auf1 expression. We also showed that CTCF binds to the promoter region of AUF1. Using mouse myoblast C2C12 cells as a model system, we found that CTCF is essential for transcriptional activation of AUF1 in this muscle myoblast system as well, and for myoblast differentiation in culture. Silencing CTCF results in down regulation of AUF1 protein and mRNA levels, and delay of myoblast differentiation. We demonstrate that CTCF regulates myoblast differentiation through AUF1, and restoration of AUF1 levels by ectopic expression in a CTCF silencing background rescues normal myoblast differentiation. Our results suggest that the CTCF/AUF1 axis is important in regulating muscle stem fate and could an important therapeutic target in late-onset myopathies and age-related sacropenia.

PANCREATIC, LIVER, LUNG, OR INTESTINAL/GUT CELLS

T1093

IDENTIFYING HINDGUT SPHEROID PROPERTIES CRITICAL FOR INTESTINAL ORGANOID FORMATION FROM HUMAN PLURIPOTENT STEM CELLS

Arora, Natasha[1], Imran Alsous, Jasmin[2], Guggenheim, Jacob[1], Asada, Harry[1], Wells, James M.[3], Shvartsman, Stanislav[2], Kamm, Roger[1] and Griffith, Linda[1], [1]MIT, Cambridge, MA, U.S., [2]Princeton, Princeton, NJ, U.S., [3]Cincinnati Childrens Hospital, Cincinnati, OH, U.S.

Given the proper environment and growth factors, human pluripotent stem cells (hPSCs) will differentiate into organoids. These are in vitro cell aggregates with three-dimensional structure, which closely mimics the in vivo tissue. A mechanistic understanding of early organoid formation is essential for transitioning these technologies into robust, high-throughput methods that are suitable for commercial and therapeutic applications. We investigated the differentiation of hPSC derived intestinal organoids, focusing on parameters of hindgut spheroid emergence that are critical for successful maturation into organoids. Spheroids or cell aggregates bud off of highly confluent hindgut cultures, only a portion of which mature into intestinal organoids over a four-week period. Using confocal microscopy and image analysis, we found that the spheroids are heterogeneous by several parameters; cell number varied from 33-569 cells per spheroid; at most 46% of spheroids formed an inner mass of cells with a clear separation of epithelial and mesenchymal cells; diameter of the inner mass ranged from 24-95 µm. We hypothesized that size, morphology, and mix of cell types were key parameters for maturation. We sorted spheroids based on diameter and presence of an inner mass using a system that positioned a glass capillary over the spheroids using three linear actuators (for x, y, and z) and gently aspirated the spheroid. The sorting system then dispensed the spheroid into the correct tube based upon size or morphology. Sorted spheroids were then embedded in matrigel and monitored for organoid formation. We discovered that only spheroids with a diameter greater than 75 µm matured into organoids and almost all spheroids with an inner mass are greater than 75 µm. These findings are an essential step to engineering a system that robustly and consistently produces high quality intestinal organoids, which have future use in drug discovery and development.

T1095

REPROGRAMMING OF MONOCYTES INTO HEPATOCYTE-LIKE CELLS: A PROMISING AUTOLOGOUS CELL BASED THERAPY FOR HEPATITIS B PATIENT

DAS, Barun[1], Bhattacharjee, Jashdeep[1], Sahay, Preeti[1], Jain, Kshama[1], Mishra, Alaknanda[1], Iyer, Srikanth[1], Kesarwani, Ashwani[1], Tyagi, Rohit[1], Sinha, Prakriti[1], Sharma, Disha[2], Scaria, Vinod[2], Nagarajan, Perumal[1] and Upadhyay, Pramod[1], [1]National Institute of Immunology, New Delhi, India, [2]Institute of Genomics and Integrative Biology, New Delhi, India

Cell based therapy has gained ground as an emerging trend for the treatment of liver diseases like acute liver failure, end stage chronic liver failure and non-metastatic liver cancer. The use of autologous cells would be even more advantageous for clinical purposes. Here, in this studies hepatocyte like cells (NeoHeps) have been generated from peripheral blood monocyte of Healthy donor blood (H) as well as Hepatitis B surface antigen (HBSAg) NAT positive blood (HNP) from chronic hepatitis patient in a two-step process. The first step commences with the priming of monocyte in the presence of recombinant cytokines to acquire a state of plasticity, termed as Reprogrammed Monocyte (RM). RM is then differentiated in the presence of recombinant growth factors to generate Hepatocyte like cells in vitro, which is free of exogenous genetic material. RM has been characterised at molecular and functional level. Genes responsible for chromatin remodelling complexes were found to be up-regulated. We have further studied the process of RM generation with respect to markers of monocyte, macrophage and pluripotency. The results suggest reprogramming of terminally differentiated monocyte in the first step followed by hepatic lineage specific re-differentiation through up regulating the cMET signalling pathway in the second step generating NeoHeps. NeoHeps from both the healthy blood and HNP blood have been extensively studied in phenotypic, molecular and functional level for different Hepatocyte specific markers suggesting its similarity more with hepatocyte than with monocyte. NeoHeps are found to have drug metabolising enzyme activity and its inductive potential by another drug suggesting its probable role in drug screening test system also. Moreover, the transplanted NeoHeps were able to engraft in partially hepatectomised NOD.CB17-Prkdc^{scid}/J mouse liver and secreted human albumin in serum. Interstingly, NeoHeps from HNP blood were found to be void of hepatitis B virus signifying the complete elimination of virus element during the culture. On the basis of above mentioned studies the NeoHeps derived from HNP blood seems to have the potential to be used in cell based therapy.

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T1097

HEPATOGENIC DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS ON COLLAGEN-COATED POLYETHERSULFONE NANOFIBERS

kabir-Salmani, Maryam[1,2], Mahmoodinia, Maryam[1], Soleimanpour-lichaei, Hamid Reza[1], Masoud, Soleimani[3] and Ardeshirylajimi, Abdolreza[4], [1]National Institute for Genetic Engineering and Biotechnology, Tehran, Iran, [2]Geneocell Ideal Co, Tehran, Iran, [3]Tarbiat Modares University, Tehran, Iran, [4]Stem Cell Technology Research center, Tehran, Iran

Induced pluripotent stem cells (iPSCs) have attracted many surgeons and scientists' attention for cell replacement therapies. Nanofibrous biocompatible scaffolds have been shown to promote better cell adhesion and improve stem cell differentiation. In the present study, after Polyethersulfone (PES) nanofibrous fabrication by electrospinning technique and surfaces modifications, characterization was performed using scanning electron microscopy (SEM), ATR-FTIR and MTT assay. Then the hepatogenic potential capacity of iPSCs after cultured on collagen coated polyethersulfone (PES/COL) scaffolds was evaluated using Real-Time RT-PCR and immunocytochemistry (ICC). After scaffolds characterization, analyses of two important definitive endoderm specific markers such as Sox17 and Foxa2 using Real-Time RT-PCR and ICC indicated that these mRNA and protein levels were increased after 5 days of hepatogenic induction. In addition, to determine hepatic differentiation of iPSCs cultured on PES/COL, the expression of Albumin and α-FP evaluated by ICC after 20 days. Real-Time RT-PCR analysis showed increased expression of Albumin, TAT, Cytokeratin19 and Cyp7A1 genes over the course of the differentiation program. In conclusion, our results demonstrated that PES/COL nanofibrous scaffolds could be a proper substrate for hepatogenic differentiation of iPSCs to significantly increase in their potential and it could also be introduced as a promising candidate for liver tissue engineering applications.

T1099

LINEAGE SPECIFICATION AND REGENERATION OF INTRA-HEPATOPANCREAS DUCTS

Marquez, Isaac[1], Marquez, Isaac Si[2], Gates, Keith[2] and Lancman, Joseph[2], [1]sanford burnham, San Diego, CA, U.S., [2]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, U.S.

The hepatopancreatic ductal system functions to transport hepatic bile and pancreatic enzymes. However, its role as developmental multipotent progenitors is controversial. Also unclear is the mechanism of ductal paucity

in Alagille Syndrome, a disease associated with JAGGED1 and NOTCH2 mutations. Leveraging the zebrafish vertebrate model to generate viable embryos with compound homozygous mutations in two of the Notch ligand genes, we demonstrate that Jagged1b (Jag1b) and Jagged2b (Jag2b) are the primary ligands required for induction of all detectable canonical Notch signaling and for the specification of all intra hepatic ducts, intra pancreatic ducts, and neogenic pancreas endocrine cells during organongenesis. The acinar and hepatocyte compartments are surprisingly not lost in these double mutants, and together with results from extensive lineage tracing of Notch active cells, we conclude that ducts do not function as a significant source of progenitors for the developing acinar and hepatocyte lineages. Further, genetic mosaic analysis suggests that insufficient Jagged signaling from endoderm derived cells can lead to failure in duct specification. This study resolves longstanding questions regarding the specification of the intra hepatopancreatic ducts and their requirement as progenitors, and suggests an alternative mechanism for Alagille syndrome ductal paucity.

T1101

MODELING OF CYTOCHROME P450 3A4 INDUCTION BY USING HUMAN IPS CELL-DERIVED ENTEROCYTE-LIKE CELLS

Negoro, Ryosuke[1], Takayama, Kazuo[2], Tachibana, Masashi[2], Sakurai, Fuminori[2] and Mizuguchi, Hiroyuki[2],
[1]Graduate School of Pharmaceutical Sciences,Osaka University., Suita, Japan, [2]Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan

It is known that expression of cytochrome P450 3A4 (CYP3A4) in enterocytes is induced by various drugs, such as dexamethasone, rifampicin, and 1α, 25-dihydroxyvitamin D3 (VD3). The induction of CYP3A4 expression in enterocytes by such drugs might affect the pharmacokinetic of concomitant drugs which are orally administered. Although Caco-2 cells (a human colon carcinoma cell line) are widely used as a model of human intestinal absorption of drugs, it is difficult to evaluate drug-mediated CYP3A4 induction by using a Caco-2 cell monolayer model because of low expression levels of CYP3A4 and pregnane X receptor (PXR, nuclear receptor which is necessary for CYP3A4 induction) in Caco-2 cells. To the best of our knowledge, an in vitro model which can accurately evaluate drug-mediated CYP3A4 induction in enterocytes has not been established. In this study, we attempted to generate enterocyte-like cells from human induced pluripotent stem (iPS) cells for drug-mediated CYP3A4 induction test. The enterocyte differentiation from human iPS cells was performed by sequential treatment with various cytokines and compounds including Activin A, glycogen synthase kinase-3β inhibitor, γ-Secretase inhibitor, SB431542, epidermal growth factor, and Wnt3A. By overlaying Matrigel in the maturation process of enterocyte-like cells, the gene expression levels of intestinal markers (VILLIN, sucrase-isomaltase, intestine-specific homeobox, caudal type homeobox 2, and intestinal fatty acid protein) were significantly enhanced. This result suggests that the enterocyte-like cells were maturated by Matrigel overlay. The percentage of VILLIN-positive cells in the enterocyte-like cells was 55.6%. To evaluate the drug-mediated CYP3A4 induction, the cells were treated with various drugs. Treatment with dexamethasone, phenobarbital, rifampicin, and VD3 resulted in 5.8-fold, 13.4-fold, 9.8-fold, and 95.0-fold induction of *CYP3A4* expression levels, respectively. On the other hand, dexamethasone, phenobarbital, or rifampicin did not elevate *CYP3A4* expression in Caco-2 cells. These results suggest that the enterocyte-like cells differentiated from human iPS cells are a useful model for evaluation of drug-mediated CYP3A4 induction in enterocytes.

T1103

DEVELOPMENT OF A NOVEL, SERUM-FREE HEPATIC ORGANOID EXPANSION MEDIUM TO STUDY MOUSE LIVER BIOLOGY IN 3D

Segeritz-Walko, Charis[1], Riedel, Michael J.[1], Thomas, Terry E.[1], Eaves, Allen C.[1,2] and Louis, Sharon A.[1],
[1]STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Hepatic organoids act as functional three dimensional (3D) in vitro models of the liver epithelium and serve as a novel platform to address diverse research questions pertinent to hepatic development, regeneration, metabolism, drug toxicity and drug development studies, liver disease modelling and adult stem cell biology. Hepatic organoids grow as a monolayer of epithelial cells arranged in individual spheres, preserve key physiological features of developing hepatocytes and are obtained through isolation and expansion of stem and progenitor cells from hepatic stem cell niches in bile ducts.We are developing Hepatic Organoid Expansion Medium, a serum-free, defined medium that allows the rapid generation of hepatic organoids from wild-type, uninjured mouse liver tissue. Mouse livers were enzymatically digested to remove mature hepatocytes. The remaining hepatic ductal tissue was resuspended in a 1:1 mixture of Corning® Matrigel® and Hepatic Organoid Expansion Medium, and plated as domes onto pre-warmed 12-well culture plate wells. These Matrigel domes were solidified for 10 minutes at 37°C, then wells were flooded with Hepatic Organoid Expansion Medium. The organoid cultures were maintained for 5-7 days at 37°C with full medium changes every other day. As early as 24h after plating, 3D liver organoids were visualized budding from embedded ducts, indicating the presence of a population of liver stem and progenitor cells. New hepatic organoid formation over multiple passages was

also assessed. Hepatic organoids could be maintained long-term for >30 passages through mechanical disruption into organoid fragments or enzymatic digestion into single cells. Organoids were passaged every 5-7 days at 1:4 to 1:10 split ratios. Cells within organoids expressed Lgr5, Axin2, Sox9, Krt7, Krt19, Epcam and Hnf4α, but not markers of mature hepatocytes, such as Alb and Cyp3a11, as assessed by immunostaining and qPCR. These data show that organoids derived and maintained in Hepatic Organoid Expansion Medium are capable of maintaining a progenitor phenotype over extended culture periods. Hepatic Organoid Expansion Medium will therefore allow rapid and efficient generation and expansion of hepatic organoids from mouse livers and find applications in a physiologically-relevant context for multiple liver research fields.

T1105

TREAT ACUTE LIVER FAILURE RAT WITH HEPATOCYTE-LIKE CELLS DIFFERENTIATED FROM HUMAN WHARTON'S JELLY MESENCHYMAL STEM CELLS

Tsai, Pei-Jiun, Department of Critical Care Medicine , VGHTPE, Taipei, Taiwan, Hsiao, Chen-Yuan, National defense medical center, Taipei City, Taiwan, Wang, Hwai-Shi, Institute of Anatomy and Cell Biology, School of Medicine, National Yang Ming Un, Taipei, Taiwan, Shyu, Jia-Fwu, National Defense Medical Center, Taipei, Taiwan and Chen, Tien-Hua, Division of General Surgery, Department of Surgery, Taipei, Taiwan

Liver transplantation is now the ultimate treatment for liver failure. However, its limitation is shortage of donor organs as well as numerous medical problems postoperatively. Thus, cell therapy as other treatment choice of liver failure is considered. We have evaluated the capacity of hepatocyte-like cells differentiated from Wharton's jelly mesenchymal stem cells (WJ-MSCs) to prevent or ameliorate acute liver failure in a rat model. WJ-MSCs were isolated from human umbilical cords and differentiated into hepatocyte-like cells by incubation with a combination of liver-tissue culture medium and growth factors. The differentiated cells were evaluated with immunocytochemistry, RT-PCR, ammonia metabolism test, and glycogen storage for characteristics of functioning hepatocytes. The hepatocyte-like cells were transplanted into the portal vein of rats with acute liver failure induced by carbon-tetrachloride injection. Survival, serum liver function tests, and body weight of the animals were recorded. Livers of the rats 30 days after transplantation were harvested. The differentiated hepatocyte-like cells had morphologic features and functional characteristics of mature hepatocytes: hepatocyte-related genes, conversion of ammonia to urea, albumin synthesis, glycogen storage, and declining values of alpha-fetoprotein. Transplantation of the hepatocyte-like cells into the rats with carbon tetrachloride-induced liver failure prevented death of the animals and prevented rising values of serum alanine transaminase (ALT), aspartate transaminase (AST), and bilirubin. Immunohistochemical study of rat liver tissues 30 days after transplantation revealed that the WJ-MSCs had survived and were producing albumin. Therefore, we concluded that WJ-MSCs could differentiated into hepatocyte-like cells in vitro. Transplantation of these hepatocyte-like cells into the portal vein of rats with carbon-tetrachloride acute liver injury prevented death of the animals and irreversible liver injury. These findings may serve as a reference for future research in cell therapy of liver failure.

ENDOTHELIAL CELLS/ HEMANGIOBLASTS

T1109

LOSS OF CELLULAR COMMITMENT OF FOP IPS CELL-DERIVED ENDOTHELIAL CELLS

Barruet, Emilie C.[1], Morales, Blanca M[1], Lwin, Wint[1], Theodoris, Christina[2], Kim, Hannah[1], Urrutia, Ashley[1], Srivastava, Deepak[2] and Hsiao, Edward[1], [1]University of California, San Francisco, San Francisco, CA, U.S., [2]Gladstone Institutes, University of California, San Francisco, San Francisco, CA, U.S.

Patients with mutations in the Activin A Type I receptor (ACVR1), a bone morphogenetic protein (BMP) receptor, develop the debilitating disease fibrodysplasia ossificans progressiva (FOP) with massive heterotopic ossification. Studies have suggested that human endothelial cells (ECs) carrying the ACVR1 R206H mutation may contribute to the formation of FOP lesions by undergoing endothelial-mesenchymal transition (EndMT). Our overall hypothesis is that activated BMP signaling caused by the ACVR1 R206H mutation in ECs increases heterotopic bone formation by activating osteogenesis. Since primary ECs cannot be isolated from FOP patients we used a series of human iPSCs created from normal control and FOP donors. Here we show that the mineralizing FOP cultures have increased numbers of ECs but no differences in mesenchymal stem cells (MSCs). To determine if the ACVR1 R206H mutation could lead to increased ECs formation we created iPSC-derived endothelial progenitor cells (iECs). CD31^{+}/KDR^{+} iECs formed at equal yields from both control and FOP iPS lines. However, we found that FOP iPSCs could form iECs at lower BMP4 concentrations compared to control iPSCs but found no significant differences in the ability of WT or FOP iECs to form at lower concentration of Activing A. FOP iECs also showed differential SMAD1/5/8 and SMAD2 levels upon BMP4 or Activin A stimulation suggesting that BMP and Activin A ligands may differentially activate separate SMADs in FOP cells as

compared to WT cells. FOP iECs also expressed increased level of fibroblastic (FSP-1), chondrogenic (COL2A1), and osteogenic (COL1A and ALPL) markers and showed significantly increased alkaline phosphatase staining when cultured in osteogenic condition but showed little difference in mineralization. Our results indicate that the ACVR1 R206H mutation may increase the ability to form endothelial cell lineages that might serve as osteoprogenitors, and cause FOP ECs to produce more COL1A and COL2A as a contributor to tissue fibrosis. These studies show that endothelial cells derived from FOP hiPSCs are useful tools for dissecting the cellular and molecular mechanisms that underlie the pathogenesis of the abnormal bone formation in FOP.

T1111

PERIVASCULAR PROGENITOR CELLS DERIVED FROM HUMAN EMBRYONIC STEM CELLS EXHIBIT FUNCTIONAL CHARACTERISTICS OF PERICYTES, AND IMPROVE THE RETINAL VASCULATURE IN A RODENT MODEL OF DIABETIC RETINOPATHY

Hong, Ki-Sung, Konkuk University, Seoul, Korea

Diabetic retinopathy (DR) is the leading cause of blindness in working-age people. Pericyte loss is one of the pathologic cellular events in DR, which weakens the retinal microvessels. Damages to the microvascular networks are irreversible and permanent, thus further progression of DR is inevitable. In this study, we hypothesize that multipotent perivascular progenitor cells derived from human ESCs (hESC-PVPCs) improve the damaged retinal vasculature in the streptozotocin (STZ)-induced diabetic rodent models. We describe a highly efficient and feasible protocol to derive such cells using a natural selection method without cell sorting processes. As a cellular model of pericytes, hESC-PVPCs exhibited marker expressions such as CD140B, CD146, NG2, and functional characteristics of pericytes. Following a single intravitreal injection into diabetic Brown Norway (BN) rats, we demonstrate that the cells localized alongside typical perivascular regions of the retinal vasculature, and stabilized blood-retinal barrier (BRB) breakdown. Findings in this study highlight a therapeutic potential of hESC-PVPCs in DR by mimicking the role of pericytes in vascular stabilization.

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T1113

POU3F2 REGULATES ENDOTHELIAL CELL DEVELOPMENT AND LINEAGE SPECIFICATION

Matrone, Gianfranco[1], Tian, Xiaoyu[2], Blau, Helen M.[3], Wong, Wing Tak Jack[2] and Cooke, John P.[2], [1]Houston Methodist Research Institute, Houston, TX, U.S., [2]Center for Cardiovascular Regeneration, Houston Methodist Research Institute, Houston, TX, U.S., [3]Stanford University School of Medicine, Stanford, CA, U.S.

We have developed bi-species heterokaryons [generated by fusion of mouse embryonic stem cells (mESC) and human endothelial cells (hEC)] as a model system for discovery of novel factors required for endothelial lineage. Our RNAseq data suggests that the determinants of endothelial phenotype in the hEC act on the mESC to recapitulate endothelial ontogeny. Furthermore, novel transcription factors (TF) in endothelial specification were implicated, such as POU domain-containing TF (POU3F2), also called BRN2 or N-Oct3. This study assessed the role of POU3F2 in endothelial cell differentiation and in zebrafish vascular development. EC differentiation was induced by culture of mESC with growth factors (VEGF, bFGF and BMP4). POU3F2 loss-of-function was induced by lentiviral shRNA in mESCs. FACS was used to analyze cell lineage. Tg(Fli1a:eGFP) zebrafish were used to analyze vascular phenotype. For POU3F2 knockdown in zebrafish, caged morpholino was injected in embryos at 1-2 cell stage and activated at 6 or 24 hour post fertilization by embryo exposure to UV light. Zebrafish GFP^+ cells were enzymatically purified from embryos and quantified by FACS. In situ hybridization with RNA sense (control) and antisense probes was used to localize POU3F2 mRNA in the zebrafish embryo. Real time PCR and western blotting were used for a semi-quantitative analysis of gene and protein expression respectively. POU3F2 knockdown in mESCs reduced the $Flk1^+CD144^+$ cell population during differentiation of mESCs. POU3F2 knockdown also reduced endothelial cell markers in these mESC derived $Flk1^+CD144^+$ cells, including Kdr, Cdh5, Nos3, Tie2 and Lmo2; and reduced EC tube formation in matrigel. In zebrafish embryos, injection of MOs targeting POU3F2 reduced POU3F2 mRNA localization, detected by in situ hybridization, protein at 24 and 48 hpf, and GFP^+ cells. This was associated with an embryo phenotype characterized by severe vascular aberrations. Our heterokaryon studies implicated the transcription factor POU3F2 in endothelial cell development. We validated the role of POU3F2 in the reprogramming of pluripotent stem cells to EC lineage. We provide data that POU3F2 is required for normal vascular development in zebrafish.

T1115

EFFECTS OF HUMAN ENDOTHELIAL COLONY FORMING CELLS ON OXYGEN INDUCED RETINOPATHY IN VARIOUS IMMUNE COMPETENT CELL-DEPLETED MICE.

Sakimoto, Susumu[1], Aguilar, Edith[1], Marchetti, Valentina[2] and Friedlander, Martin[3], [1]The Scripps Research Institute, La Jolla, CA, U.S., [2]STEMCELL Technologies Inc., Vancouver, BC, Canada, [3]Scripps Research Institute Friedlander Lab, La Jolla, CA, U.S.

Human endothelial colony forming cells (ECFCs) are a homogeneous subpopulation of endothelial progenitor cells that can be isolated from human cord blood. We and others have shown that ECFCs enhance vascular repair in a murine model of oxygen induced retinopathy (OIR). Past studies all used C57Bl/6 mice as the recipient and, thus, it is unclear if the rescue effect is dependent on the immune response due to interspecies cross-reaction. In this study we assessed the efficacy of human ECFCs in rescuing OIR using mice lacking various immune-competent cell types. Human ECFCs were derived from cord blood. We used the following mice: Rag2 KO mice, lacking mature lymphocytes; LCK cre mice crossed with transgenic mice expressing human diphtheria toxin receptors (DTR), for LCK+ T-lymphocyte ablation (LCK-DTR mice); and LysM-DTR mice, for LysM+ myeloid cell ablation. We induced OIR in these mice and injected 1.0 x 10^5 ECFCs or vehicle intravitreally at P12. Immune cell depletion in DTR transgenic mice was subsequently started by intraperitoneal injection of 25 ng/g DT for 5 consecutive days. We analyzed the percentage of area of neovascular tufts (NV) and vascular obliteration (VO) at P17 (n>8). Rag 2 KO mice injected with ECFCs showed 65.6 % reduction in NV ($p<0.001$) and 36.3 % reduction in VO when compared to vehicle control ($p<0.001$). These results were similar to our previous reports using C57Bl/6 wild type mice. In LCK-DTR mice after receiving ECFCs, the areas of NV or VO did not show any statistically significant change either between cre (+) and cre (-) mice (NV: 0.42 ± 0.24 % and 1.39 ± 0.75 %, respectively (p=0.26). VO: 3.68 ± 1.13 % and 4.91 ± 1.95 %, respectively (p=0.60).). LysM-DTR mice injected with ECFCs also showed no statistically significant change between cre (+) and cre (-) mice (NV: 0.28 ± 0.25 % and 0.20 ± 0.16 %, respectively (p=0.80). VO: 2.73 ± 0.95 % and 1.68 ± 0.53 %, respectively (p=0.35).). We did not detect any statistically significant difference in OIR rescue between immune-cell depleted and control mice following ECFC injection. These results suggest the rescue effect of ECFCs in OIR is not a simple consequence of interspecies immune reaction induced by ECFCs.

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EPITHELIAL CELLS (NOT SKIN)

T1117

EFFICIENT ESTABLISHMENT AND LONG-TERM MAINTENANCE OF 3-DIMENSIONAL MOUSE SMALL INTESTINAL AND COLONIC ORGANOIDS USING A NOVEL DEFINED AND SERUM-FREE INTESTICULT™ ORGANOID GROWTH MEDIUM

Conder, Ryan Kingsley[1], Elstone, Fisal[1], Lankhorst, Marianne[1], Riedel, Michael J.[1], Thomas, Terry E.[1], Eaves, Allen C.[1,2] and Louis, Sharon A.[1], [1]STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

The intestinal epithelium is rapidly renewed by $Lgr5^+$ stem cells located at the crypt base. Recently, new protocols have allowed the establishment and maintenance of organoid structures in vitro that recapitulates the mammalian intestine and can be used to model mammalian intestinal development. Isolated intestinal crypts are cultured in a semi-solid 3D environment consisting of specialized culture medium in the presence of Corning™ Matrigel™. We developed IntestiCult™ Organoid Growth Medium (OGM), a defined medium that supports efficient establishment and long-term maintenance of organoids from mouse small intestine. Here we demonstrate the application of IntestiCult™ OGM to also support the establishment and maintenance of organoids from colonic tissue, which differs both structurally and functionally from the small intestine. Both intestinal and colonic crypts were cultured and passaged as recommended in the IntestiCult™ OGM protocol, except the colonic crypt densities for primary cultures from a single mouse colon (low) or 4 pooled colons (high) were tested. Efficiency of organoid formation from small intestinal primary cultures was 64 ± 8% (mean ± SD; n=6) by day 5 and increased to 86 ± 4% (n=4) after the first passage and remained consistently >85% over at least 12 passages (90 ± 3%, n=3) as previously reported. The presence of $Lgr5^+$ stem cells and differentiated cells within the small intestinal derived organoids were confirmed by immunocytochemistry and qPCR. Unlike the crypts of the small intestine, the colonic crypts contain no Paneth cells that support the $Lgr5^+$ stem cells, however colonic organoids were successfully established, albeit at a lower efficiency, 38 ± 9% (mean ± SD; n=3) by day 5, with efficiencies increasing to levels similar to the small intestine (84 ± 2%; n=3) over 5 passages, indicating IntestiCult OGM can support the long term culture of colonic organoids. We found plating crypts pooled from 4 mouse colons in primary cultures was required to improve efficiency of colonic organoid establishment resulting in an increase to 51 ± 7%; n=3 in colonic organoid formation efficiency.

In summary, IntestiCult™ OGM promotes efficient formation and expansion of both small intestinal and colonic crypts and thus can be used to study intestinal function or stem cell biology ex vivo from both these regions.

T1119

PNEUMACULT™: AN INTEGRATED CULTURE MEDIUM SYSTEM FOR IN VITRO HUMAN AIRWAY MODELING

Hou, Juan[1], Brown, Tyler[1], Riedel, Michael J.[1], Thomas, Terry E.[1], Eaves, Allen C.[1,2] and Louis, Sharon A.[1], [1]STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Primary human bronchial epithelial cells (HBECs) can be cultured at the air-liquid interface (ALI) using specialized media, resulting in differentiated cultures that morphologically and functionally 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. We recently launched PneumaCult™-ALI, a serum- and Bovine Pituitary Extract (BPE)-free medium to support efficient mucociliary differentiation of HBECs in ALI culture. Here, we report the development of PneumaCult™-Ex, a serum- and BPE-free medium that supports rapid expansion of HBECs in monolayer culture. Commercially available HBECs (passage 1) were thawed and seeded into culture flasks containing PneumaCult™-Ex or control medium (BEGM™) at 3.5 x 103 cells/cm2. The culture medium was fully exchanged every other day and cells were passaged once cultures reached ~80% confluence. At each passage, cells were enzymatically dissociated and re-plated at 1 x 104 cells/cm2 in PneumaCult™-Ex or control medium for further expansion, or plated in PneumaCult™-ALI to assess differentiation potential. Cultures in expansion media were characterized by expansion rate and expression of basal cell markers by immunocytochemistry (ICC). Differentiated cultures were characterized for marker expression by qPCR, flow cytometry and ICC at 28 days after ALI establishment. Barrier function was measured weekly by trans-epithelial electrical resistance (TEER) using EVOM2. Average fold expansion over 4 passages was similar between cells cultured in PneumaCult™-Ex or control medium (7.1 ± 1.4 vs. 7.2 ± 1.9; mean ± SD, n=7, p = 0.9). Cells cultured in either control medium or PneumaCult™-Ex showed cobblestone morphology and uniform expression of basal cell markers p63 and p75NTR. Cells expanded in either PneumaCult™-Ex or control medium were successfully differentiated at passages 1 to 3 to generate a pseudostratified mucociliary epithelium containing goblet cells expressing MUC5AC and ciliated cells expressing FoxJ1 and AC-tubulin. Cultures differentiated using PneumaCult™-ALI showed stable TEER (200 - 600 Ω-cm2) for at least 25 weeks (n=4). Together, PneumaCult™-Ex and PneumaCult™-ALI create a fully integrated BPE-free culture system for in vitro human airway modeling.

T1121

CLONAL ANALYSIS IN THE MOUSE EMBRYONIC MAMMARY GLAND REVEALS THE EXISTENCE OF UNIPOTENT STEM CELLS

Rodilla, Veronica, **Lilja, Anna M.**, Huyghe, Mathilde and Fre, Silvia, Institut Curie, Paris, France

During mammary gland development, undifferentiated cells in the embryonic mammary bud are gradually specialized to form a ductal tree, composed of an epithelium with luminal cells facing the duct, and an outer basal cell layer in contact with the basal membrane. The luminal compartment can be further separated into luminal cells with and without expression of hormonal receptors estrogen (ER) and progesterone (PR). Previous studies have led to conflicting results regarding the plasticity and multipotency of stem cells in the mammary gland, and knowledge about cell fate commitment in the embryo is still sparse. We used the Notch1 receptor, expressed in luminal ERneg cells, as a marker to genetically track mammary cells through lineage tracing, to define their cellular hierarchy from embryo to early puberty. By defining the critical time windows when cellular lineages are specified during development, we showed that luminal and basal lineages are separated perinatally, while luminal lineages expressing or lacking hormone receptors are resolved later during early puberty. Using a multicolor reporter mouse and wholemount imaging of the ductal tree, we revealed that while cells in the embryonic mammary bud are yet undifferentiated, the majority of them already show a unipotent committment towards a luminal or basal lineage. To assess if cells are intrinsically regulated, we explored the effect of constitutive Notch1 activation in the embryo. This resulted in an exclusive luminal ERneg progeny, proving that Notch1 is essential for cell fate commitment in the mammary bud. In addition, ectopic activation of Notch1 in basal cells leads to a progressive cell fate switch towards luminal ERneg commitment. Our results show that Notch1 dictates cell fate in mammary cells at any given time point during development, demonstrating the importance of intrinsic cues for lineage specification in the mammary gland epithelium.

T1123

PRIMARY CILIA REGULATE PROLIFERATION AND CILIATED CELL FATE OF MOUSE AND HUMAN AIRWAY BASAL STEM CELLS THROUGH IGF-1/PI3K/AKT AND WNT/β-CATENIN SIGNALING

Paul, Manash K., University of California Los Angeles, Los Angeles, CA, U.S.

The proximal airway epithelium has a robust response for repair after an injury that is vital for host defense. This response occurs in two phases: an initial expansion phase of the airway basal stem cells (ABSCs) followed by a phase of differentiation, which ultimately leads to the repair of the pseudostratified epithelium with the optimal proportion of ABSCs and differentiated mucociliary cells. Aberrant stem cell self renewal/proliferation and/or abnormal differentiation results in airway disease, thus understanding the homeostatic mechanisms is critical. We show that paracrine IGF-1 mediated PI3K/Akt and autocrine canonical Wnt/β-catenin signaling pathways interact to promote ABSC expansion after injury by inhibiting GSK3β. β-catenin activation is mediated by a site-specific phosphorylation of β-catenin that is critical for its nuclear localization. The active IGF-1/PI3K/Akt and Wnt/β-catenin signaling phase is followed by an activation of GSK3β which inhibits the Kif24, Cep110, Cep97 protein complex, thereby promoting primary ciliogenesis. The primary cilium on ABSCs inhibits proliferation, whereas the primary cilium of intermediate cells directs a GSK3β/c-Myb mediated multi-ciliated cell program. This study identified a regulatory mechanism for proliferation of ABSCs and differentiation to the ciliated cell fate during airway epithelial repair which has significant implications for stem cell biology, lung repair and premalignancy of the airway epithelium.

T1125

A NOVEL LNCRNA TARGETS microRNA TO REGULATE COLON CANCER STEM CELL ASYMMETRY AND PLASTICITY

Shen, Xiling, Duke University, Durham, NC, U.S.

Emerging reports show that microRNAs, such as miR-34a, can act as cell fate determinants to initiate asymmetric division in colon cancer stem cells (CCSCs) (Bu et al, Cell Stem Cell, 2013, Bu et al, Cell Stem Cell, 2016). However, what initiates microRNA asymmetry and whether such mechanisms play any role in regulating normal intestinal/colon stem cells? (1) Here we show that a novel long non-coding RNA (lncRNA) regulates CCSC asymmetric division by directly targeting miR-34a. Segregated in the daughter stem cell compartment, the lncRNA recruits epigenetic regulators to methylate and deacetylate the miR-34a promoter simultaneously. The lncRNA promotes CCSC self-renewal and tumor formation in xenograft models, and is upregulated in late-stage clinical colorectal cancers (CRC), contributing to miR-34a silencing and CRC proliferation. The fact that lncRNA can target microRNA for spatial asymmetry highlights the regulatory complexity of non-coding RNAs (ncRNAs), which occupy the bulk of the "dark" genome. (2) We further discovered that the lncRNA, miR-34a, the cell fate determinant Numb, and Notch form nested feedforward and feedback loops that commits to cell fate asymmetry. Quantitative analyses revealed that this unique regulatory scheme enforces permanent separation of cell fates. Perturbation to this motif leads to a new population of cells with more plastic and ambiguous identity, displaying both CCSC and differentiation behaviors. RNA-seq analysis revealed that they reside in a new, intermediate transcriptional state between stem and non-stem states. (3) We show that proinflammatory stress caused by DSS or TNFa treatment can silence the lncRNA and activate miR-34a in normal Lgr5+ intestinal and colon stem cells, causing some of them to switch to asymmetric division to curb excessive proliferation. Restoration of the lncRNA exacerbates stem cell proliferation and eventually leads to oncogenic transformation under chronic inflammation. Therefore, ncRNA-mediated asymmetric division provides a safeguard against excessive stem cell self-renewal.

Funding Source: NIH R01GM095990, R01 GM114254, NSF 1350659

EPIDERMAL CELLS

T2001

FOXC1 GOVERNS MURINE HAIR FOLLICLE STEM CELL QUIESCENCE AND NICHE MAINTENANCE TO PRESERVE LONG-TERM TISSUE-REGENERATING POTENTIAL

Lay, Kenneth and Fuchs, Elaine, Rockefeller University, New York, NY, U.S.

Adult stem cells (SC) are critical for making and repairing tissues throughout the lifetime of the organism. This is exemplified by SCs located within the bulge (Bu) of the murine pelage hair follicle (HF), where they are kept quiescent and activated only periodically to fuel cycles of hair regeneration. How such tight regulation of SC activity is essential to conserve their long-term tissue-regenerating potential remains poorly understood. We addressed this by conditionally ablating a key transcription factor FOXC1 expressed in Bu-HFSCs. We show that FOXC1-deficient HFs are perturbed in two ways: 1) their Bu-HFSCs exit quiescence to make new hairs much earlier than their WT counterparts; and 2) they lose their old Bu while making a new one in the regenerative process. To understand mechanisms underlying these defects in SC quiescence and Bu architecture upon FOXC1 loss, we performed

RNA-sequencing of Bu-HFSCs. Transcriptomic changes suggest that perturbation of SC quiescence may cause downstream changes in cell adhesion. We found that both WT and FOXC1-deficient Bu-HFSCs down-regulate E-cadherin when they are proliferating to make new hairs. However, FOXC1-deficient Bu-HFSCs fail to re-establish quiescence on time and are unable to restore E-cadherin levels. As a result, they cannot withstand the mechanical pressure exerted by the newly growing hair, resulting in the gradual loss of the Bu. We further demonstrate that overall levels of SC-inhibitory factors are reduced with Bu loss, thereby lowering the threshold for SC activation. This altered SC-extrinsic niche, coupled with the SC-intrinsic propensity to proliferate, causes further aberrant SC cycling and excessive rounds of hair regeneration. Consequently, these FOXC1-deficient Bu-HFSCs are unable to preserve their numbers and fail to maintain a full hair coat for the animal as it ages. Together, our findings suggest that Bu-HFSCs conserve their limited potential by coupling quiescence to adhesion-mediated niche maintenance in order to achieve long-term tissue homeostasis.

T2003

CALORIC RESTRICTION PROMOTES FUR REMODELING AS A THERMOREGULATORY MECHANISM

Forni, Maria Fernanda[1], Peloggia, Julia[1], Braga, Tárcio Teodoro[2], Ortega Chinchilla, Jesus Eduardo[3], Navas Iannini, Carlos Arturo[3], Olsen Saraiva Camara, Niels[2] and Kowaltowski, Alicia Juliana[1], [1]Dep. Bioquímica, IQ-USP, SP, Brazil, São Paulo, Brazil, [2]Dep. Imunologia, ICB-USP, SP, Brazil, São Paulo, Brazil, [3]Dep Fisiologia, IB-USP, SP, Brazil., São Paulo, Brazil

Caloric restriction (CR), the limitation of dietary calories without lack of essential nutrients, extends the lifespan of a variety of species, including yeast, worms, flies and mice. Among the many effects of this diet, recent results show that it affects stem cells, responsible in mammals for the maintenance and replacement of tissues throughout life. Furthermore, many aspects of mammalian aging can be related to a decline in the replicative function of stem cells. In order to investigate the impact of CR on stem cells we submitted swiss female mice to 6 months ad libitum (AL) or 60% of the total calories (40% CR) and observed the impact on the skin and associated stem cells. The first observation was that CR backskin fur coat was more even and long due to an increase in guard hair follicle density and length ($p \leq 0.001$). The thickness of the epidermis was also increased in the CR group, in detriment of the hypodermis ($p \leq 0.05$). Hair clipping experiments also demonstrated that both synchronized and unsynchronized hair follicles show increased growth rates ($p \leq 0.05$), most probably due to early stem cell recruitment. In accordance, the pool of β1-integrin^{+} interfollicular (IFSC) and β1-integrin^{HIGH}/CD34^{+} hair follicle-associated epidermal stem cells (HFSC) was significantly increased ($p \leq 0.05$ and $p \leq 0.001$, respectively). The bioenergetic profile of both epidermis and dermis was evaluated. CR epidermis presented increased glycolysis, while the dermis displayed a significant increase in spare and maximal mitochondrial respiration. This metabolic shift culminates in phenotypic alterations that are significant for thermoregulation. Remarkably, when the fur was removed, CR animals displayed defective thermoregulation associated with rupture of locomotion patterns and lean weight loss ($p \leq 0.001$). In brief, these findings unveil not only a striking effect of CR upon stem cells, but also an important evolution-selected adaptative mechanism to cope with reduced insulation derived from a thinner hypodermis.

Funding Source: FAPESP

T2005

NICOTINAMIDE PREVENTS DIFFERENTIATION OF HUMAN PRIMARY KERATINOCYTES

Tan, Chye Ling[1], Chin, Wai Kiat[1], Quek, Ling Shih[1], Rovito, Holly[2], Oblong, John E.[2] and Bellanger, Sophie[1], [1]A*STAR, Singapore, Singapore, [2]The Procter and Gamble Company, Cincinnati, OH, U.S.

Nicotinamide (NAM) is a member of the vitamin B family (B_3, niacinamide) and a precursor of NAD+, an essential co-factor involved in major metabolic pathways. During ageing and UV exposure, NAD+ levels have been shown to diminish, this depletion being associated with a global drop of metabolism in aged cells. An effect of NAM in maintaining pluripotency of human embryonic stem cells has been suggested, while NAD+ seems to positively regulate both self-renewal and differentiation of neural stem/progenitor cells. Regarding skin, NAM has been found to maintain metabolism levels in dermal fibroblasts under oxidative stress conditions and to facilitate reprogramming of human foreskin fibroblasts to a pluripotent state. However, the effect of NAM on epidermal keratinocyte stem cells remains unknown. Our lab aims at understanding the role of NAM and bioenergetics pathways in stemness maintenance versus differentiation in human skin. Our preliminary data show that, in vitro, not only does NAM globally lower expression of early (keratins 1, 10 and 13) and late (involucrin and filaggrin) differentiation markers in a heterogeneous population of human primary keratinocytes, but it also prevents induction of differentiation over a 12-day time-course experiment. Interestingly, this negative effect on differentiation seems to occur in all human primary keratinocytes tested regardless of the age of donors. Colony forming efficiency assays show more holoclones (presumably epidermal stem cells) in NAM-treated populations compared to untreated cells. Current efforts aim at elucidating the NAM-driven metabolic pathway involved in the maintenance of epidermal stem cell proliferation. This should help to sort out the

puzzling mechanism underlying the proliferation to differentiation transition in the human skin epithelium.

Funding Source: This work is co-funded by A*STAR Singapore and The Procter & Gamble Company.

EYE OR RETINAL CELLS

T2009

STEPWISE DIFFERENTIATION OF DENTAL PULP STEM CELLS TOWARD RETINAL-PIGMENTED EPITHELIUM CELLS

Gerami-Naini, Behzad[1], Chan, Benjamin[2], Tozzi, Lorenzo[3], Ghezzi, Chiara[3], Kaplan, David[3] and Rowan, Sheldon[4], [1]Department of Diagnostic Sciences, Tufts University, Boston, MA, U.S., [2]Department of Orthodontics and Dentofacial Orthopedics, Tufts University, Boston, MA, U.S., [3]Department of Biomedical Engineering, Tufts University, Medford, MA, U.S., [4]USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA, U.S.

Age-related macular degeneration (AMD) is the leading cause of blindness in the aging American population. Stem cells hold promise for personalized cell replacement therapy, as the affected cell type, retinal pigment epithelial cells (RPE) can be efficiently generated from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). Clinical studies are underway using hESC-derived RPE, however the required immunosuppressive therapy is a significant obstacle for many patients. iPSC-derived RPE may overcome some of these concerns, but present distinct technical challenges. We propose here using human dental pulp stem cells (DPSCs) as a potential source for patient-matched RPE cells. DPSCs are neuroectodermal-derived cells that can be easily obtained from a patient's extracted tooth. DPSCs express many stem cell markers and their ability to differentiate toward numerous cell types without requiring viral transduction is well established. Directed differentiation of DPSCs toward RPE was attempted using a characterized protocol with Noggin, Dkk1, and Activin. However, DPSCs were not viable under these conditions. To establish a rational step-wise differentiation protocol, we screened for culture and differentiation conditions to induce neuroectodermal stem cell fate by modulating media, serum, growth factor, and small molecules. Two conditions showed early neural stem cell differentiation. These cells were characterized as expressing SOX2, PAX6, SIX3, and NESTIN. In addition, they expressed NANOG and/or OCT4 pluripotency markers. In order for stem cell-derived RPE cells to be utilized clinically, they need to be engineered into a functional 3D polarized tissue that can be transplanted into a patient. We established a 3D polarized tissue model using iPSC-derived RPE and bioengineered silk fibroin as a bio-mimetic membrane. Silk fibroin is non-immunogenic and its degradation, mechanical properties, and surface patterning are easily tunable. We will compare the function of 3D tissue engineered DPSC-derived RPE versus iPSC-derived RPE to ensure that our cells mimic the properties of normal RPE tissue that we will eventually use to replace diseased RPE seen in AMD. We propose that DPSC-derived RPE cells could offer safe and autologous cells for tissue replacement therapies.

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T2011

TOWARD IN VIVO RETINAL STEM CELL ACTIVATION: INHIBITION OF BMP OR SFRP2 PROTEINS IN THE ADULT MOUSE EYE INDUCES CILIARY BODY-SPECIFIC PROLIFERATION AND EXPANDS THE RETINAL STEM CELL POPULATION

Grise, Kenneth Neil, Balenci, Laurent, Wonders, Carl P., Coles-Takabe, Brenda and van der Kooy, Derek J., University of Toronto, Toronto, ON, Canada

Adult retinal stem cells (RSCs) are rare cells that reside in the pigmented ciliary epithelium of the mammalian eye. In culture, RSCs readily proliferate to form clonal, free floating spheres after 7 days, with the capacity to self-renew and differentiate into all of the cell types of the neural retina and retinal pigmented epithelium. However, RSCs do not proliferate or generate new retinal cells in adult mammals in vivo. Previously, we identified BMP and sFRP2 proteins as potential mediators of RSC quiescence with in vitro experiments. Here, we investigated whether BMP and sFRP2 inhibition could disinhibit RSC quiescence in vivo in mice. In one eye, we injected noggin or α-sFRP2 antibody intravitreally 3 times, once every 24hrs, at 2 doses (1.5ng/μL or 2.5ng/μL). The contralateral eye was injected with PBS. During injection, all mice received EdU in their drinking water. One group received no injections to serve as naïve controls. Mice were euthanized at 24hrs or 4 weeks after the last injection and EdU+ cells were quantified in the ciliary body (CB) and neural retina (NR). Noggin or α-sFRP2 did not affect the number of EdU+ cells in NR 24hrs or 4 weeks post-injection. 24hrs post-injection: There were increased EdU+ cells in the CB of the 2.5ng/μL α-sFRP2 treated eyes, and both the 1.5ng/μL and 2.5ng/μL noggin treated eyes had increased EdU+ cells in the CB. 4 weeks post-injection: α-sFRP2 treated eyes had increased EdU+ cells in the CB, and noggin treated eyes had increased EdU+ cells in the CB compared to naïve control but not PBS control. To determine if noggin or α-sFRP2 could expand the RSC pool in vivo, we injected a broad dosage range (7 doses, ranging 0.5-62.5ng/μL) of either molecule. 7 days after the last injection, we performed a clonal sphere assay. Noggin had an effect at

2ng/µL, where it over doubled the number of primary RSC spheres (2.38x). α-sFRP2 had an effect at 2.5ng/µL and also doubled the number of primary RSC spheres (2.17x). These results demonstrate RSC proliferation can be disinhibited in the adult mouse eye and present a first step toward a pharmacological intervention to endogenously regenerate the retina. The future goals of this investigation will be to determine if this expanded RSC population can differentiate into new retinal neurons, migrate into the neural retina and improve visual function.

Funding Source: Canadian Institutes of Health Research, Foundation for Fighting Blindness, Natural Sciences and Engineering Research Council of Canada CREATE M3 Scholarship, Vision Science Research Program Scholarship

T2013

PATIENT-SPECIFIC 3D RETINAL CUPS AS FAITHFUL HEREDITARY RETINAL DISEASE MODEL

Lukovic, Dunja, Artero Castro, Ana and Erceg, Slaven, CIPF, Valencia, Spain

Retinitis pigmentosa (RP), the most common form of hereditary retinal degeneration causing blindness, has high social and healthcare burden and currently has no cure. Understanding RP is highly hampered by high genetic heterogeneity (over 60 genes implicated) and inaccessible disease tissue.

We used iPS cell technology in order to obtain patient specific retinal 3D tissue in which to depict the retinal homeostasis and the pathogenesis of retinal degenerative disease and thus provide new scenarios for studies of molecular mechanism and therapy in RP. We have collected biopsies from RP patients with identified disease causing mutations and derived fibroblasts from which we generated retinal tissue through reprogramming. Patient specific retinal organoids are compared to their healthy sibling controls at molecular and cellular level.

T2015

TUMORIGENICITY AND OTHER SAFETY STUDIES FOR HESC-DERIVED RPE CELLS: A STEP FORWARD IN AGE-RELATED MACULAR DEGENERATION TREATMENT

Padrell Sánchez, MSc, Sara, Petrus-Reurer, Sandra, Plaza Reyes, Alvaro, Kvanta, Anders and Lanner, Fredrik, Karolinska Institute, Stockholm, Sweden

Dry Age-related Macular Degeneration (AMD) is the leading cause of blindness in people aged 65 and older in the Western world. This disease is untreatable at present and its main feature is the degeneration of Retinal Pigment Epithelium (RPE), leading to a progressive photoreceptor degeneration and vision loss. We have recently established a novel xeno-free and robust hESC in vitro differentiation protocol using human recombinant laminin 521. The hES-RPE cells have been transplanted into a large-eyed preclinical model, the rabbit, with promising functional results. Exhaustive characterizations have demonstrated that the cellsobtained by this protocol share the main morphological, histological, physiological and functional features with native RPE cells, which makes these cells a good candidate for a possible AMD treatment. Since the obtained cells have been differentiated from hESC, it cannot be ignored that a tiny fraction of undifferentiated and possibly tumorigenic cells may still reside in the final product. Therefore, before these cells could be considered for further clinical applications, exhaustive tumorigenicity tests on mice and other safety studies have to be performed in order to assess the purity and safety of the final product. To perform the tumorigenicity studies, several groups of NOG mice have been subcutaneously injected, with increasing number of hESC (ranging from 10 to 1 million cells) to establish how many lingering cells could generate a tumorogenic growth. In parallel, 10 million cells from three time points along the differentiation protocol have been injected. All groups are being monitored for a possible teratoma formation. The experiment is still on going and so far 10/10 mice injected with 1 million of hESC and 6/10 mice injected with 100.000 hESC developed teratomas, whereas no sign of teratoma formation has been observed in any of the mice injected with mature hES-RPE cells. Furthermore, we are undertaking whole genome sequencing of the original hESC and the mature hES-RPE cells to evaluate the possible introduction of harmful alterations of the genome. Finally, bio-distribution of transplanted hES-RPE cells is currently undertaken in transplanted rabbits. If all these safety tests have a positive outcome, our hES-RPE cells would take a step forward into further clinical trials.

T2017

SENSING THE SUCCESS OF LIMBAL STEM CELL TRANSPLANTATION IN LSCD: IMPLICATION OF EXPLANT SIZE, SOURCE AND ITS CELL CHARACTERISTICS IN CLET/SLET

Singh, Vivek[1,2], Kethiri, AbhinavR[1,3], Shukla, Sachin[1,3], Basu, Sayan[3] and Sangwan, Virender S[1,3], [1]Prof. Brien Holden Eye Research Center, Hyderabad, India, [2]LV Prasad Eye Institute (HERF), Hyderabad, Hyderabad, India, [3]Tej Kohli Cornea Institute, L. V. Prasad Eye Institute, Hyderabad, India., Center for Regenerative Ophthalmology, L. V. Prasad Eye Institute, Hyderabad, India, Hyderabad, India

A major challenge of simple limbal epithelial transplantation (SLET) or the cultivated limbal epithelial transplantation (CLET) is the inability to decide the amount of donor tissue essential for ensuring safety of the donor eye. The

aim of this study was to explore the expansion capability and biological properties of the limbal explants with respect to the source, size, proliferation capacity and corneal and stem cell markers characterisation. Limbal explants from live (n=7) and cadaveric (n=10) tissues were cultured and expansion of the explants was measured using ImageJ. Proliferation of the cells was analyzed by BrdU assay at 3, 5, 7 and 9 days and expression of different stem cell and epithelial markers were analyzed using IHC. Explants from both live (donor mean age-53.2±11.4 years) and cadaveric (mean age- 43.7±26.9 years) could be expanded and % expansion into epithelium was greater when live tissue was used (80%) compared to cadaveric (62.5%). The mean area of growth was 190±38.82 mm^2 in live and 208±93.76 mm^2 in the cadaveric tissues at end point. The mean area of growth in both live and cadaveric limbal tissues was dependend on the size of the explants. In live limbal explants in the size range of 0.2-0.4 mm^2 the mean area of growth was 180.39±44.02 mm^2 and increased to 199.6±33.63 mm^2 in explants of 0.4-0.9 mm^2. In contrast, in cadaveric tissue explants the mean area of the tissue decreased with increase in explant size. Explants of 1.0-1.5mm^2 show a mean expansion of 231.02±87.24 mm^2 in comparison to 185.12±100.28 mm^2 in explants of 1.5-2.0 mm^2. However, the percentage of proliferating cells was neither dependent on the nature of the explants (live or cadaveric) or size of the explant . IHC analysis revealed that irrespective of whether the tissue were expanded from live or cadaveric sources, the expression of stem cell and corneal epithelial markers like ABCG2, Vimentin, E-cadherin and CK 3+12 was similar. We demonstrates that majority of the cadaveric limbal biopsies (62.55) can be expanded and could thus be used as a source for expansion of limbal stem cells. The mean area of expansion of the epithelium was comparable in the live and cadaveric explants. In addition, proliferation rates and expression of stem cell and corneal epithelium markers were also similar in live and cadaveric explants.

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NEURAL CELLS

T2019

EFFICIENCY OF NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS UNDER THE TREATMENT WITH RETINOIDS

Sperling, Laura Elena[1], Reis, Karina[2], Girardi, Carolina[2] and Pranke, Patricia[3], [1]UFRGS, Porto Alegre, Brazil, [2]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Stem Cell Laboratory, Fundamental Health Science Institute, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [3]Institute for Research with stem cells, Porto Alegre, Brazil

The central nervous system lacks a good capacity of regeneration; therefore, differentiated embryonic stem cells are a valuable source of cells for therapy of CNS diseases and injuries. Retinoids are important biologically active molecules that comprise a group of natural and synthetic analogues of all-trans-retinoic acid (ATRA), a metabolite of the fat-soluble vitamin, retinol. Retinoic acid (RA) is a widely used factor for the differentiation of embryonic stem cells (ESCs) into neural precursors. Active RA signaling suppresses a mesodermal fate during in vitro culture and leads to neuroectodermal differentiation in ESCs. EC23 is a chemically and light stable alternative that does not degrade and has biological activity similar to ATRA. ATRA is produced from the metabolism of retinol to retinaldehyde. In this study, the effects of retinoid supplementation (ATRA, EC23, retinal) on neural differentiation of mouse ESCs was investigated. ESCs were induced to differentiate into neuronal lineage by formation of embryoid bodies (EBs), followed by the treatment of embryoid bodies with 10-6M retinoic acid, EC23 or retinal for 2 days, and selection of neural precursors in a serum free medium containing insulin, transferrin, selenium and fibronectin. The neurogenic effect of the three-abovementioned retinoids was compared by analyzing the expression of stem cell (Oct4 and Nanog), neural precursor (nestin, Pax6) and mature neuronal and glial marker (TUJ, O4, GFAP) by flow cytometry and real time PCR. Our results show that the addition of exogenous retinoids enhances neural differentiation of mouse ESCs. While retinal increases the generation of neural precursor cells as seen by the expression of nestin and Pax6, both ATRA and EC23 induced increased maturation and stabilization of the neuronal phenotype (neuronal marker β-III-tubulin). No significant changes were observed in the expression of GFAP. All retinoids increased the expression of the oligodendrocyte marker O4. Immunocytochemical staining was performed to analyse the expression of the nestin and β-III-tubulin. Treatment with retinoids resulted in an increased homogeneity of cells, with predominant neuronal

morphology. In conclusion, retinoid supplements improve the reliability and efficiency of neural differentiation.

Funding Source: CNPq, CAPES, FAPERGS, Stem Cell Research Institute

T2021

ROBUST DIRECT REPROGRAMMING GENERATES MATURE INDUCED MOTOR NEURONS THAT RECAPITULATE ALS DISEASE PHENOTYPES IN VITRO

Babos, Kimberley Nicole[1], Galloway, Kate[1], Kisler, Kassandra[2], Zitting, Madison[1], Li, Yichen[1], Shi, Yingxiao[1], Quintino, Brooke[3], Chow, Robert[1], Zlokovic, Berislav V.[1] and Ichida, Justin[1], [1]University of Southern California, Los Angeles, CA, U.S., [2]University of Southern California, Los Anglese, CA, U.S., [3]California State University Fullerton, Fullerton, CA, U.S.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by selective loss of spinal motor neurons, nerve cells that actuate voluntary muscle movement. We previously identified a collection of seven transcription factors capable of converting mouse and human fibroblasts directly into spinal motor neurons (iMNs) in vitro. This approach, called direct lineage conversion, provides a rapid route to producing patient-specific neurons for translational studies. However, reprogramming inefficiency and difficulty generating homogeneous populations of bona fide-like neuronal subtypes limits the disease modeling and translational utility of this approach. We identified several roadblocks to successful iMN conversion, including TP53. Utilizing a unique and robust method of p53 inhibition, we generated a homogeneous motor neuron population that exhibits a transcriptional profile typical of bona fide neurons. p53-inhibited iMNs acquire more complex motor neuron morphometric characteristics, as well as more mature electrophysiological properties compared to uninhibited cells. Additionally, we find that altering our reprogramming cocktail under p53 inhibition permits subtype-specification into discrete spinal motor neuron populations. We utilized this system to generate iMNs from primary healthy control and ALS patient fibroblasts. Patient iMNs generated under p53 inhibition recapitulate ALS disease phenotypes, exhibiting faster degeneration and functional hyperexcitability compared to iMNs generated with normal p53 activity, owing to their enhanced maturity and acquisition of more bona fide-like neuronal properties. Such observations indicate that p53 inhibition allows acquisition of mature, distinct, and, more important, clinically relevant, induced motor neurons for in vitro disease modeling. Collectively, these studies will allow more accurate and robust modeling of neurological disorders like ALS via highly efficient production of bona fide, homogeneous subtype-specific neurons.

T2023

LEPTIN DOES NOT ALTER PROLIFERATION OF NEURAL STEM CELL IN THE NEUROGENIC NICHES OF YOUNG ALZHEIMER'S DISEASE MICE MODEL

Calió, Michele Longoni, Loyola, Tulio, Ko, Gui Mi and Porcionatto, Marimélia, Universidade Federal de São Paulo, são paulo, Brazil

Alzheimer's disease (AD) is the most common cause of dementia. Although two of the main pathological features of AD (amyloid plaques and neurofibrillary tangles) were already recognized, the pathogenesis of the disease remains unsettled. Neurogenesis in the adult hippocampus, which is notably affected by progressive neurodegeneration and pathology, is implicated in learning and memory regulation. It has been show that leptin, an adipose tissue-derived hormone, is capable of reducing the amount of extracellular amyloid beta, tau phosphorylation in neuronal cells and can modulate neurogenesis in the adult dentate gyrus, although the mechanisms involved in this process in the AD brain are still unknown. Importantly, chronic administration of leptin resulted in a significant improvement in the cognitive performance of transgenic animal models.It is speculated that a deficiency in leptin levels or function may contribute to systemic and CNSabnormalities leading to disease progression. Here, we sought to determine if leptin is able to stimulate proliferation of ventricular/subventricular zone and hippocampus neural stem cells of three month old male double transgenic APP/PS1 and wild type mice. After 7 days of intraperitoneal administration of leptin, we estimated the number of proliferating cells. There was no significant increase in the number of Ki67-positive cells in the subgranular zone of the dentate gyrus or in the ventricular/subventricular zone, as shown by immunostaining analysis. Our results indicate that acute administration of leptin does not increase proliferation of neural progenitor cells in neurogenic niches of young mice.

Funding Source: CNPq and FAPESP

T2025

REGULATORY ROLE FOR MIRNAS DURING DEVELOPMENTAL NEUROTOXICITY RESPONSE TO SILVER NANOPARTICLE IN HUMAN EMBRYONIC STEM CELLS-DERIVED NEURONAL CELLS

Choi, Mi-Sun[1], Oh, Jung-Hwa[2,3], Song, Chang-Woo[2] and Yoon, Seokjoo[2,3], [1]Korea Institute of Toxicology (KIT), Youseong-gu, Korea, South, [2]Korea Institute of Toxicology (KIT), Daejeon, Korea, [3]University of Science & Technology, Daejeon, Korea

microRNAs (miRNAs) have an important role in organism development, cell differentiation and lineage specification, but relatively little known of their functional role in regulating developmental neurotoxicity. Human embryonic stem cells (hESCs) provide a model system to study developmental biology as well as toxicology because of its ability to differentiate into all fetal cell lineages. Neural differentiation of hESCs has been established as alternative methods to investigate in vitro developmental neurotoxicity (DNT). To identify the molecular developmental neurotoxic effects, we studied the changes of miRNAs expression in hESCs-derived neural precursor cells (NPCs) after exposure to silver nanoparticle. Silver nanoparticles with size of around 11nm evoked significant toxicity in hESCs-derived NPCs in a dose-dependent manner and induced cell cycle arrest, apoptosis following a significant increase in oxidative stress. During in vitro DNT, six miRNAs (miR-297, miR-132, miR-22, miR-27b*, miR-196b*, miR-1226) were highly regulated in hESCs-derived NPCs by silver nanoparticle treatment. In this study, we provide molecular insights into the responses to silver nanoparticle in hESCs-derived NPCs based on global mRNA and miRNA expression analyses, and key regulatory mRNAs and miRNAs involved in oxidative stress and dysfunctional neurogenesis have been suggested. We provide the potential application of hESCs-derived NPCs to evaluate the developmental neurotoxicity of nanoparticles and this approach can further lead to risk assessment of personalized toxicology for population and individuals by applying the induced pluripotent stem cells derived neural cell model. Our findings provide information for understanding the underlying molecular mechanisms of the regulatory networks at the transcriptional and post-transcriptional level and improve the understanding of the molecular responses that underlie developmental neurotoxicity.

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T2027

STUDYING HUMAN SEROTONERGIC NEURONS IN VITRO

Elkins, James[1], Vadodaria, Krishna[2] and Gage, Fred H.[2], [1]Cal State San Marcos, San Marcos, CA, U.S., [2]Salk Institute for Biological Studies, La Jolla, CA, U.S.

The serotonergic neurotransmitter system plays a major role in normal brain function and research has shown it to be compromised in some neuropsychiatric disorders. Recent publications have demonstrated that by manipulating growth factor pathways human serotonergic neurons can be generated from pluripotent stem cells (PSC). Separate studies utilized overexpression of certain transcription factors in fibroblasts to generate human serotonergic neurons directly. Our lab has recently demonstrated that fibroblasts can be transdifferentiated directly to serotonergic neurons in vitro. These serotonergic neurons displayed spontaneous activity and released serotonin in vitro. In the current study, serotonergic neural differentiation from human PSCs is achieved by manipulating growth factor pathways, specifically Wnt and Shh pathways. The differentiation process is ordered and time sensitive starting with Wnt activation followed by Shh and Fgf4. Human PSC-derived serotonergic neurons are studied for functional activity in vitro and in the context of transdifferentiated serotonergic neurons. The findings of this study would be relevant to modeling neuropsychiatric disorders in vitro.

Funding Source: Funded by California Institute for Regenerative Medicine

T2029

DERIVATION AND STABILIZATION OF NOVEL HUMAN NEURAL PRECURSOR CELLS FROM EMBRYONIC BRAIN TISSUE FOR BIOMEDICAL APPLICATIONS

Günther, Katharina[1], Wörsdörfer, Philipp[1], Thier, Marc Christian[2], Meyer, Sandra[1], Wischmeyer, Erhard[3] and Edenhofer, Frank[1,4], [1]University of Würzburg, Institute of Anatomy and Cell Biology, Würzburg, Germany, [2]German Cancer Research Center, HI-Stem, Heidelberg, Germany, [3]University of Würzburg, Institute of Physiology, Department of Neurophysiology, Würzburg, Germany, [4]University of Innsbruck, Institute of Molecular Biology, Department of Genomics, Stem Cell Biology and Regenerative Medicine, Innsbruck, Austria

Recently, major progress in cellular reprogramming for modeling neurological and neuropsychiatric diseases has been achieved. The generation of iPSCs and their differentiation into neural progenitor cells (NPCs) and the direct conversion of somatic cells into NPCs emerged into

a promising strategy to obtain patient-specific cells. Nevertheless, it remains unclear if those NPCs represent the physiological state. Primary NPCs from fetal brain tissue might serve as an alternative source for cell replacement and a bona-fide-model for comparative studies. Additionally, it could elucidate neural developmental mechanisms. Whereas earlier studies demonstrate the derivation of rosette-forming or radial-glia-like cells from primary tissue we hypothesized that the modulation of crucial developmental signaling pathways such as TGFß and SHH is instrumental for the stabilization of early progenitors. Thus, we assessed the potential of small molecules for the stable cultivation of primary early NPCs. Indeed, we identified conditions allowing robust formation of neuroepithelial (NEP) colonies displaying a homogeneous morphology and high proliferation rate. Moreover, they could be monoclonally expanded thus far for 45 passages. Characterization by immunofluorescent stainings and quantitative PCR show a neural stem cell profile including SOX1, PAX6, Nestin and SOX2. Differentiation analysis indicates a strong neurogenic potential with a high percentage of TUJ1-positive neurons in presence of glial cells marked by GFAP/S100ß. Further, patch clamp experiments reveal functional active neurons among which GABAergic, glutamatergic, dopaminergic and serotonergic subtypes can be found. Interestingly, rare neurons stain positive for the PNS neuron marker Peripherin. By applying directed differentiation protocols we increased the percentage of dopaminergic neurons along with neural crest-derived PNS neurons and mesenchymal cells. All in all, we here present a study showing the derivation of a novel embryonic-derived early NEP population by defined media conditions. These cells enable on the one hand broad biomedical applications such as cell replacement therapies, drug screening and neural tissue engineering and serve on the other hand as a comparative cell population.

T2031

FAST NEURONAL MATURATION WITHIN HUMAN INDUCED PLURIPOTENT STEM-DERIVED CORTICAL AGGREGATES

Illes, Sebastian[1,2], Iszák, Juliá[2], Vizlin Hodzic, Dzeneta[3], Zhai, Qiwei[3], Strandberg, Joakim[2], Olsson Bontell, Thomas[2], Hanse, Eric[2] and Funa, Keiko[3], [1]University of Gothenburg, Gothenburg, Sweden, [2]Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, [3]Sahlgrenska Cancer Center, University of Gothenburg, Gothenburg, Sweden

The human cortex comprises mature neurons which are functional interconnected and is able to generate synchronous activity. Human pluripotent stem cell (hPSC)-derived neural stem cells recapitulate processes of corticogenesis *in vitro*, e. g. time-dependent sequential production of cortical neurons, cortical layer development, neuron to glial transition. The electrophysiological maturation on single and network level of hPSC-derived cortical neurons are considered as a process which required several weeks up to months. We developed a novel procedure to generate hPSC-derived cortical aggregates and characterized the cellular composition by confocal laser microscopy and applied Patch-Clamp- as well as multi-electrode array (MEA) recordings to characterize the electrophysiological functionality on single cell and neuronal network level. Confocal laser microscopy revealed that hPSC-derived cortical aggregates comprise of early and late-born cortical neurons, S100-positive astrocytes, vGlut1 and PSD-95 mature synapse. Cell-attached and whole-cell recordings demonstrated that neurons within hPSC-derived cortical aggregates show mature electrophysiological properties, i.e. generation of spontaneous action potentials, bursts as well as inhibitory and excitatory spontaneous synaptic activity. MEA recordings detected synchronize cortical network activity within 3 weeks of cultivation. Cultivation of hPSC-derived cortical neurons in 3-d aggregates enhance the electrophysiological neuronal maturation on single cell and neuronal network level.

T2033

DISSECTING PATHOLOGICAL AGGREGATION OF ATAXIN-3 IN MACHADO-JOSEPH DISEASE: A CONSPIRATION OF CALCIUM, CALPAINS AND DECREASED AUTOPHAGY

Jungverdorben, Johannes[1,2], Breuer, Peter[3], Koch, Philipp[1], Wüllner, Ullrich[3], Peitz, Michael[1,2] and Brüstle, Oliver[1,2], [1]Institute of Reconstructive Neurobilogy, Bonn, Germany, [2]German Center for Neurodegenerative Diseases, Bonn, Germany, [3]Department of Neurology, Bonn, Germany

Machado-Joseph disease (MJD) or spinocerebellar ataxia type 3 is a devastating neurological disorder driven by a polyglutamine repeat expansion in the C-terminus of ataxin-3 and represents the most frequent form of inherited spinocerebellar ataxias worldwide. In a previous study we showed that protein aggregation in iPSC-derived MJD neurons could be initiated via excitatory stimulation resulting in Ca^{2+} entry and subsequent calpain-mediated cleavage of ataxin-3. However, the role of intracellular Ca^{2+} stores in ataxin-3 aggregation remained unclear. Here we set out to explore whether and to what extent intracellular Ca^{2+} stores contribute to the generation of ataxin-3 aggregates in MJD neurons. Blockage of the Ca^{2+}-induced calcium release (CICR) from the endoplasmic reticulum (ER) by ryanodine profoundly diminished ataxin-3-positive SDS-insoluble aggregates induced by glutamate, indicating an amplifying and important role of in calpain activation and ataxin-3 aggregation. Direct Ca^{2+} release from ER induced by ATP through metabotropic

P2Y receptors (P2YR) or inhibition of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) also yielded SDS-insoluble aggregates, underlining the relevance of intracellular Ca^{2+} release for the aggregation process. Since P2YR are already expressed in immature neural cells, ATP could trigger ataxin-3 aggregation even in one week-old neurons. Moreover, ATP-stimulation led to microscopically visible neuronal intranuclear inclusions (NIIs) positive for ataxin-3 and ubiquitin, a neuropathological hallmark of MJD. However, NII induction by SERCA inhibition, P2Y or ryanodine receptor stimulation showed a high variability between independent experiments. We reasoned that this variability might be due to fluctuations in proteasomal degradation and/or autophagy. Indeed, careful calibration of media conditions towards inhibition of autophagic flux resulted in reliable NII formation selectively in MJD neurons. Taken together, our data point to a complex pathological interplay where calpain activation by dysregulated Ca^{2+} homeostasis and autophagy conspire in the process of protein aggregation in MJD. The rapid induction of visible NIIs within a few days should also facilitate the study of pharmacological interference with protein aggregation.

T2035

NEURO-IMMUNE CROSSTALK THROUGH INTERLEUKIN-4 AND ITS RECEPTOR INDUCES REGENERATIVE NEURAL STEM CELL PLASTICITY AND NEUROGENESIS AFTER AMYLOIDβ42-INDUCED NEURODEGENERATION IN ADULT ZEBRAFISH BRAIN

Bhattarai, Prabesh[1], Kuriakose Thomas, Alvin[2], Cosacak, Mehmet Ilyas[1], Papadimitriou, Christos[1], Froc, Cynthia[3], Reinhardt, Susanne[4], Bally-Cuif, Laure[3], Zhang, Yixin[2] and **Kizil, Caghan**[1,5], [1]German Centre for Neurodegenerative Diseases (DZNE), Helmholtz Association, Dresden, Germany, [2]B-Cube, TU Dresden, Dresden, Germany, [3]National Center for Scientific Research (CNRS), Cedex, France, [4]Center for Regenerative Therapies Dresden, TU Dresden, Dresden, Germany, [5]German Center for Neurodegenerative Diseases (DZNE) Dresden, Helmholtz Associatio, Dresden, Germany

We cannot regenerate our neurons after neurodegeneration, in part due to difficulties in recruiting endogenous neural stem/progenitor cells (NSPCs) towards neurogenesis in vivo. Zebrafish has an extensive regenerative ability in its central nervous system, but the regenerative ability after neurodegeneration has not been extensively adressed. Therefore, with a neurodegeneration model, zebrafish could help elucidate the molecular programs of neurodegeneration-induced regeneration. Thus, we generated a novel adult zebrafish model of neurodegeneration by injecting synthetic Amyloid-β42 (Aβ42) into adult zebrafish brains using cell penetrating peptides as potent carriers. Aβ42 deposition was prominent in neurons as early as 1 day after injection and persisted more than a month as determiend by electron microscopy and mmunological stainings. This accumulation led to phenotypes reminiscent of human amyloid pathophysiology: apoptosis, inflammation, synaptic degeneration, and persistent learning deficits. Interestingly, zebrafish responded to Aβ42-mediated neurodegeneration by inducing proliferation of NSPCs and neurogenesis, which yielded in neurons that survived over long-periods of time. To understand the molecular basis of such a regeneration response, we performed a transciptome analysis using next-generation sequencing after sorting NSPCs, neurons and microglia. We found that immune-related pathways, especially Interleukin-4 (IL4) is highly upregulated in non-NSPC population, and its receptor IL4R is expressed only in NSPCs. When we injected human IL4 into healthy zebrafish brains, NSPC proliferation and neurogenesis increased significantly. Also, blocking IL4R in NSPCs during neurodegeneration significantly reduced NSPC proliferation and neurogenesis through regulating Stat6 phosphorylation. Our results indicate that IL4 is sufficient and necessary to directly activate NSPCs, exemplifying a novel neuro-immune cross talk for imposing a neuro-regenerative capacity to endogenous neural stem cells. Our Aβ42-mediated neurodegeneration model and transcriptome analysis in the adult zebrafish can therefore help us to identify how a regenerative response can be elicited, and could progress to neuronal repair in human brains by modifying the inflammatory milieu.

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T2037

DYSFUNCTION OF B-CELL CLL/LYMPHOMA 9 RESULTS IN ABNORMAL CORTICAL DEVELOPMENT AND NEURAL EXCITABILITY

Yang, Beimeng, Liu, Furong, Zhou, Ying, Yu, Tao and **Li, Weidong**, Shanghai Jiao Tong University, Shanghai, China

BCL9 located on human chromosome 1q21.1 locus,was originally identified as an oncogene in a precursor B cell acute lymphoblastic leukemia. Further studies showed that BCL9 has been associated with tumor invasion, stem cell traits and cell migration through the Wnt signaling pathway. Recently, human genetic studies suggested that common variants in the BCL9 gene confer risk of schizophrenia, bipolar disorder and autism. However, the function and mechanisms of BCL9 in the brain is poorly

understood. Here, we demonstrated that BCL9 is highly expressed in mouse brain during the embryonic and early postnatal period. We found that knockdown of BCL9 in E13.5 can severely disturb the migration of cortical neurons and significantly increase of ultrasonic vocalization rate of pups at postnatal day 3. Importantly, dysfunction of BCL9 by knockdown or knockout increased voltage-dependent Na+ channels expression and changed neuronal excitability in the cortical neuron. Our findings indicated a possible causative role of BCL9 in the pathogenesis of neurodevelopmental disorders.

T2039

ADMINISTRATION OF ZONISAMIDE PROMOTES SURVIVAL RATE OF POST-TRANSPLANT DOPAMINERGIC NEURONS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

Miyawaki, Yoshifumi[1], Samata, Bumpei[2], Nishimura, Kaneyasu[3], Morizane, Asuka[4] and Takahashi, Jun[4], [1]Center for iPS Cell Research and Application, kyoto, Japan, [2]CiRA, Kyoto University, Kyoto, Japan, [3]Karolinska Institutet, Stockholm, Sweden, [4]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Parkinson's disease (PD) is characterized by progressive degeneration of dopaminergic neuron (DA) at substantia nigra. Recently dopaminergic neuronal progenitors (DPs) derived from iPS/ES cells are considered as promising donor source for cell therapy for PD on behalf of fetal tissues. One of the problems of the cell therapy for PD is the low survivability of the grafted cells. We tired to improve their survival by administration of previously approved drugs. In our previous study, we found that valproic acid (VPA) and zonisamide (ZNS) improved survival rate of mouse iPSC-derived DPs. In this study we observed the effect of VPA and ZNS on human iPSC-derived DPs (hiPSC-DPs). We transplanted hiPSC-DPs into the striatum of immune-deficient (X-SCID) rats and administrated VPA or ZNS everyday intraperitoneally for 4 weeks or 16 weeks. An immunofluorescent study revealed that administration of ZNS (60mg/kg/day) increased the number of survived dopamine neurons that were positive for tyrosine hydroxylase (TH) and nuclear receptor related-1 (NUUR1) in the grafts both 4 and 16 weeks after transplantation. ZNS has been reported to have various neuroprotective effects. We are now studying the mechanisms how ZNS promotes the survival of hiPSC-DPs after transplantation.

T2041

THE INHIBITION OF BOTH WNT SIGNALING PATHWAYS INDUCED THE CORTEX INCLUDING MOTOR NEURONS FROM HUMAN PLURIPOTENT STEM CELLS

Motono, Makoto[1], Ogura, Takenori[2], Ioroi, Yoshihiko[2] and Takahashi, Jun[2,3], [1]Center for iPS Cell Research and Application, Kyoto Univ, Kyoto, Japan, [2]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [3]Kyoto University Graduate School of Medicine, Kyoto, Japan

The recapitulation of human cortical development in a controlled, defined manner from pluripotent stem cells (PSCs) has considerable potential for studies of human neural development, circuit formation and function, and for the construction of in vitro models of neurological diseases. Furthermore, PSCs are a promising donor cell source for transplantation therapy. In this study, we present the differentiation method of cortical neuron from PSCs for clinical application in the future. The inhibition of Wnt signaling is important to differentiate cortical neurons from PSCs. Therefore, we compared various types of small molecules to induce cortical neurons from PSCs in serum-free embryoid body-like aggregate culture. DKK1, which is often used for induction of cortical neurons, and one type of small molecules inhibited only the canonical pathway of Wnt signaling, whereas another type of ones inhibited both the canonical and non-canonical Wnt pathways. The small molecules of Porcupine inhibition, WNT-C59 and LGK794, which inhibit both Wnt signaling pathways, efficiently induced CTIP2^{+}/COUP-TF1^{-} cells, which are characteristic of the cells found in anterior cortex including motor neurons. In addition, when grafted into the cortex of adult mice, the cells induced using WNT-C59 showed abundant axonal fiber extension toward the spinal cord. These results raise the possibility of this differentiation protocol contributing to cell replacement therapy for motor neuron diseases or insults.

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T2043

FUNCTIONAL MATURATION AND LONG-TERM SURVIVAL OF HUMAN PLURIPOTENT STEM CELL-DERIVED RETINAL GANGLION CELLS

Ohlemacher, Sarah[1], Sridhar, Akshayalakshmi[1], Xiao, Yucheng[2], Cummins, Theodore[2] and Meyer, Jason S.[1,2], [1]Indiana University-Purdue University Indianapolis, Indianapolis, IN, U.S., [2]Indiana University, Indianapolis, IN, U.S.

Retinal ganglion cells (RGCs) play an essential role in transmitting visual information from the eye to the visual thalamus in the brain. They are also the primary cell type affected in traumatic retinal injuries as well as optic neuropathies. Human pluripotent stem cells (hPSCs) provide an attractive source of cells that can be used for patient-specific cell replacement therapies, drug screening, and disease modeling. However, little is known about the functional maturation of these hPSC-derived RGCS. As such, the development of these neurons was evaluated over time, and methods in which to influence their maturation and functionality were tested. hPSCs were differentiated toward a neural lineage in a stepwise fashion as previously described, yielding highly enriched populations of optic vesicle-like neurospheres. Within 40 days of differentiation, presumptive RGCs differentiated in a temporally-appropriate manner and expressed a compliment of RGC-associated features as well as proper morphological features. In prolonged culture, subtypes of RGCs emerged over time, including melanopsin-expressing intrinsically photosensitive RGCs. The maturation of hPSC-derived RGCs in extended cultures was observed by extensive neurite outgrowth, and the development of synaptic-like structures was further examined by microscopy and electrophysiology. These results will facilitate the use of hPSC-derived RGCs to study the progression of neuronal development and degeneration, the development of drug therapeutics and the use of hPSC-derived RGCs for cell replacement therapies.

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T2045

MODELING ALZHEIMER'S DISEASE USING PATIENT IPSC-DERIVED NEURAL CELLS

Cusulin, Carlo[1], Schumacher, Björn[2], Graf, Martin[1], Baumann, Karlheinz[1] and **Patsch, Christoph**[1], [1]Roche Innovation Center Basel, Basel, Switzerland, [2]University of Cologne, Cologne, Germany

Alzheimer's disease (AD) is a neurodegenerative disorder, characterized by β-amyloid deposits, neuronal death and dementia. The disease is prevalently sporadic, but the hereditary cases provided a great insight into its etiology. In the familial forms, mutations typically involve genes encoding amyloid precursor protein (APP) or presenilin, which both result in abnormal production of the amyloidogenic peptide Aβ42 over Aβ40. The advent of iPSCs allowed for the modeling and investigation of disease using patient-derived cells. Nonetheless, researchers face major challenges in obtaining fast, reproducible and robust differentiation and, especially, disease relevant phenotypes. This is particularly true for an inherently heterogeneous tissue like the brain and complex disorders such as AD. Therefore, we first established a robust and scalable differentiation protocol into neural precursor cells and neurons. We then proceeded to use iPSCs derived from four familial AD patients to investigate disease mechanism. For this we differentiated neurons from these cells and measured the production of amyloid peptides (Aβ42 and Aβ40); in all patient-derived the ratio of Aβ42 over Aβ40 was increased compared to cells obtained from healthy controls. These changes were reflected in the expression of disease relevant genes, such as MAPK1, PRKCB and SERPINA3. We went on to analyze other pathological hallmarks of AD, such as phosphorylated Tau. While the relevance of β-amyloid production in the context of neuronal death needs to be elucidated further, our model provides a robust platform to investigate this disease and to test therapeutically relevant compounds in vitro.

T2047

MORPHOLOGICAL CHARACTERIZATION OF IPSC-DERIVED NEURONS FROM MONOZYGOTIC TWINS DISCORDANT FOR SCHIZOPHRENIA

Roth, Julien George[1], Baul, B.S., Tithi[1], Sarkar, Anindita[2], Marchetto, M. Carolina[1], Wright, Rebecca[1] and Gage, Fred H.[3], [1]Salk Institute for Biological Sciences, La Jolla, CA, U.S., [2]Salk Institute for Biological studies, San diego, CA, U.S., [3]Salk Institute for Biological Studies, La Jolla, CA, U.S.

Schizophrenia (SCZ) is a debilitating neurodevelopmental disorder with a worldwide prevalence of approximately 1%. To examine the morphology and connectivity of SCZ neurons on a cellular level, human-induced pluripotent stem cells (hiPSCs) were used to develop neurons from monozygotic twins discordant for SCZ. Such cell models share the genetic background of the patient, and have the potential to recapitulate the faulty neurodevelopmental mechanisms that may provide the molecular and cellular foundations of the disorder. In particular, neural components of the hippocampal circuit were generated from the hiPSC twin lines and characterized in order to investigate SCZ pathology originating in the hippocampus. The efficacy with which hiPSC-derived neural progenitor cells differentiated into dentate granule (DG) neurons as well

as the branching complexity and neurite length of the DG neurons were compared across monozygotic twin lines. There was no significant difference in the ability of healthy control and SCZ cell lines to generate PROX 1 positive DG neurons. Additionally, the extent to which such neurons formed synaptic connections was assessed via labeling with a monosynaptic rabies-tracing virus. The analysis of the rabies tracing paradigm is currently underway; however, the neurons have expressed the requisite fluorescent markers to demonstrate neural connectivity within the cultures. Preliminary data has revealed that neurons derived from SCZ cell lines demonstrate decreased neurite length and complexity when compared to both the control-line from the discordant twin line and healthy concordant twin lines. Furthermore, neurons derived from the control line of a discordant twin pair also demonstrate decreased neurite length and complexity when compared to healthy concordant twin cell lines. Taken together these results may point to a quantifiable morphological difference between control and SCZ neurons at early developmental stages which, in turn, may contribute to disease onset later in life.

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T2049

HUMAN PLURIPOTENT STEM CELLS SERVE AS AN EFFECTIVE IN VITRO MODEL FOR STUDIES OF EARLY STAGES OF RETINOGENESIS

Sridhar, Akshayalakshmi[1], Ho-A-Lim, Kimberly[2], Cooke, Jessica[2] and Meyer, Jason S.[1], [1]Indiana University-Purdue University Indianapolis, Indianapolis, IN, U.S., [2]IUPUI, Indianapolis, IN, U.S.

Human pluripotent stem cells (hPSCs) allow for the unprecedented ability to recapitulate early stages of human development which are otherwise inaccessible to investigation. This is especially true for one of the earliest events in human development, the establishment of a neuroretinal fate from an unspecified pluripotent population. To test the ability of hPSCs to serve in this capacity, these cells were directed to differentiate using a 3D approach to analyze their ability to successfully recapitulate early events in human development in a temporal and developmentally-appropriate fashion. To do so, hPSCs were first directed to an anterior neural phenotype, which was marked by the appearance of 3D neural rosettes. These rosettes were indicative of the neural tube closure in vivo, which was confirmed by analysis of stage-specific neural transcription factors via immunocytochemistry and quantitative RT-PCR. Next, the cells were directed to an optic vesicle-like stage, where the presumptive retinal cells were identified by the expression of appropriate transcription factors. Finally, 3D optic vesicle-like neurospheres were identified, isolated, and further analyzed by immunocytochemistry for the expression of markers associated with some of the differentiated cell types of the neural retina. Upon establishment of the 3D differentiation protocol, this system was further utilized to study the expression patterns of retinal progenitor-associated genes and examine their mechanism of action in retinal fate determination via gene overexpression and knockdown experiments. Overall, the results of this study help to demonstrate the suitability of hPSC-derived 3D differentiation as an effective tool to model critical stages of early human development.

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T2051

CULTURED SCHWANN CELLS DEMONSTRATED LINEAGE SWITCHING CAPABILITY AND ACQUIRED OLIGODENDROCYTE PHENOTYPES

Tsui, Y.P., Tam, K.W., Shum, D.K.Y. and Chan, Y.S., The University of Hong Kong, Hong Kong, Hong Kong

Schwann cells and oligodendrocytes are myelin-forming glia found in the peripheral and central nervous systems, respectively. Despite being similar in function, the two cell types have distinct developmental origins. In cultured Schwann cells, however, we serendipitously observed expression of the oligodendrocyte lineage fate determining factor Olig2. Purified Schwann cell cultures were prepared from neonatal rat sciatic nerves. Olig2-positive Schwann cells (OL2-SCs) were detected at 25-30 DIV. By 50-60 DIV, OL2-SCs acquired polydendritic morphology typical of oligodendrocyte precursors. This was accompanied by a decline in Schwann cell marker expression. The OP-like cells were termed Schwann cell-derived oligodendrocyte precursors (SC-OP). We therefore hypothesized that the peripheral nervous system environment is essential for the maintenance of Schwann cell identity. Co-culture of OL2-SCs with dorsal root ganglia neurons prevented conversion in SC-OPs. In contrast, SC-OPs co-cultured with dorsal root ganglia neurons continued differentiation into mature oligodendrocyte-like cells with myelin basic protein-positive segments along multiple axons. Our results revealed "lineage switching" capability of Schwann cells isolated from the peripheral nervous system. Preservation of Schwann cell identity in vitro requires signaling cues derived from peripheral neurons.

T2053

MATURATION OF HUMAN IPS CELL-DERIVED BRAIN MICROVASCULAR ENDOTHELIAL CELLS FOR IN VITRO BLOOD BRAIN BARRIER MODEL

Yamaguchi, Tomoko, Nishijima, Misae and Kawabata, Kenji, National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan

Blood brain barrier (BBB) is formed by brain microvascular endothelial cells (BMECs) working together with astrocytes and pericytes. BMECs 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 BMECs 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 BMECs are different between human and animals. To properly predict the disposition characteristics of drugs, human BBB models have also been developed using primary BMECs isolated from freshly resected brain specimens. However, it is difficult to obtain a large amount of BMECs for the construction of in vitro BBB models. To overcome, we previously succeeded in the generation of BMECs-like cells from human induced pluripotent stem cells (iPSCs). However, maturation levels of iPS cell-derived BMECs are still insufficient because the transendothelial electric resistance (TEER) values and the expression levels of transporter genes in iPS cell-derived BMECs are low in comparison with those in primary BMECs. Therefore, in this study we attempted to maturation of iPS cell-derived BMECs. The TEER values were elevated by the addition of Wnt proteins to iPS cell-derived BMECs. In addition, the expression of tight junction related genes, such as claudin-5 and ZO-1, were elevated by Wnt protein. On the other hand, sodium butyrate could reduce the TEER values. These results showed that Wnt could promote the maturation of iPS cell-derived BMECs. Human iPSC-derived BMECs would be a cell source for generation of in vitro BBB models, and also expected to application to drug-screening.

T2055

SCALABLE PRODUCTION OF NEURO-SPHERES FROM HUMAN PLURIPOTENT STEM CELLS IN A 3D CULTURE SYSTEM CONTAINING FUNCTIONAL POLYMERS

Zhang, Haixin[1], Akamatsu, Wado[2,3], Miyazaki, Takamichi[1], Aiba, Kazuhiro[1], Okano, Hideyuki[3] and Nakatsuji, Norio[1], [1]Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto, Japan, [2]Center for Genomic and Regenerative Medicine, Juntendo University School of Medicine, Tokyo, Japan, [3]Department of Physiology, Keio University, School of Medicine, Tokyo, Japan

Differentiation of neural stem/progenitor cells (NS/PCs) from human pluripotent stem cells (hPSCs) is an important procedure for the cell therapy and high-throughput drug screening. Then, scalable production of neural lineage cells is required for such biomedical applications. We previously reported a simple 3D hPSC sphere culture system that incorporates mechanical passaging and suspension by functional polymers. Such 3D culture system has advantages for scalable production of NS/PCs from hPSCs. Here, we have developed a simple, efficient and scalable 3D culture system for differentiation of hPSC sphere into neuro-spheres, which were kept suspended without stirring by the gellan gum polymer. This system may be optimal for multiple future applications.

T2057

INDUCTION OF HUMAN NEURONS FROM ADULT PERIPHERAL BLOOD T CELLS THROUGH DIRECT REPROGRAMMING

Tanabe, Koji[1], Ang, Cheen Euong[1], Chanda, Soham[1], Levinson, Douglas[2], Südhof, Thomas C.[2] and Wernig, Marius[2], [1]Institute for Stem Cell Biology and Regenerative Medicine, Stanford, CA, U.S., [2]Stanford University, Stanford, CA, U.S.

Recent developments in gene editing techniques have allowed to generate powerful human cell models of disease-causing mutations with isogenic controls. However, large scale sequencing and genetic linkage studies have revealed that most of the genetic risk for neuropsychiatric diseases like schizophrenia, bipolar disease and autism is explained by combinations of common small effect variants. Therefore, experimental introduction of such genetic variants in animal or human cell models are expected to yield no detectable phenotypes. Instead, it will be important to obtain relevant cell types such as neurons with the patients' genetic background carrying all disease-contributing variants. Given the magnitude of the human genome, cells will have to be studied from large numbers of individuals. Induced pluripotent stem (iPS) cells repre-

sent one way to generate such cells, but are complicated by line-to-line variability and are difficult to obtain from many donors. Induced neuronal (iN) cells directly reprogrammed from skin fibroblasts can be obtained easier from many donors but donor fibroblasts are ill-defined and require invasive biopsies.Here, we show that human, adult, peripheral blood mononuclear cells (PBMCs) as well as purified T lymphocytes can be directly converted into fully functional iN cells demonstrating that terminally differentiated, human cells can be efficiently transdifferentiated into a distantly related lineage. T cell-derived iN cells, generated by non-integrating gene delivery, showed stereotypical neuronal morphologies and expressed multiple pan-neuronal markers, fired action potentials and were able to form functional synapses. Small molecule addition and optimized culture systems have yielded conversion efficiencies up to 4% resulting in the generation of up to about 40,000 iN cells from one milliliter of peripheral blood in one step. Thus, our method allows the generation of sufficient neurons for experimental interrogation from a defined, homogeneous, and easily accessible donor cell population.

REPROGRAMMING

T2061

PHARMACOLOGICAL CONVERSION OF HUMAN FIBROBLASTS TOWARDS A CARDIAC FATE

Cao, Nan[1], Huang, Yu[1], Zheng, Jiashun[2], Spencer, C. Ian[1], Zhang, Yu[3], Fu, Ji-Dong[4], Zhang, Mingliang[5], Srivastava, Deepak[1] and Ding, Sheng[1], [1]Gladstone Institutes of Cardiovascular Disease, San Francisco, CA, U.S., [2]University of California, San Francisco, San Francisco, CA, U.S., [3]the J David Gladstone Institutes, San Francisco, CA, U.S., [4]Heart and Vascular Research Center, MetroHealth Campus, Case Western Reserve University, Cleveland, OH, U.S., [5]Gladstone Institutes, San Francisco, CA, U.S.

Reprogramming of somatic fibroblasts directly into alternative lineages represents a promising regenerative strategy. However, transdifferentiating human cells to generate homogeneous, functional cells remains challenging and may benefit from a chemical reprogramming approach. Here we show that highly reprogrammed, functional cardiomyocyte-like cells can be generated by treating human fibroblasts with a combination of nine compounds (9C) without genetic materials. The chemically induced cardiomyocyte-like cells (ciCMs) uniformly had well-organized sarcomeres, were contractile, and closely resembled human cardiomyocytes in their transcriptome, epigenetic, and electrophysiological properties. Single cell transcriptional analyses demonstrated that individual ciCMs exhibited similar expression levels of cardiac genes and are highly reprogrammed and homogeneous. Global epigenomic analyses revealed that 9C treatment in human fibroblasts resulted in induced a rapid opening of previously closed chromatin in developmentally important genes, particularly in key cardiac developmental regulators, thus enabling the promoters/enhancers of these genes to bind effectors of major cardiogenic signals. When transplanted into infarcted mouse hearts, 9C-treated fibroblasts were efficiently converted to cardiomyocyte-like cells. The current study not only achieves significantly higher quality human iCMs than previously reported, but also provides an example of an approach free of foreign genetic material that may be adapted for reprogramming multiple cell types and may have important implications in regenerative medicine.

T2063

TRANSDIFFERENTIATION OF HUMAN FIBROBLASTS TO INDUCED SEROTONERGIC NEURONS

Xu, Zhimin[1], Jiang, Houbo[1], Zhong, Ping[1], Yan, Zhen[1], Chen, Shengdi[2] and **Feng, Jian**[1], [1]State University of New York at Buffalo, Buffalo, NY, U.S., [2]Ruijin Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China

Serotonergic (5HT) neurons exert diverse and widespread functions in the brain. Dysfunction of the serotonergic system gives rise to a variety of mental illnesses including depression, anxiety, obsessive compulsive disorder, autism and eating disorders. Here we show that human primary fibroblasts were directly converted to induced serotonergic (i5HT) neurons by the expression of Ascl1, Foxa2, Lmx1b and FEV. The transdifferentiation was enhanced by p53 knockdown and appropriate culture conditions including hypoxia. The i5HT neurons expressed markers for mature serotonergic neurons, had Ca^{2+}-dependent 5HT release and selective 5HT uptake, exhibited spontaneous action potentials and spontaneous excitatory postsynaptic currents. Application of serotonin significantly increased the firing rate of spontaneous action potentials, demonstrating the functional utility of i5HT neurons for studying serotonergic neurotransmission. Furthermore, i5HT neurons transplanted in rat brains produced extensive neuronal processes. The availability of human i5HT neurons will be very useful for research and drug discovery on many serotonin-related mental disorders.

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T2065

USING DEFINED TRANSCRIPTION FACTOR INDUCED MICROGLIA TO MODEL NEURODEGENERATIVE DISORDERS

Hor, Pooja[1], Hennes, Valerie[1] and Ichida, Justin[2],
[1]University of Southern California, Los Angeles, CA, U.S., [2]USC, Los Angeles, CA, U.S.

Microglia are the resident immune cells in the brain and spinal cord. Recent studies indicate that genetic variants that lead to several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD), can act via microglia to initiate these diseases. As shown in recent large-scale GWAS studies, AD-associated variants for one-third of the AD linked loci reside in gene regulatory enhancers in immune cells, not neurons. Moreover, some of the most common disease-causing mutations occur in genes such as TREM2 (AD) or C9ORF72 (ALS), which are most highly expressed in microglia amongst cells of the central nervous system. However, lack of approaches for generating a renewable source of patient-specific microglia has limited efforts to identify how genetic variants change human microglial function that lead to neurodegeneration. To enable mechanistic and translational studies on patient-specific microglia, we identified a set of 6 transcription factors that convert mouse and human fibroblasts or iPSCs into functional microglia. Mouse and human induced microglia (iMG) possess a gene expression profile highly similar to primary microglia, including expression of key markers such as CX3CR1, IBA1 and CD11b. Both mouse and human iMGs robustly phagocytose several biologically relevant targets such as b-amyloid oligomers and degenerating neurons, and rapidly activate inflammatory pathways in response to lipopolysaccharide stimulation, indicating that they possess the salient functions of bona fide microglia. Importantly, iMGs carrying ALS-causing mutations in the SOD1 gene cause toxicity towards motor neurons, indicating that iMGs recapitulate ALS disease phenotype previously observed in primary microglia both in vitro and in vivo. Moreover, we performed the motor neuron survival assay with iMGs generated from controls and C9ORF72 ALS patients. We observed similar neuronal toxicity caused by iMGs harboring ALS-causing C9ORF72 mutation suggesting that the C9ORF72 repeat expansion may cause neurodegeneration in part due to microglial induced neurotoxicity. Together, these results indicate that 6 transcription factors can convert mouse and human fibroblasts or iPSCs into functional microglia enabling successful disease modeling and future translational studies.

T2067

MERGING EPIGENETIC AND METABOLIC REPROGRAMMING IS A UNIQUE APPROACH TO GENERATE FUNCTIONAL ENDOTHELIAL CELLS FOR REGENERATIVE MEDICINE

Kelaini, Sophia, Grieve, David, Stitt, Alan W and Margariti, Andriana, Queen's University Belfast, Belfast, U.K.

Damage to endothelial cells (ECs) is a hallmark for the onset of cardiovascular disease. Development of fast and robust new methodologies that produce well-characterised, homogenous, clinical-grade cells suitable for tissue repair/re-modelling would have great utility. Recently, our group have shown a new paradigm of direct reprogramming strategy into ECs without traversing pluripotency, which could be useful for regenerative medicine. Although, these findings highlight the remarkable process of EC reprogramming, the limitation of the extremely low efficiency (1-4%) still remains. Development of fast and robust new methodologies that produce well-characterised, homogenous, clinical-grade cells suitable for tissue repair/re-modelling would have great utility. The present study has developed a remarkable novel approach based on the concept of merging epigenetic and metabolic reprogramming establishing a unique strategy to reprogram ECs. Our data reveals, for the first time, a unique combination of factors mediating epigenetic and metabolic alterations induce EC reprogramming in a fast and highly efficient approach. Human fibroblasts have been forced to over-express one pluripotency inducer such as OCT4, one epigenetic factors, one lineage specific factor in the presence of a metabolic modulator and cultured for 6-10 days in EC-Specific Media (ECM) in fibronectin-coated dishes. Under these conditions, human fibroblasts change morphology when compared to control cells (treated with a mock plasmid and cultured in identical conditions). Gene expression profiling data reveals the induction of EC markers such as CD31, CD144 and KDR. These results have also been confirmed by flow cytometry and immunofluorescent staining which provides evidence of EC-specific marker induction. Importantly, Matrigel plug assays in vitro have demonstrated that the combination of these key factors induced the formation of vascular-like structures. The underlying mechanisms of this unique approach are being fully elucidated and evaluated in hind limb ischemia model. Together, these findings may establish the therapeutic potential of reprogrammed ECs which would have transforming consequences for regenerative and personalised medicine.

Funding Source: This work was supported by Grants from BBSRC and British Heart Foundation.

T2069

INDUCED CARDIAC PROGENITOR CELLS DIFFERENTIATE IN CARDIAC EXTRACELLULAR MATRIX PATCHES TRANSPLANTED POST-MYOCARDIAL INFARCTION

Lalit, Pratik[1], Shmuck, Eric[2], Hacker, Timothy[2], Raval, Amish[2] and Kamp, Timothy J.[2], [1]UW-Madison, madison, WI, U.S., [2]University of Wisconsin-Madison, Madison, WI, U.S.

We recently reported lineage reprogramming of fibroblasts into expandable and multipotent induced cardiac progenitor cells (iCPCs). Adult fibroblast derived from a Nkx2.5-EYFP/rtTA reporter mouse from three tissue sources (cardiac, lung and tail-tip) were directly reprogrammed into iCPCs using a combination of five defined factors (Mesp1, Tbx5, Gata4, Nkx2.5, Baf60c) and activation of Wnt (BIO) and JAK/STAT (LIF) signaling. The purpose of this study was to test the applicability of iCPC technology for cardiac regenerative medicine. At first, we injected iCPCs in mice with experimentally induced myocardial infarction (MI) via intramyocardial injection and monitored for 4 wks. iCPCs significantly improved survival ($p<0.01$ Mantel-Cox test) in treated animals (75%) as compared to control (11%). Immunohistochemistry (IHC) revealed that iCPCs localized to the scar area and differentiated into aligned, rod-shaped cardiomyocytes (CMs) and vascular cells including smooth muscle cells (SMs) and endothelial cells (ECs). However, only a small fraction of the injected cells were retained within the scar after 4 wks. To increase long-term cell retention in vivo and empower functional cardiac repair, we reasoned that iCPC delivery via a tissue-engineered cardiac patch would hold benefit. Cardiac extracellular matrix (ECM) patches were generated by a novel method of high-density culture of human cardiac fibroblasts followed by decellularization. iCPCs seeded on cardiac ECM patches and cultured in differentiation medium migrated within the patch in 24 hrs and could be cultured in vitro for at least 2 months. IHC 45 days after seeding revealed that iCPCs differentiated into aligned CMs in the cardiac ECM patch. Next, cardiac ECM patches seeded with iCPCs (24hr seeding) were transplanted to the epicardial surface of the heart 2 days after MI. The mouse hearts were analyzed after 6 wks, and IHC revealed that iCPCs differentiated within the patch into cardiac lineage cells including CMs (cardiac actin), SMs (SMA) and ECs (CD31). iCPC-derived CMs were not only retained within the patch but also colonized majority of the patch. Moreover, the patch was highly vascularized both by host and iCPC-derived SMs and ECs. These results indicate that iCPC cardiac ECM patches provide a promising new tool for cardiac regenerative therapy.

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T2071

PARTICIPATION OF OCT4 MODULATED ASH2L ISOFORM B-MEDIATED HISTONE 3 LYSINE 4 METHYLATION IN SOMATIC CELL REPROGRAMMING

Liao, Bing[1] and Jin, Ying[1,2], [1]Shanghai Jiaotong University School of Medicine, Shanghai, China, [2]Institute of Health Sciences, Shanghai, China

Reprogramming somatic cells into induced pluripotent stem cells (iPSCs) utilizing defined factors represents a groundbreaking achievement and big leap for both academic stem cell research and regenerative medicine. To achieve the goal of clinical application, elucidation of the mechanisms underlying induced reprogramming are crucial. Previous studies in our laboratory demonstrated that Wwp2-catalyzed ubiquitinated Oct4 proteins are subject to degradation by 26S proteasomes during the differentiation process of pluripotent stem cells. Herein, we identified 5 lysine residues of Oct4 as ubiquitin-conjugation sites using mass spectrometry. Mutation of the 5 lysine residues abrogated Wwp2-catalyzed Oct4 ubiquitin modifications and enhanced the protein stability of Oct4 in differentiated cells. Subsequently, a mutant form of Oct4 (Oct4-5R, the 5 lysine residues were mutated to 5 arginine) or wild-type Oct4 (Oct4-WT) was introduced into MEF (mouse embryonic fibroblasts) cells together with Sox2 and Klf4 to induce reprogramming. Intriguingly, the Oct4-5R containing cocktail led to a reprogramming efficiency 10 times higher than that of the cocktail containing Oct4-WT. Consistently, Wwp2 null MEF cells had a significantly higher efficiency in pluripotency induction than wild type MEF cells, suggesting that Wwp2-mediated Oct4 ubiquitination acts as a barrier for pluripotency induction in MEF cells, probably through down-regulating the level of ectopically expressed Oct4 proteins. However, further investigation showed that the enhancement in the protein stability of the Oct4-5R mutant could not entirely account for the improvement of the iPSC generation. Instead, the higher pluripotency induction efficiency elicited by Oct4-5R could be partially attributed to the enhancement of Ash2l isoform b-mediated histone 3 lysine 4 methylation. Collectively, this study not only opens a new way to improve iPSC generation but also provides new insights into the mechanisms by which reprogramming event occurs involving Oct4 and Ash2l isoform b. More importantly, our results represent an important step toward the understanding how the reprogramming factor coordinates with the epigenetic regulator to control the induction of pluripotency.

Funding Source: Function and mechanism study on E3 ubiquitin ligases of transcriptional factor Oct4 in the pro-

cess of pluripotency establishment and maintenance. National Natural Science Foundation of China 31471231

T2073

OPTIMISATION OF A METHOD FOR GENERATING DOPAMINE NEURONS FROM DIRECTLY REPROGRAMMED ADULT HUMAN NEURAL PRECURSOR CELLS

Playne, Rebecca Grace[1], Jones, Kathryn[2], Firmin, Erin[2] and Connor, Bronwen J.[2], [1]University of Auckland, Auckland, New Zealand, [2]University of Auckland, Department of Pharmacology, Centre for Brain Research, Auckland, New Zealand

Parkinson's disease (PD) is a common neurological disease that affects 1-2% of the population over 65. The brain pathology of PD is characterised by progressive degeneration of midbrain dopamine neurons as well as the deposition of proteinacious plaques called Lewy bodies. The advent of reprogramming technology has provided an exciting new way to study PD. Live human neurons can be obtained from cells donated by PD patients and used to model disease progression. Additionally, this may provide a platform for drug identification and screening. To date, induced pluripotent stem cells have received much attention for modelling PD, while direct reprogramming strategies have taken a back seat, largely due to a lack of robust protocols for the generation of high yields of authentic human ventral midbrain dopamine (vmDA) neuronal cells. We have previously demonstrated that adult human dermal fibroblasts (HDFs) can be directly reprogrammed to induced neural precursors (iNPs) using SOX2 and PAX6. The present study aims to modify this method for the generation of iNPs with a bias towards a dopaminergic fate (induced dopaminergic precursors; iDAPs). HDFs were transiently transfected with SOX2 in conjunction with either PAX6, LMX1A and/or FOXA2 and cultured in reprogramming media supplemented with SHH-C24II, purmorphamine, CHIR99021 and/or FGF8 in order to mimic in vivo ventral midbrain developmental cues. Under select conditions, iDAPs were generated as demonstrated by enhanced expression of the vmDA progenitor markers NURR1 and PITX3 when compared with iNPs generated under standard reprogramming conditions. It was found that early, limited exposure to CHIR99021 during reprogramming was necessary in order to enhance the expression of vmDA progenitor markers. Induced DAPs could subsequently be differentiated into neuronal cultures in which up to 80.7% of cells co-expressed the dopaminergic marker TH and the neuronal marker TUJ1. Additionally, TH+ cells co-expressed the dopaminergic/A9 markers AADC, GIRK2, DAT and VMAT2. Further studies are underway to examine functional characteristics of these cells.

T2075

ACTIVATION OF THE ER UNFOLDED PROTEIN RESPONSE INCREASES THE CELLULAR REPROGRAMMING EFFICIENCY

Simic, Milos[1], Schinzel, Robert Thomas[1], Moehle, Erica[2] and Dillin, Andrew[3], [1]University of CaliforniaBerkeley, Berkeley, CA, U.S., [2]University of California, Berkeley, Berkeley, CA, U.S., [3]Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, CA, U.S.

Cellular reprogramming is achieved by the forced expression of 4 transcription factors: OCT4, SOX2, KLF4 and cMYC. This process theoretically requires a global remodeling of the organelles and a drastic change in metabolism. We therefore hypothesized that the early steps of reprogramming would result in the activation of a variety of stress pathways in the cell, which might in turn impact the efficiency of reprogramming. We focused in particular on the endoplasmic reticulum unfolded protein response (ER UPR). Interestingly we found that the expression of the reprogramming factors results in the activation of two branches of the ER UPR, as measured by mRNA levels. Furthermore, the secretion capacity of the ER was reduced. Unexpectedly, we found that a key downstream target, BIP, was downregulated on a protein level by using a translational reporter during the early phases of reprogramming, suggesting that the cell was not able to trigger a proper ER UPR. In order to initiate a proper stress response we overexpressed XBP1s. This resulted in the activation of the ER UPR, as expected, but also an increase of the efficiency of reprogramming.These results suggest that the low efficiency of cellular reprogramming is the result of the cell's inability to initiate a proper stress response to cope with the newly expressed load of protein that will eventually change the fate of this cell.

T2077

GATA6 POTENTLY INITIATES REPROGRAMMING OF PLURIPOTENT AND DIFFERENTIATED CELLS TO EXTRAEMBRYONIC ENDODERM STEM CELLS

Wamaitha, Sissy E.[1], del Valle, Ignacio[1], Cho, Lily T.Y.[1], Wei, Yingying[2], Fogarty, Norah[1], Blakeley, Paul[1], Sherwood, Richard I.[3], Ji, Hongkai[2] and Niakan, Kathy[1], [1]The Francis Crick Institute, London, U.K., [2]Johns Hopkins University, Baltimore, MD, U.S., [3]Harvard University, Cambridge, MA, U.S.

Transcription factor-mediated reprogramming is a powerful method to study cell fate changes. We find that the transcription factor Gata6 can initiate reprograming of multiple cell types to induced extraembryonic endoderm (iXEN) cells. Intriguingly, Gata6 is sufficient to drive iXEN cells from both mouse pluripotent cells and differentiated neural cells. Profiling transcriptional changes following

Gata6 induction in mouse ES cells reveals step-wise pluripotency factor disengagement alongside step-wise activation of extraembryonic endoderm genes. Chromatin immunoprecipitation and subsequent high-throughput sequencing analysis shows Gata6 enrichment near both pluripotency and endoderm genes, suggesting that Gata6 functions as both a direct repressor and activator.

Furthermore, GATA6 induction in human ES (hES) cells also downregulates pluripotency gene expression and upregulates extraembryonic endoderm genes, revealing a conserved function in mediating this cell fate switch. GATA6-induced human cells exhibit a distinct molecular signature when compared to definitive endoderm cells, and are similar to single cells isolated from the primitive endoderm compartment of the human blastocyst. Altogether, this demonstrates that GATA6 is a versatile and potent reprogramming factor that has the potential to establish stable human XEN cells.

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T2079

REGENERATION OF CARDIOMYOCYTES FROM FIBROBLASTS IN VIVO IN A PIG MODEL

Yu, Pengzhi (Palmer)[1], Radzinsky, Ethan[1], Chin, Albert[2], Huang, Yu[1], Metzler, Scott A.[1], Saeed, Maythem[3] and Srivastava, Deepak[1], [1]Gladstone Institutes, University of California, San Francisco, San Francisco, CA, U.S., [2]Pavillion Medical Innovations, Boston, MA, U.S., [3]University of California, San Francisco, San Francisco, CA, U.S.

We previously reported that the forced expression of three transcription factors (Gata4, Mef2c and Tbx5) 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 more transcription factors, ESRRG and MESP1, were sufficient to initiate cardiac reprogramming in vitro. In this study, we investigated whether these five factors could induce conversion of fibroblasts to cardiomyocytes in vivo in pigs as a pre-clinical model. Two days after myocardial infarction created by catheter occlusion of the left ventricular descending artery, we did magnetic resonance imaging (MRI) to assess the damage to cardiac function. We intra-myocardially delivered retroviral vectors expressing DsRed only, or together with genes encoding the human cardiac reprogramming factors, in the peri-infarct region four days post MI. 8 weeks after the retroviral infection, we did MRI to assess cardiac function again and explanted the heart for histological analysis. 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 structures and morphology. Ejection fraction calculated from our preliminary MRI data showed potential recovery of cardiac function from pigs injected with human cardiac reprogramming factors. But we still need to operate on more pigs to validate this. 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.

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IPS CELLS

T2083

COMPARISON OF DIFFERENTIATION POTENTIAL OF HUMAN NUCLEAR TREANSFER-EMBRYONIC STEM CELLS AND ISOGENIC INDUCED PLURIPOTENT STEM CELLS

An, Borim[1], Heo, Hye-Ryeon[1], Kim, Eunbi[1], Kim, Woo Jin[1], Yang, Se-Ran[2] and Hong, Seok-Ho[1], [1]Kangwon National University, Chuncheon, Korea, South, [2]Kangwon National University, Chuncheon, Korea

Induced pluripotent stem cells (iPSCs) generated from somatic cells by introduction of reprogramming factors and embryonic stem cells (ESCs) generated by nuclear transfer (NT) into an unfertilized oocytes have attracted as an ideal source for personalized regenerative medicine. However, functions, phenotypes and gene expressions according to developmental stages of derivatives from human iPSCs and NT-ESCs have not been compared. In this study, we employed human iPSCs and NT-ESCs derived from the same donor cells and generated alveolar epithelial progenitor type II (AETII) cells using a stepwise induction protocol. We analyzed the morphology, functions (surfactant protein B and C), and phenotypes (carboxypeptidase M, NKX2.1, and EPCAM) of AETII cells using immunocytochemistry, real-time PCR, and flow cytometry. AETII cells derived from human iPSCs and human NT-ESCs exhibited a typical cuboidal morphology and expressed similar level of AETII cell specific markers including carboxypeptidase M, NKX2.1 and EPCAM. Surfactant protein B and C were detected as the AETII cells mature. This study is the first to compare functional, phenotypic, and genetic similarity and difference of AETII cells derived from human NT-ESCs and isogenic iPSCs, and provides better understanding of how derivation approaches contribute to differentiation potential. Further study will be needed to compare the genetic and epigenetic signatures between NT-ESCs and isogenic iPSCs, which may clari-

fy how reprogramming approaches are reflected in outcomes of differentiation.

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T2085

EFFECT OF EXTRACELLULAR MATRICES ON THE COLONY FORMING EFFICIENCY OF HUMAN PLURIPOTENT STEM CELLS CULTURED IN STEMFIT™AK

Chang, Jessica, Agung, Eviryanti, Matsumoto, Takuya, Senda, Sho and Kobayashi, Tsuyoshi, Institute for Innovation, Ajinomoto Co., Inc., Kanagawa, Japan

We have developed a defined, xeno-free culture medium, StemFit™AK, capable of robust feeder-free maintenance of undifferentiated human pluripotent stem cells (hPSCs). When used in combination with recombinant human laminin-511 E8 fragment, hPSCs could be easily and stably passaged as single cells, allowing for standardized expansion [Nakagawa et al., 2014]. At ISSCR 2015, we reported that the iPS cell line, 201B7, could be successfully cultured on commercially available extracellular matrixes (ECM) using StemFit™AK after transition from on feeder to feeder-free culture. We also reported that the medium also supports efficient growth and colony formation, even at very low seeding densities required for cloning and single-cell analysis. To maximize the efficiency of colony formation using StemFit™AK, we investigated the effect of ECMs on growth and colony forming efficiency. First, we confirmed that hiPSCs cultured in StemFit™AK could be maintained using different ECMs for at least four weeks. We next examined the effect of ECMs on the characteristics of hiPSCs by evaluating colony forming efficiency at very low seeding densities on different ECMs after single cell dissociation of hiPSC colonies. To do this, we examined the expression of cell adhesion and signaling molecules by quantitative PCR and immunochemistry. From these studies, we have identified characteristics of hiPSCs during growth and maintenance, and how these characteristics could be controlled through the manipulation of culture conditions.

T2087

CURCUMIN WITH THERAPEUTIC POTENTIAL IN BEST DYSTROPHY DISCOVERED BY USING PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS-DRUG SCREENING SYSTEM

Chiou, Shih-Hwa, Department of Med Res & Education, Taipei Veterans General Hosp, Taipei, Taiwan

Best dystrophy (BD), also termed best vitelliform macular dystrophy (BVMD), is a juvenile-onset form of macular degeneration and central visual loss. Unfortunately, there is no definite therapy for BD or improving the visual functional on this progressive disease. Human induced pluripotent stem cell (iPSC) system present unique opportunities that are starting to be successfully explored for genetic and chemical screening. This could be crucial in moving forward iPSC-based therapies. 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. Compared with unaffected sibling-derived iPSC-derived RPE cells (ctrl-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. Using the iPSC-based drug screening system, we further found the curcumin could significantly improve the mRNA expression levels of Best gene in BD-iPSC-derived RPE cells. Notably, we discovered curcumin to enhance the expression of antioxidant enzymes, which was accompanied by a decrease in intracellular ROS production and hydrogen peroxide induced oxidative stress. In summary, these data suggest that curcumin provide a potential therapeutic value of cytoprotection by regulating the anti-oxidative capabilities of degenerated RPEs from Bestrophy disease.

T2089

HO-1 AND NRF-2 DEFICIENCY AFFECTS GENERATION AND DIFFERENTIATION OF INDUCED PLURIPOTENT STEM CELLS

Stepniewski, Jacek, Pacholczak, Tomasz, Skrzypczyk, Aniela, Jozkowicz, Alicja and **Dulak, Jozef**, Jagiellonian University, Krakow, Poland

Activation of p53, a major roadblock for successful iPSCs generation, can be triggered by increased oxidative stress. Therefore, here we evaluated the role of heme oxygenase-1 (HO-1, encoded by Hmox1 gene) and Nrf2 (encoded by Nfe2l2 gene), two major anti-oxidative and cytoprotective factors, in reprogramming process. Addi-

tionally, their effect on iPSCs differentiation was investigated. Hmox1$^{-/-}$ fibroblasts demonstrated elevated level of p53 and p53-regulated miR-34a and 14-3-3σ protein, which can halt cells in the G2/M phase of cell cycle, what was also observed in Hmox1$^{-/-}$cells. Additionally, the lack of HO-1 resulted in increased expression of miR-29a, let-7d as well as Hdac5 and Hdac7. Interestingly, reprogramming provided a decreased number of iPSCs colonies generated from Hmox1$^{-/-}$ cells. Similarly impaired reprogramming was observed in Nrf2-lacking fibroblasts, which demonstrated lower level of HO-1 in comparison to their control counterparts, although miR-34a was decreased in Nfe2l2$^{-/-}$ cells. Importantly, increased reprogramming was observed upon stimulation of fibroblasts with sulforaphan (Nrf2 activator) or cobalt protoporphyrin (CoPPIX) (HO-1 activator). Interestingly, valproic acid (VA) shown previously to enhance reprogramming, in our hands decreased expression of HO-1 in murine fibroblasts, what was followed by less efficient iPSCs generation.

Hmox1$^{+/+}$ and Hmox1$^{-/-}$ iPSCs, as well as Nfe2l2$^{+/+}$and Nfe2l2$^{-/-}$ cells demonstrated similar expression of pluripotency markers such as Oct-4, Sox2 and miRNAs belonging to miR-290 family. Additionally, Hmox1$^{+/+}$ and Hmox1$^{-/-}$ iPSCs were able to spontaneously differentiate via embryoid bodies to cells originating from three germ layers. However, lower number of contracting clusters was observed in HO-1-lacking cells. Interestingly, no teratomas were formed by Hmox1$^{-/-}$ iPSCs upon subcutaneous injection into immunocompromised mice (0 of 8 injections) in contrast to their control counterparts (5 of 8). Similar effects were not observed in case of Nfe2l2$^{-/-}$ iPSCs since their pluripotency was confirmed both in vitro and in vivo. These results indicate that HO-1 and Nrf2 deficiency attenuates reprogramming efficacy whereas the lack of HO-1 may additionally affect differentiation potential of iPSCs.

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T2091

SUMATRAN ORANGUTAN INDUCED PLURIPOTENT STEM CELL GENERATION AND CEREBRAL ORGANOID FORMATION

Field, Andrew, LaMontagne, Erin, Phillips, Alex, Reyes-Ortiz, Andrea, Whitehead, Lila, Meng, Vincent, Salama, Sofie and Haussler, David H., University of California, Santa Cruz, Santa Cruz, CA, U.S.

The orangutan was the first Great Ape species to diverge from the main hominid line making it the evolutionary bridge between highly complex and neurologically developed apes and more distant primates. Because of its critically endangered status, research on the orangutan has been limited, thus, we have created orangutan induced pluripotent stem cell (iPSC) lines that allow us to research early developmental processes without harming any animals. When used as a model system, these cells can be an infinite source of tissue for studying early embryonic development enabling detailed molecular analysis of these processes.Using Sumatran orangutan fibroblast cells acquired from Oliver Ryder at the San Diego Frozen Zoo, we generated multiple iPSC lines. Due to their origin, we have the knowledge of the donor animals' medical histories, quality of life, and other biological data deeming them a valid standard for genomic and developmental studies. These cell lines were reprogrammed using Sendai virus, an integration-free technique that allows the reprogramming factors to clear from the cells after a heat treatment and several mitotic divisions. Pluripotency genes were confirmed active in the cell lines. Further, these lines successfully formed teratomas *in vivo,* and differentiated into cell types of all three embryonic germ layers *in vitro.* We are currently focused on using two orangutan iPSC lines for further analysis.The first direction we are pursuing with the orangutan iPSCs is studying primate brain development. The cerebral cortex has been the most rapidly expanding component of the brain throughout primate evolution. To better understand gene expression patterns involved in complex cortical development, we are directing differentiation of the orangutan iPSCs into 3D neural cortical organoids to trace transcriptomic changes as they relate to tissue formation throughout development. The developmental data will contribute to a cross-species comparison examining transcription throughout corticogenesis in humans, chimpanzees, and rhesus macaques to identify genes involved in the advanced cortical development of the Great Apes.

Funding Source: Howard Hughes Medical Institute, National Institutes of Health, California Institute for Regenerative Medicine

T2093

CREATING PATIENT-SPECIFIC STEM CELL THERAPIES FOR HEMOPHILIA B VIA CRISPR/CAS9 GENE CORRECTION

Green, Kevin[1], Ramaswamy, Suvasini[1], Tonnu, Nina[1], Menon, Tushar[1,2] and Verma, Inder[1], [1]Salk Institute, La Jolla, CA, U.S., [2]Vertex Pharmaceuticals, La Jolla, CA, U.S.

Hemophilia B is a rare, X-linked genetic disorder, affecting an estimated 20,000 individuals in US and is caused by a defect in a clotting protein, Factor IX (FIX). This defect in FIX function, caused by mutation in the FIX gene, leads to excessive bleeding, internal hemorrhages and joint arthropathies. Current therapies are transient and fraught with issues: increased risk of blood-born infections, high costs, limited availability, and short half-life of the admin-

istered proteins. This project aims to correct FIX deficiency by creating a stem cell based therapy utilizing patient-specific induced pluripotent stem cells (iPSCs) and CRIPSR/Cas9 gene editing technologies for treatment for Hemophilia B. My main focus of the project will be to implement a gene correction designed to circumvent patient-specific sequencing and to develop a CRISPR/Cas9 based method of correction that can be used universally for all patients to restore levels of functional FIX. We propose to do this by knocking-in a functional copy of the FIX cDNA downstream of the endogenous promoter but upstream of the translation start site. This way, irrespective of where a mutation is present in the coding region, a fully functional FIX protein will be produced under its endogenous promoter. Currently, we have initiated the screen by nucleofecting the donor DNA along with the Cas9 and the targeting guides into iPSC lines from a patient. After a PCR based screen, we have identified a number of putative corrected clones for further testing and characterization. These if corrected, will be directionally differentiated into hepatocytes through an optimized differentiation protocol for both in vitro and in vivo efficacy studies with the eventual goal of creating a safe, inexpensive, and permanent treatment for Hemophilia B.

Funding Source: Funding for this project is provided by the California Institute for Regenerative Medicine Early Translational Grant TR4-06809 under Dr. Inder Verma at the Salk Institute for Biological Studies.

T2095

AN IMPROVED PROTOCOL FOR GENERATION AND IDENTIFICATION OF IPS CELL LINES FOLLOWING SIMULTANEOUS REPROGRAMMING AND GENE EDITING OF HUMAN FIBROBLASTS

Howden, Sara E Emily[1], Bateman, John[1], Lockhart, Paul[1], Petrou, Steven[2], Elefanty, Andrew George[1], Stanley, Ed[1] and Thomson, James A.[3], [1]Murdoch Childrens Research InstituteThe Royal Children's Hospital, Parkville, Australia, [2]University of Melbourne, Parkville, Australia, [3]Morgridge Institute for Research, Madison, WI, U.S.

Autologous cell therapies based on genetically repaired patient-derived induced pluripotent stem cells hold great promise for the treatment of many inherited and acquired diseases. We recently reported that the processes of gene correction and reprogramming could be performed together in a simple one-step procedure using episomal-based reprogramming vectors and the Cas9/CRISPR gene targeting system. We have since modified our protocol to include transfection of mRNA (rather than a plasmid) encoding Cas9 to facilitate more transient and uniform expression of the Cas9 protein. Additionally, we now use plasmid DNA rather than single-stranded oligonucleotides as a DNA repair template following the revelation that incorrectly synthesized oligonucleotides can potentially introduce undesirable mutations at frequencies of up to 30% in targeted clones. We have also found that transfection of supercoiled plasmid DNA is significantly more effective than linearized DNA for gene targeting by homologous recombination, as determined by reporter expression following introduction of EGFP into the endogenous DNMT3B locus. We have further demonstrated the robustness of our revised protocol through the generation of multiple genetically repaired iPS cell lines from numerous human fibroblast lines, including those derived from patients with skeletal dysplasia, cardiac dysfunction and epilepsy caused by mutations in the COL1A1, ALPK3 and SCN2A genes respectively. We have also used this approach to effectively introduce specific mutations into normal cells. Lastly, we describe a simple and rapid allele-specific PCR-based protocol that is free of genomic DNA extraction and purification steps for screening and identification of gene-edited iPS cell colonies.

Funding Source: NHMRC

T2097

PATIENT MOTOR NEURONS UNVEIL EARLY MITOCHONDRIAL DYSFUNCTION AND AMINO ACID METABOLIC PATHWAY PERTURBATIONS IN C9orf72 ALS

Kaus, Anjoscha Samija, Manfredo, Berhan, Kerl, Alexis, Gross, Andrew, Yang, Wei and Sareen, Dhruv, Cedars-Sinai, Los Angeles, CA, U.S.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting upper and lower motoneurons (MNs). While ALS is largely sporadic, intronic G_4C_2 repeat expansion mutations in C9orf72 have been identified in a subset of familial and sporadic patients. Clinically, sporadic and familial ALS appears similarly with a rapid course of disease progression. Current therapeutics are largely ineffective in significantly prolonging life for ALS patients. Thus there is a great need for developing early presymptomatic ALS signatures and biomarkers. We identified metabolic signatures in C9orf72 ALS patient-derived induced pluripotent stem cells (iPSCs) MN cultures via quantitative proteomic analysis suggesting early pre-symptomatic signs of impaired mitochondrial functioning in bioenergetics handling and amino acid metabolism. Among the 5,433 protein groups analyzed, 208 displayed highly significant differences in ALS patient derived lines, with 89 of those being mitochondrial protein groups. Aside from impaired protein functions in oxidative phosphorylation, TCA cycle, and tRNA charging, functional alterations in amino acid synthesis stood out. Specifically, the candidates Ornithine Aminotransferase (OAT), an enzyme crucial for Proline synthesis, and Glycine Amidinotransferase (GATM), essential for creatine synthesis, were dramatically up-regulated in ALS samples, as con-

firmed by Protein detection via Western blotting analysis. The two candidates closely intersect as GATM produces ornithine as a by-product which is the crucial substrate for OAT to synthesize Proline. This finding was in agreement with elevated levels of tRNA ligases, suggesting that an early C9orf72 ALS phenotype may be an exceedingly high demand of G_4C_2 repeat peptides, such as Proline, which could lead to an imbalance in protein-synthesis and supply and triggering of stress pathways. Screening for dysregulated elements of protein synthesis may serve as an early marker of MN dysfunction in C9orf72 ALS mitochondrial metabolic related pathways and can identify factors increasing MN longevity towards discovery of novel targeted therapeutics for ALS.

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T2099

AN EFFICIENT CONSTRUCTION OF LENTIVIRAL VECTORS THAT IDENTIFY AND ELIMINATE TUMORIGENIC CELLS DERIVED FROM PLURIPOTENT STEM CELLS

Kosai, Ken-ichiro, Ide, Kanako and Mitsui, Kaoru, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan

Human pluripotent stem cells (hPSCs) are promising sources for cell transplantation therapy. However, incomplete abolition of tumorigenicity, including teratomas and cancers, causes potential safety concerns in their clinical trials. Importantly, most previous approaches focused on “INDIRECT” inhibition of tumorigenicity by reducing the reprogramming-associated oncogenic potential of iPS cells. Because they cannot completely eliminate tumorigenic potentials due to the intrinsic characteristics of hPSCs, innovative safety approaches should be developed. Here we present the antitumorigenic strategy by a novel methodology that can efficiently generate diverse “Tumorigenic Cell-targeting Lentiviral Vectors (TC-LVs). TC-LVs has a recombination cassette for comprehensive analysis of the candidate promoters, upstream to the unit consisting of fluorescent genes, 2A sequence and suicide genes. The different promoter types of TC-LVs are able to make easily and rapidly using recombinase. Tumorigenic cells in the transduced hPSCs can be specifically identified and efficiently killed by fluorescent genes and suicide genes, respectively, under the candidate promoters, which should be specifically and strongly activated in the undifferentiated and/or the transformed hPSCs, but not in the differentiated one. To test the efficacy of this system, hPSCs were transduced with TC-LVs composing Venus (EGFP) and Herpes Simplex Virus Thymidine Kinase (HSV-tk) genes driven by either of the ubiquitously active CA or the cancer-specific survivin promoter. All of the hPSCs in the undifferentiated status were almost perfectly killed by an addition of ganciclovir (GCV) in the GCV dose-dependent and the promoter activity-dependent manners, whereas they were viable in the differentiated status. The results importantly suggest the necessity of the best combination of the promoter and the suicide and the fluorescent genes. In conclusion, we have developed the novel method for a rapid generation of TC-LVs that can systematically identify the best promoter and suicide gene to surely eliminate tumorigenic cells, without harmful effect to the differentiated cell. This methodology may facilitate the safe clinical application of hPSCs-based cell therapy.

T2101

STEM CELL THERAPY AFTER WHITE MATTER STROKE

Llorente, Irene L.[1], Miller, David Ryan[1], Mazzitelli, Jose Alejandro[1], Cinkornpumin, Jessica[2], Lowry, William[1] and Carmichael, S Thomas[3], [1]UCLA, Los Angeles, CA, U.S., [2]University of California, Los Angeles, Long Beach, CA, U.S., [3]University of California Los Angeles, Department of Neurology, Los Angeles, CA, U.S.

Subcortical white matter stroke (WMS) constitutes up to 30% of all stroke subtypes. There is no medical therapy that promotes recovery in this disease. Human induced pluripotent stem cells (iPSs) have shown benefit in some pre-clinical stroke models, but have never been tested in white matter stroke. In this study two stem/progenitor cell lines were used: human iPS- neuronal progenitor cells (NPCs) and human iPS- glial enriched progenitor cells (GEPs)- this cell line is differentiated from an iPSC-NPC line into an immature glial cell. iPS-GEPS may be uniquely suited to repairing the brain after white matter stroke, as it produces immature glial cells that may both stimulate the injured cerebral white matter, and differentiate into replacement cells that have been lost in the stroke. We tested the effects of iPS-NPC and iPS-GEP transplantation in NSG mice in a subacute stage (7 days post-stroke) and performed tissue, MRI and behavioral outcome measures up to 6 months after transplantation. Both stem/progenitor cell lines presented a good engraftment and survival after 2 months. iPS-NPCs and iPS-GEPs behave very differently after transplantation. iPS-GEPs migrate widely in white matter after transplant, while the iPS-NPCs remained cluster in the infarct area. WMS. iPS-GEPs treatment stimulated endogenous neurogenesis, defined as the production of new neurons and glial cells, at a level 4 times than that seen with iPS-NPCs. Both iPS-NPC and iPS-GEP treatment led to remyelination in the stroke area 2 months post-transplantation (assessed by MBP staining). Motor testing showed that iPS-GEPs uniquely improved motor recovery after stroke compared to the stroke-alone and stroke + iPS-NPCs. This white matter repair can be tracked in the living animal in real time with MRI, providing a biomarker for brain repair with this cell

line in this disease. These findings suggest that transplanted iPS progenitor cells could potentially replace lost cells and circuitry in ways that are unique to the subtype of the iPS progenitor: GEP vs NPC

T2103

ASSESSMENT OF GENETIC INSTABILITY IN HUMAN INDUCED PLURIPOTENT STEM CELLS DURING LONG-TERM CELL CULTURE

Miura, Takumi[1], Okamura, Kohji[2], Yasuda, Satoshi[1], Umezawa, Akihiro[2] and Sato, Yoji[1], [1]National Institute of Health Sciences, Tokyo, Japan, [2]National Research Institute for Child Health and Development, Setagaya, Japan

Somatic mutations are thought to contribute to tumorigenicity or immortalization of normal cells. Recent studies indicate that human-induced pluripotent stem cells (iPS cells) have DNA changes involving a wide variety of somatic mutations in coding regions. Therefore, a better understanding of genetic basis of iPS cell-based products is expected to benefit their assessments of safety or efficacy in future regenerative medicine. However, it is controversial whether these mutations may have been introduced during the reprogramming process, either due to replication errors in culture or due to clonal expansion of pre-existing somatic mutations in iPS source cells. In this study, to monitor the patterns of somatic mutation accumulation during long-term culture in human iPS cells, we evaluated the somatic mutation rate during human iPS cell passaging by whole exome sequencing. The raw data of 15, 30, 40 Gb per exome from iPS cells collected at every 3 passages (until 15 passages) was mapped to the haploid human reference sequence hg19 (GRCh37). In 40Gb sequencing, we compared the performance of three somatic single nucleotide variant (SNV) calling algorithms (Strelka, Virmid, GATK) for exome sequencing data. As a result, even SNVs at low allele frequency (< 5%) was detectable with high accuracy by Strelka and GATK. Chromosomal aberrations such as trisomy 12 were also predicted based on their pattern of SNVs accumulated in the whole exome sequences at all points of passage. Furthermore, we found a few protein coding mutations accumulated during passaging of iPS cells, suggesting that they possibly involve the acquisition of growth advantage. Our results suggest that the accurate determination of spontaneous mutation rates during cell culture provides strong insights into phenotypic changes in a cell population, which would be useful for establishment, quality control, storage and distribution of iPS cell banks/ stocks, as well as for our understanding of these processes.

T2105

AN ETHNICALLY DIVERSE PANEL OF HUMAN PLURIPOTENT STEM CELLS FOR PHARMACOGENOMIC STUDIES

Nguyen, Thomas[1,2], Fakunle, Eyitayo S.[2], Zakinova, Angela[2], Pivaroff, Cullen G.[2], Pratola, Victoria[2], Schell, John Paul[3], Peterson, Suzanne E.[2] and Loring, Jeanne F.[2], [1]California State University San Marcos, San Marcos, CA, U.S., [2]The Scripps Research Institute, San Diego, CA, U.S., [3]Karolinska Institutet, Stockholm, Sweden

Drug development is a lengthy and expensive process that can cost upward of several billion dollars and require 10-15 years. One reason for these exorbitant costs is “post-marketing drug failure”, in which drugs are recalled after billions of dollars have been spent getting the drugs to market. The primary reason for post-marketing drug failure is idiosyncratic drug induced liver injury (DILI). There is extensive evidence showing DILI may frequently be caused by ethnicity-based variations in drug metabolizing enzymes and drug targets (e.g., receptors) that affect drug toxicity and efficacy. No practical means currently exist to screen drugs in vitro for genome variation-associated toxicity. Current methods use in vitro testing and animal studies, and human clinical trials, all of which cannot efficiently and accurately capture 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 and have derived iPSCs from primary cells obtained from a multiethnic cohort of healthy individuals, including several Africans, African Americans, Asians, Middle Easterners, Latin Americans, Native Americans, and Caucasians. Using immunocytochemistry, embryoid body-based differentiation techniques, and PluriTest® we have demonstrated that the ethnically diverse iPSC lines analyzed thus far are pluripotent. In addition, characterization by whole genome single nucleotide polymorphism (SNP) analysis indicates that the reported ethnicity of the lines generally 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.

T2107

GENERATION OF INDUCIBLE HUTCHINSON-GILFORD PROGERIA SYNDROME MODEL USING HUMAN PLURIPOTENT STEM CELLS

Park, Kyeyoon, National Institutes of Health, Bethesda, MD, U.S. and Robey, Pamela G., NIDCR/NIH/DHHS, Bethesda, MD, U.S.

Hutchinson-Gilford Progeria Syndrome (HGPS) is a genetic disorder characterized by an appearance of accelerated aging which includes scleroderma-like skin, limited growth, full-body alopecia, atherosclerosis, kidney failure, and cardiovascular problems. HGPS is caused by mutations in LMNA gene. LMNA encodes prelamin A protein which becomes lamin A after its C-terminal domain including farnesyl group is truncated. Mutant prelamin A, also called progerin, does not contain the truncation signal and retains the farnesyl group. In HGPS patients, progerin accumulation causes progressive molecular defects including nuclear shape abnormalities and DNA damage. The vascular phenotype in HGPS patients shows similarity to those seen with age, suggesting the potential importance of HGPS as a model for normal aging. Here, human induced pluripotent stem cells (iPSCs), derived from skin fibroblasts from normal donors were modified to express a progerin-RFP fusion protein in a doxycycline-inducible manner. In order for this, targeted genome engineering was employed to integrate the Tet transactivator expression unit in a safe-harbor site, the CLYBL (citrate lyase beta like) locus on chromosome 13 in the human genome, and the retrovirus was used to introduce TRE-LMNA-RFP expression unit. The resulting iPSC lines enable us to manipulate the progerin protein level, and study its effect on cell survival and function under different conditions in various types of cells such as pluripotent cells, cardiomyocytes, and vascular endothelial cells.

T2109

AKT/GSK3B SIGNALING PATHWAY IS CRITICALLY INVOLVED IN HUMAN PLURIPOTENT STEM CELL SURVIVAL

Romorini, Leonardo[1], Garate, Ximena[1], Neiman, Gabriel[1], Luzzani, Carlos[1], Furmento, Verónica[1], Guberman, Alejandra S.[2], Sevlever, Gustavo[1] and Miriuka, Santiago[1], [1]LIAN-FLENI, Buenos Aires, Argentina, [2]UBA/CONICET, Buenos Aires, Argentina

Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are self-renewing pluripotent stem cells (PSC) that can differentiate into a wide range of specialized cells. Basic fibroblast growth factor (bFGF) has been proved to be essential to both hESCs and hiPSCs survival, stemness and self-renewal. One of its downstream targets, PI3K, and its most prominent effector, AKT, regulate cell viability and apoptosis in many different cell types. Although it is well known that PI3K/AKT activation by bFGF is relevant for PSC stemness maintenance, the role of this signaling pathway on PSC survival has not yet been fully elucidated. The aim of this work was to explore the relevance and molecular mechanisms involved in the regulation of hESCs (WA09/H9 and WA01/H1 lines) and hiPSCs (FN2.1 line) survival by AKT. We found that pharmacological inhibition of AKT with three non-structurally related inhibitors (GSK690693, AKT inhibitor VIII and AKT inhibitor IV) decreased cell viability in a concentration dependent manner. We next analyzed several aspects of programmed cell death and found an increase in the translocation of phosphatidylserine (PS) to the outer leaflet of the plasma membrane and in the extent of DNA fragmentation as soon as 8 hours after AKT inhibitors addition in PSC. Moreover, Western blot analysis revealed the activation of the initiator Caspase-9, the effector Caspase-3, and PARP cleavage at different time points after AKT inhibition. However, no relevant changes in BCL-2 family members BCL-XL, BCL-2 and BAX protein products were found. Importantly, we observed that GSK3β signaling is responsible, at least in part, of the apoptosis induction triggered by AKT inhibition. Importantly, pharmacological inhibition of GSK3β with CHIR99021 decreased basal apoptosis rates and induced proliferation of PSC cultured under standard conditions. Collectively, in this study we demonstrated that AKT signaling activation, partly mediated by GSK3β inhibition, prevents apoptosis and thus results relevant for human PSC survival.

T2111

MODELLING SPINAL AND BULBAR MUSCULAR ATROPHY (SBMA) WITH MOTOR NEURONS DERIVED FROM PATIENT-SPECIFIC IPS CELLS

Sheila, Marianne, Genome Institute of Singapore, Singapore

Spinal bulbar muscular atrophy (SBMA), also known as Kennedy's Disease, is an X-linked recessive neuromuscular disorder characterized by the selective degeneration of lower motor neurons within the spinal cord and brainstem. SBMA results from the abnormal expansion of the CAG repeat in exon 1 of the androgen receptor (AR) gene and thus is categorized as one of a group of nine trinucleotide repeat disorders. The selective loss of motor neurons in SBMA results in limb and bulbar muscle atrophy and weakness, mobility problems, swallowing and speech difficulties, gynaecomastia and reduced fertility. Although there is widespread expression of AR in various tissues and organs, the exact mechanism of selective motor neuron loss within the spinal cord and brain stem remains elusive. Several lines of evidence point to transcriptional dysregulation and the consequent perturbation in the gene regulatory network controlled by AR to be contributing factors in the pathology of SBMA. To further understand the pathophysiology of SBMA and the dysregulation in

the genomic network, our research in SBMA is focused on two main areas: (1) modelling the selective death of motor neurons in SBMA using patient-specific iPS cells which are induced to differentiate into motor neurons; and (2) adopt a transcriptomic approach in identifying genes that are involved in motor neuron degeneration. This approach aims to shed light on the molecular basis and pathways dysregulated in SBMA resulting in motor neuron degeneration and the eventual goal of establishing novel therapeutic strategies for SBMA treatment.

T2113

HYBRID CELLULAR METABOLISM COORDINATED BY ZIC3 AND ESRRB SYNERGISTICALLY ENHANCES SOMATIC CELL REPROGRAMMING

Sone, Masamitsu[1], Yamanaka, Shinya[2] and Yamamoto, Takuya[1], [1]Kyoto University, Kyoto, Japan, [2]Gladstone Institutes, San Francisco, CA, U.S.

Naïve pluripotent stem cells utilize both glycolysis and oxidative phosphorylation (OXPHOS) pathways to satisfy their metabolic demands. However, it is unclear how somatic cells acquire this hybrid energy metabolism during reprogramming toward naïve pluripotency. Here, we show that Zic3 and Esrrb synergistically enhance the reprogramming efficiency by regulating cellular metabolic pathways when transduced with Oct4, Sox2 and Klf4 (OSK) into murine fibroblasts. Zic3 recruited Esrrb to its own binding sites, some of which are proximal to glycolysis-related genes, thereby cooperatively upregulating these genes to activate glycolytic metabolism. On the other hand, the regulatory modes of these transcription factors (TFs) on OXPHOS were antagonistic: Zic3 repressed OXPHOS, while Esrrb activated it. Therefore, when introduced with Zic3, Esrrb restored OXPHOS activity, which was essential for efficient reprogramming. Our study suggests that an appropriate balance of metabolic pathways is achieved by the combinatorial function of TFs in the process of somatic cell reprogramming.

T2115

DYSFUNCTIONAL SODIUM CURRENTS AND ALTERED PHARMACOLOGY OF SCN8A MUTANT HUMAN iPSC-DERIVED NEURONS

Tidball, Andrew, Lopez-Santiago, Luis, Isom, Lori and Parent, Jack, University of Michigan, Ann Arbor, MI, U.S.

De novo mutations in SCN8A have been found to cause a newly characterized early infantile epileptic encephalopathy, EIEE13. More than 100 patients with these mutations have been identified in recent years with the disease typically manifesting with intractable seizures, intellectual and physical disabilities in later life as well as high rates of SUDEP (Sudden Unexpected Death in Epilepsy). The goal of this research project is to use patient-derived cells to characterize electrophysiology alterations caused by SCN8A mutations associated with early-onset epilepsy and to develop a novel platform for identifying effective pharmacological agents for this debilitating disease. SCN8A encodes for the voltage-gated sodium channel (VGSC) $Na_v1.6$, the most abundant Na_v channel. We have identified two EIEE13 patients with SCN8A missense mutations (Arg1872>Leu and Val1592>Leu). Fibroblasts from the patients' skin biopsies were reprogrammed into induced pluripotent stem cells (iPSCs) and then differentiated into excitatory cortical-like neurons. Using whole-cell patch-clamp recordings, we discovered an increase in the percentage of peak transient sodium current density that is persistent or non-inactivating (from 2.5% to 6.25%) and decreased peak transient sodium current density (from -284 pA/pF in control to -127 pA/pF) in the patient neurons. The same iPSC-derived neurons were also cultured on multi-well multieclectrode array plates to measure spontaneous action potentials before and after acute exposure to anti-epileptic drugs (AEDs). We used two AEDs known to have efficacy in the patient population (oxcarbazepine and lamotrigine), one AED thought to be ineffective (levetiracetam), and one compound shown to preferentially bind to VGSCs in the inactivated state (riluzole). All of the drugs, except levetiracetam, partially inhibited activity at concentrations found in patient CSF and showed clear dose-dependent inhibition. Interestingly, at low concentrations, riluzole had a significantly greater effect on control cells (72% inhibition) than patient cells (23% inhibition) potentially indicating dysfunctional inactivation in the mutant channels. This finding suggests a possible mechanism for the observed increase in the percentage of sodium current density that is persistent current.

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T2117

INDUCED PLURIPOTENT STEM CELL GENERATION FROM DIABETIC PATIENTS MONONUCLEAR CELLS AND DIFFERENTIATION TOWARDS FUNCTIONAL ENDOTHELIAL CELLS TO STUDY ENDOTHELIAL CELL DYSFUNCTION

Vilà González, Marta, Kelaini, Sophia, Amstrong, David, Lois, Noemi, Grieve, David, Stitt, Alan and Margariti, Andriana, Queen's University Belfast, Belfast, U.K.

Diabetes affects around 300 million people worldwide. People who suffer from diabetes very often develop macro and/or microvascular complications such as cardiovascular diseases and diabetic retinopathies that high-

ly increase its morbidity/mortality. Endothelial cell (EC) dysfunction is the triggering event of these complications, although the exact mechanisms that underlie this impairment are still not completely understood. Induced pluripotent stem cell (iPSC) technology is widely used for disease modelling, in addition to drug screening and, potentially, regenerative medicine. However, the existing methods to obtain iPSCs still need optimisation in order to be more efficient, less time consuming, more cost effective and more patient compliant. In our lab, we have generated and characterised iPSCs from peripheral blood mononuclear cells (PBMCs) obtained from both healthy donors and diabetic patients.

With the ultimate goal to study the EC dysfunction in these patients, iPSCs were subjected to EC differentiation. The iPSCs were cultured on feeder-free conditions in StemPro-34 (Invitrogen) for 4 days in the presence of bone morphogenic protein 4, Activin A and basic fibroblast growth factor (bFGF) to induce differentiation towards cardiac mesoderm. The embryoid bodies which had formed were then cultured with VEGF and bFGF for 2 further days before selection for KDR^+ cells was performed using magnetic activated cell sorting (MACS). KDR^+ cells were further differentiated towards the EC phenotype, with the cells by this stage showing an EC-like morphology. Quantitative real time PCR analysis also found that expression of EC progenitor markers CD34 and KDR were significantly increased at day 6 of differentiation. These EC-like cells were designated as hiPS-ECs and maintained in culture.

These functional and stable hiPS-ECs have provided the basis for in vitro assessment of patient-specific microvascular EC function. Moreover, the development of this protocol has allowed us to generate iPSCs from diabetic patients' PBMCs, which is a more patient compliant cell source, in an efficient way. We believe that this method will lead to successful generation of iPSCs from other types of patients and to the elucidation of the EC dysfunction in diabetic patients, and will bring us closer to personalised medicine.

T2119

HUMANITY IN A DISH: UNCOVERING THE COMMON GENETIC BASIS FOR HUMAN METABOLIC DISEASE WITH IPSCS

Warren, Curtis Robert[1,2], Friesen, Max[2,3], Becker, Caroline[4], O'Sullivan, John[5], Choi, Jihoon[4], Xia, Fang[2,4], Zhang, Xiaoling[6], Peters, Derek[2,4], Wakabayashi, Ph.D., Yoshi[6], Florido, Mary H. C.[2,4], Shay, Jennifer[2,4], Daheron, Laurence M.[4], Zhu, Jun[6], Gerszten, Robert E.[5], Deo, Rahul[7], Ramachandran, Vasan[8,9], O'Donnell, Christopher J.[9] and Cowan, Chad[2,5], [1]Harvard University, Somerville, MA, U.S., [2]Harvard University, Cambridge, MA, U.S., [3]Harvard Stem Cell Institute, Somerville, MA, U.S., [4]Harvard Stem Cell Institute, Cambridge, MA, U.S., [5]Massachusetts General Hospital, Boston, MA, U.S., [6]National Heart, Lung, and Blood Institute, Bethesda, MD, U.S., [7]University of California, San Francisco, San Francisco, CA, U.S., [8]Boston University, Boston, MA, U.S., [9]The Framingham Heart Study, Framingham, MA, U.S.

The NHLBI Framingham Heart Study (FHS) is a long-term, ongoing cardiovascular study that has been following three generations of residents of Framingham, Massachusetts, including ~5,000 individuals in the Framingham Offspring Cohort since 1971. Carefully assessed clinical phenotypes are collected over routine examination cycles, genome-wide genotyping data has been obtained on these individuals, and exome and whole genome sequencing are underway. As such, the FHS cohort represents perhaps the most intensively studied collection of people in the world. Like all other cohort studies, FHS has an important limitation - the lack of availability of human tissues with which to make a link between genetic variation and whole-organism clinical phenotypes. Induced Pluripotent Stem Cell (iPS) technology offers the ability to reprogram and engineer patient-specific tissues ex vivo. These tissues offer the prospect of observing cellular- and organ-level phenotypes to provide critical insights into disease pathophysiology driven by genetic variation. To this end, we collected peripheral blood cells from 2,503 consenting individuals of the FHS Offspring Cohort and reprogrammed a subset ascertained at a genetic variant rs12740374 to iPSCs. We differentiated 68 (34 homozygous major; 34 homozygous minor for the SNP) patient-specific iPSCs into hepatocytes and adipocytes to investigate how rs12740374 affects metabolic disease phenotypes via gene expression and metabolomic profiling. We previously performed (in collaboration with Dr. Sekar Kathiresan) expression quantitative trait locus (eQTL) analyses of genotype vs. gene expression in surgical liver and adipose tissue samples from patients; we found a strong association between the LDL-C- and MI-associated SNP defining our iPSC cohort - rs12740374 - and hepatic expression of the SORT1 gene (PMCID:

PMC3062476). Similarly, we observe a strong association between rs12740374 and SORT1 expression in iPSC-derived hepatocytes but not adipocytes. Thus, large cohorts of iPSCs accurately model disease mechanisms arising from common genetic variants (Warren et al. in preparation). We plan to expand this study to discover novel genotype-phenotype associations relevant to metabolic disease and use these insights as entry points for developing new therapeutics.

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T2121

DEVELOPMENT OF XENO-FREE MEDIUM FOR HUMAN IPS CELL CULTURE SYSTEMS

Yanagihara, Kana[1], Shoji, Shinichiro[1], Furue, Miho Kusuda[2] and Tsukahara, Masayoshi[3], [1]Kyowa Hakko Bio Co., Ltd., Tokyo, Japan, [2]National Institutes of Biomedical Innovation, Health and Nutrition, Ibaraki Osaka, Japan, [3]Kyowa Hakko Kirin Co.,Ltd., Tokyo, Japan

Given their proliferation and differentiation potential, human pluripotent stem cells (hPSCs) are an attractive cell source for mass generation of lineage- and potentially patient-specific progenies in regenerative medicine such as cardiac regeneration therapy. Clinical and industrial applications of hPSCs, however, may require large cell mass to be produced under the well-defined and economically viable conditions according to GMP guidelines. Recently, several feeder-free culture systems have developed with serum-free and xeno-free (XF) media. Although these materials are available for hPSC maintenance, it is unsuitable for large-scale culture because practiced hand and high cost are necessary. To address these issues, we aim to develop a novel and efficient medium for large-scale hPSC culture. Here, we have developed a xeno- and albumin-free medium for hPSC maintenance. We screened a number of compounds that maintain pluripotency when albumin was removed from the culture medium. Combinations of factors were found effective on the proliferation of human iPS cell line 253G1. These cells highly expressed pluripotency marker, OCT3/4. Quality tests of hPSCs cultured under the albumin-free medium are ongoing now.

Funding Source: xeno-free medium for human iPS cell culture systems

IPS CELLS: DIRECTED DIFFERENTIATION

T2127

CHARACTERIZATION AND POTENTIAL IMMUNOMODULATORY PROPERTIES OF HUMAN INDUCED PLURIPOTENT STEM CELL (hiPSC)-DERIVED TROPHOBLAST CELLS

Malysheva, Svitlana, Frau, Hannover, Germany, **Martin, Ulrich**, REBIRTH Cluster of Excellence, Hannover Medical School, Hannover, Germany and Wunderlich, Stephanie, Hannover Medical School, Hannover, Germany

Pregnancy is a unique situation in which the mother and the semi-allogeneic fetus are able to coexist, obviously through mechanisms that induce and maintain peripheral immunological tolerance of the mother towards the fetus. Trophoblast cells are thought to play a key role in induction and maintenance of this tolerance. Recently it was shown that trophoblast cells can be generated from human embryonic and induced pluripotent stem cells (hiPSCs) in vitro. hiPSCs-derived trophoblast cells may provide a valuable basis for development of in vitro models for investigation of early fetal-maternal interactions. In the current study we compared the efficiency of trophoblast differentiation from hiPSCs through the formation of embryoid bodies (EBs), and by the induction with BMP4. EB-based differentiation as well as targeted differentiation through BMP4 yielded cells with epithelial morphology that expressed trophoblast markers cdx2, cytokeratin 7 (CK7), eomes and chorionic gonadotropin. BMP4 induction yielded 93,7 % of CK7-positive cells already at day 4, compared to 33,8 % in EB-mediated differentiation on day 18. Moreover, during BMP4-induced differentiation we detected expression of placental lactogen, which was not detected in the EB-based approach. However, during differentiation through EBs we observed the formation of CK7-positive cystic structures, obviously formed by fluid-pumping epithelium. Finally, we have investigated expression of immunomodulatory molecules by hiPSCs-derived trophoblast cells. Indeed, various isoforms of non-classical class I histocompatibility antigen HLA-G could be detected. Indoleamine 2,3-dioxygenase (IDO) could not be detected, but was inducible by interferon γ (IFN-γ). Some other co-stimulatory molecules like TRAIL (TNF-related apoptosis-inducing ligand) and PD-L1 (programmed death-ligand 1) were also expressed in hiPSCs-derived trophoblast cells. Further studies concerning targeted generation of defined trophoblasts subsets, upregulation of immunomodulatory molecules and functional analysis of resulting cell lineages are ongoing. The derivation of defined trophoblasts sublineages will enable the investigation of early fetal-maternal interactions including

mechanisms of tolerance induction and maintenance in novel human in vitro models.

Funding Source: German Research Foundation (Cluster of Excellence REBIRTH, EXC 62/3)

T2129

GENETICALLY ENCODED FLUORESCENT VOLTAGE INDICATOR REPORTS ELECTROPHYSIOLOGICAL CHANGES IN HIPSC-CMS WITH MATURATION IN LONG-TERM CULTURE

Biendarra, Sherri Marie, Li, Xing, Brandt, Emma and Nelson, Timothy J., Mayo Clinic, Rochester, MN, U.S.

One major challenge in the application of the human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM) platform for basic research and therapeutic applications is the need to appropriately evaluate and control for stage of cardiac maturation and cardiomyocyte subtype in heterogeneous populations. Numerous differences can be seen in hiPSC-CM phenotype based on the stage of maturation, including gene expression, electrophysiology, calcium transients, beating activity, size and morphology, and metabolism. Furthermore, hiPSC-CM maturation status may vary between studies and within studies for different cell lines and culture conditions. We sought to obtain non-invasive electrophysiological readings and functionally gauge maturation of hiPSC-CMs through the application of the fluorescent genetically encoded voltage indicator (GEVI) Arclight. GEVIs are emerging as a potentially valuable tool in this field due to the possibility for large-scale and long-term phenotypic assessments with lower toxicity and higher photostability than dyes. To this end, hiPSC-CM cell lines from three healthy adults were allowed to mature over the course of ~6 weeks in culture following cardiac differentiation with a chemically defined media protocol. Arclight was introduced to these cell lines via lentiviral transduction and subsequently used to monitor action potential properties of individual spontaneously beating cardiomyocytes. We detected statistically significant increases in action potential amplitude and maximum upstroke velocity on a population level between early (< Day 17) and later time points. Additionally, we saw a higher proportion of cells with an APD_{90}/APD_{50} ratio of >1.4 after 30 days of differentiation. This ratio has been used previously in the identification and quantification of distinct cardiomyocyte subtypes. Overall, this system allowed for us to functionally profile heterogeneity within cell populations and between differentiations at multiple stages of development. These results suggest that this approach could be used to monitor action potential parameters that have historically been associated with cellular maturation on either a single cell or population level.

Funding Source: This work was supported by the Todd and Karen Wanek Family Program for Hypoplastic Left Heart Syndrome.

T2131

QUAKING IS A KEY REGULATOR OF ENDOTHELIAL CELL DIFFERENTIATION, NEOVASCULARIZATION AND ANGIOGENESIS

Cochrane, Amy[1], Kelaini, Sophia[1], Bojdo, James[1], Vilà González, Marta[2], Hu, Yanhua[3], Grieve, David[1], Stitt, Alan[1], Zeng, Lingfang[3], Xu, Qingbo[3] and Margariti, Andriana[1], [1]Queen's University Belfast, Belfast, U.K., [2]Centre for Experimental Medicine QUB, Belfast, U.K., [3]King's College London, Belfast, U.K.

Cardiovascular disease is a major cause of death worldwide and characterized by progressive endothelial cell (EC) dysfunction. The capability to derive ECs from induced Pluripotent Stem (iPS) cells holds huge therapeutic potential. Elucidation of the molecular mechanisms underlying EC differentiation will ultimately advance stem cell regenerative therapy towards reality which would represent a paradigm shift in the treatment of cardiovascular disease. In this study, using the model of iPS cells differentiation towards ECs, the RNA binding protein QKI was found to be an important regulator of the VE-cadherin stabilisation and VEGFR2 transcriptional activation during the EC differentiation process. iPS cells have been generated based on a highly efficient approach, fully characterized and forced to differentiate towards ECs. The role of QKI has been further elucidated in EC differentiation derived from iPS cells. QKI was found to be induced during EC differentiation from iPS cells, and its expression was shown to be maintained at high levels in mature ECs. Notably, QKI overexpression induced the activation of EC markers, whilst knockdown by shRNA suppressed EC differentiation. It has been demonstrated that QKI plays a role in the induction and stabilization of VE-Cadherin and activation of VEGFR-regulatory binding sites AP1 and STAT3 and STAT3 phosphorylation. Importantly, QKI modulated the transcriptional activation of VEGFR through STAT3 signaling. The notion that QKI indeed played an important role during EC differentiation was further supported from additional data which clearly demonstrated that knockdown of QKI resulted in inhibition of angiogenesis in vivo. Remarkably, ECs derived from iPS overexpressing QKI improved neovascularization and blood flow recovery (almost 100%) in a hind limb ischemia model by showing an enhanced engraftment capacity when compared to non-modified iPS-ECs or PBS control groups. Notably, human iPS cells overexpressing QKI induced angiogenesis on Matrigel plug assays in vivo only seven days after subcutaneously injection in SCID mice. These results demonstrate that QKI holds a key role

in EC differentiation highlighting a clear functional benefit in neovascularization, blood flow recovery and angiogenesis.

T2133

PRODUCTION AND CHARACTERIZATION OF DOPAMINE NEURONS FROM BABOON PLURIPOTENT STEM CELLS

Grow, Douglas Alvin[1], Simmons, DeNard[1], Gomez, Jorge[1], Wanat, Matthew[1], McCarrey, John R.[1], Paladini, Carlos[1] and Navara, Christopher[2], [1]University of Texas at San Antonio, San Antonio, TX, U.S., [2]University of Texas, San Antonio, San Antonio, TX, U.S.

The progressive death of dopamine producing neurons in the substantia nigra pars compacta (SNc) is the principle cause of symptoms of Parkinson's disease (PD). The derivation of dopaminergic neurons from induced pluripotent stem cells brings new hope for a patient-specific, stem cell-based replacement therapy to treat PD and related neurodegenerative diseases. To facilitate development of this approach, we seek to establish a large nonhuman primate preclinical model for testing the efficacy and safety of stem cell-based transplantation. To this end, we differentiated baboon fibroblast-derived induced pluripotent stem cells (biPSCs) into dopaminergic neurons with the application of specific morphogens and growth factors. We confirmed that biPSC-derived dopaminergic neurons resemble those found in the human midbrain based on cell-type specific expression of dopamine markers TH and GIRK2. Using RT-qPCR, we also show that biPSC-derived dopaminergic neurons express PAX6, FOXA2, LMX1A, NURR1, and TH, genes characteristic of this cell type *in vivo*. We used perforated patch-clamp electrophysiology to demonstrate that biPSC-derived dopaminergic neurons fired spontaneous rhythmic action potentials. We also found that injection of depolarizing current would illicit high-frequency action potential firing with spike frequency adaption. Finally, we show that biPS-derived neurons released catecholamines in response to electrical stimulation. These results demonstrate the utility of the baboon model for testing and optimizing the efficacy and safety of stem cell-based therapeutic approaches for the treatment of PD.

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T2135

GENE-EDITING ENABLES ISOLATION OF IPSC-DERIVED ALVEOLAR TYPE II CELLS FOR DEVELOPMENT AND DISEASE MODELING

Jacob, Anjali[1], Hawkins, Finn[2], Benson, Katherine[2], Serra, Maria[2] and Kotton, Darrell[2], [1]Boston University, Boston, MA, U.S., [2]Boston University School of Medicine, Boston, MA, U.S.

Alveolar epithelial type II cell (AEC2) dysfunction has been implicated as a primary cause of pathogenesis in many poorly understood lung diseases that lack effective therapeutics, including idiopathic pulmonary fibrosis and COPD. AEC2s are inaccessible to study in the developing human embryo and difficult to study in patients. They proliferate poorly and rapidly transdifferentiate into other cell types when isolated and cultured, severely limiting research in alveolar development and disease. Using iPSC technology and directed differentiation to generate AEC2s de novo would provide novel opportunities to study normal AEC2 development as well as the pathogenesis of monogenic alveolar diseases. Current methods to derive AEC2s from iPSCs are limited by heterogeneous cell populations and long differentiation times. We took advantage of the fact that surfactant protein C (SFTPC) is specifically expressed in AEC2s in-vivo and used TALENs technology to generate a fluorescent (tdTomato) reporter that enables isolation of a pure population of SFTPC+ putative AEC2s after only 20-30 days of directed differentiation. Mouse models have shown that all cells of the alveolar epithelium develop from an early NKX2.1+ progenitor, but this finding has yet to be verified in humans. By sorting early Day 15 NKX2.1+ or NKX2.1- cells using a knockin NKX2.1^{GFP} reporter and replating them in 3D culture, we found that the entire SFTPC$^{tdTomato+}$ putative AEC2 population derives from mesenchyme-depleted NKX2.1^{GFP+} sorted cells, the first evidence of alveolar cells deriving from primordial NKX2.1+ progenitors in an in-vitro human system. On day 30 of our AEC2 differentiation protocol, a population of SFTPC$^{tdTomato+}$ cells emerged, which when sorted to purity and analyzed by RT-qPCR, expressed AEC2-specific genes at levels similar to week 21 fetal distal lung alveolar epithelial cells. Pure sorted populations of Day 30 SFTPC$^{tdTomato+}$ cells can also be replated and maintained in 3D culture for several weeks, suggesting the first steps toward an in-vitro platform for alveolar disease modeling and drug screening. Ongoing studies involve using RNA-Seq to perform whole transcriptome analysis of iPSC-derived SFTPC$^{tdTomato+}$ cells compared to primary AEC2 controls, as well as protein and ultrastructural analysis of these cells.

T2137

EFFECTIVE HEPATIC DIFFERENTIATION FROM INDUCED PLURIPOTENT STEM CELLS WITH SUFFICIENT OXYGEN SUPPLY THROUGH OXYGEN PERMEABLE MEMBRANES

Kimura, Keiichi, Horiguchi, Ikki, Kido, Taketomo, Miyajima, Atsushi and Sakai, Yasuyuki, The University of Tokyo, Tokyo, Japan

Although primary human hepatocytes are ideal cell sources for constructing in vitro liver models, there is a constant donor shortage of these cells. To solve this problem, hepatocytes derived from human induced pluripotent stem cell (hiPSC) are highly expected as alternative cell sources. However, hiPSC-derived hepatocytes have much poorer function than normal primary hepatocytes because of incomplete maturation during differentiation. In this study, we found that direct oxygenation to the cell layer during differentiation improved hepatic function. Polydimethylsiloxane (PDMS) plates, which enable direct oxygenation to the cell layer, were used in a cell culture in addition to conventional tissue culture treated polystyrene (TCPS) plates. Differentiation of iPSCs was performed with several different oxygen concentrations and oxygen supply fluxes (TCPS and PDMS). Comparing the different methods of differentiation revealed that the levels of hepatic gene expression, such as these of ALB, CYP3A4 and CYP1A2, were upregulated in cells differentiated on PDMS plates. Moreover, ELISA analyses showed that these cells exhibited 7 to 10 times higher albumin production ability than those on conventional plate (TCPS/20%-O2). Interestingly, cells differentiated on a low oxygen supply condition (TCPS/5%-O2) exhibited significantly low hepatic functions and remained expression of pluripotent marker such as Oct4, which implied they failed hepatic differentiation on a low oxygen supply. From these results, oxygen supply to the cell layer is important to hepatic maturation. In addition, to quantify the amount of oxygen consumed by cells during hepatic differentiation, we measured the oxygen concentration above the cell layer and calculated the oxygen diffusion flux to cell layer by using Fick's Law. Since the oxygen flux to the cell layer was 10 times higher with PDMS than with TCPS, cells on PDMS plates could utilize more oxygen for respiration, differentiation and maturation. In summary, direct oxygenation with PDMS membrane is a better alternative culture method over conventional TCPS plates for the maturation of hiPSCs derived hepatocytes in vitro.

T2139

DISSECTING THE MECHANISMS UNDERLYING ADOLESCENT-ONSET SCHIZOPHRENIA USING PATIENT-DERIVED CELLS

Li, Yichen[1], Haenseler, Walther[2], Cowley, Sally[2], James, Anthony[2], Martinez-Devesa, Pablo[3], Nivet, Emmanuel[4], Féron, François[4], Mackay-Sim, Alan[5] and Szele, Francis[1], [1]University of Oxford, Oxord, U.K. of Great Britain and Northern Ireland, [2]University of Oxford, Oxford, U.K. of Great Britain and Northern Ireland, [3]John Radcliffe Hospital, Oxford, U.K. of Great Britain and Northern Ireland, [4]Aix-Marseille University, Marseille, France, [5]Griffith Univ, Brisbane QLD, Australia

Schizophrenia is a devastating mental disorder that affects about 1% of the total population. Patients with adolescent-onset schizophrenia (AOS) suffer from more severe symptoms and have a worse prognosis. The aetiology of schizophrenia is largely unknown. However, it is widely accepted, in part, as a neurodevelopmental disorder with a multifactorial genetic and environmental (e.g. inflammation) basis. It has been difficult to produce adequate animal models. Therefore we are using in vitro stem cell strategies to study human neural development in AOS. The human olfactory mucosa harbours stem cells that give rise to neurons. Olfactory ecto-mesenchymal stem cells (OE-MSCs) in vitro may provide an accessible resource for studying the molecular mechanisms that underlie the abnormal neurodevelopment seen in schizophrenia. Accordingly, we have isolated OE-MSCs from twelve patients with AOS and six age- and gender-matched healthy controls. Using this material, we assess the stem cell properties, and performing functional assays addressing proliferation and migration, which were previously shown as aberrantly regulated in adult-onset schizophrenia. From the same subjects, we also obtained skin biopsies for generating patient-derived induced pluripotent stem cells (iPSCs) with the ultimate aim of differentiation of cortical projection neurons and microglia. The microglia will be made following an unpublished directed differentiation protocol from collaborators (WH, SC). The differentiation of iPSCs into cortical projection neurons has been shown to recapitulate neurodevelopment in vivo, and generates cerebral cortex layer-specific neurons. We assess the timing, quantity, morphology, synaptogenesis and synaptic maturity of neural cells during differentiation with and without iPSC-microglia. We are also interested in the genetics of the patients. We collected blood samples from patients and their healthy parents to perform DNA sequencing, for identifying de novo mutations that link with AOS. We also collect single cells during neuronal differentiation to perform transcriptomic study longitudinally between iPSCs derived from patients with AOS and healthy controls. Our ultimate goal is to dissect

out convergent molecular pathways shared between patients with different genomic alterations.

Funding Source: British Medical Association Award, Clarendon Fund Scholarship

T2141

HIGH RESOLUTION MAPPING OF DETERMINISTIC IPSC REPROGRAMMING TO MURINE GROUND STATE PLURIPOTENCY

Zviran, Asaf, **Rais, Yoach**, Mor, Nofar, Hanna, Jacob and Novershtern, Noa, Weizmann Institute of Science, Rehovot, Israel

A Major revolution in stem cell research was achieved upon the discovery of cellular reprogramming. However, in most conventional reprogramming approaches, only a small fraction of the somatic cell population becomes pluripotent (iPSC), thus making this process hard to investigate at sufficient molecular depth and without making biased selection of certain time points or sorted sub-populations.Our group has previously identified Mbd3/NuRD chromatin remodeler complex as a major roadblock for reprogramming, and that optimized depletion (50-80%) of Mbd3 or complete ablation of Gatad2a (also known as P66a) at early stages of reprogramming, can positively alter the process and leads to a near deterministic reprogramming, where nearly all of the initial cell population became pluripotent in a synchronized manner after 8 days. We have now used the Mbd3 and Gatad2a depleted deterministic reprogramming platforms, in order to conduct a depth molecular mapping of reprogramming trajectory. We have collected samples every 24 hours throughout the 8 days until completion of reprogramming, measuring transcription, histone modifications, transcription-factors binding, chromatin accessibility (with ATAC-Seq) and DNA methylation. I will present here the main findings related to cellular programs which participate in reprogramming and their regulation, I will describe the temporal order of epigenetic changes in promoters and enhancers which are driving transcription activation, and I will present the role that DNA methylation and translational control play in the cell fate conversion process.This is the first ever conducted high-resolution mapping of authentic and continuous epigenetic dynamics occurring during deterministic somatic cell reprogramming towards ground state naive pluripotency in mice.

T2143

HIGH EFFICIENCY DIFFERENTIATION OF SKELETAL MYOBLASTS FROM HUMAN PLURIPOTENT STEM CELLS IN A SCALABLE 3D ORGANOID CULTURE

Rukstalis, Michael, Ji, Lin and Ramos-Zayas, Rebeca, Pfizer, Cambridge, MA, U.S.

Advancements in the understanding of mesoderm signaling pathways during development have led the generation of multiple protocols for the directed differentiation of skeletal myoblasts from human pluripotent stem cells. A remaining challenge is the efficient scaling of these protocols to generate sufficient differentiated cells to support both high throughput drug discovery applications and cellular replacement therapies. To address this limitation, we developed a chemically defined 3D organoid platform to produce somitic mesoderm and its derivatives at densities of 1 million cells per ml or greater in a scalable suspension culture. To minimize off-target differentiation and increase purity, we optimized the timing and concentration of the WNT, HGF, Notch, and TGFb signaling pathways to synchronously differentiate the organoids at high efficiency through each developmental stage of skeletal myogenesis. Using this platform we can reproducibly generate highly enriched populations of PAX7+ myogenic progenitors and MYOG+/Desmin+ skeletal myblasts within 21 days of culture. These pluripotent cell-derived myoblasts undergo fusion within the 3D organoids and exhibit a gene expression profile and twitching behavior similar to adult human myotube cultures. Finally, this differentiation platform can be easily scaled into pendulum-type spinner flasks for large scale production of human skeletal myoblasts to fully enable in vitro discovery efforts.

T2145

GENERATION OF MULTIPOTENT CANINE EXTRAEMBRYONIC ENDODERM-LIKE CELLS VIA THE FORMATION OF INDUCED PLURIPOTENT STEM CELLS

Unezaki, Naoya[1], Nishimura, Toshiya[1], Kanegi, Ryoji[1], Wijesekera, Daluthgamage Patsy Himali[1], Sugiura, Kikuya[1], Hatoya, Shingo[1], Kawate, Noritoshi[1], Tamada, Hiromichi[1], Imai, Hiroshi[2] and Inaba, Toshio[1], [1]Osaka Prefecture University, Izumisano, Japan, [2]Kyoto University, Sakyo-Ku, Japan

Extraembryonic endoderm (XEN) cells are a kind of stem cells, derived from primitive endoderm (PrE) which emerges on the mural surface of inner cell mass at the late blastocyst stage. They are useful cell sources in understanding the mechanism of early embryonic development due to their differentiation potency into an extraembryonic endoderm lineage, such as the visceral endoderm (VE) and parietal endoderm (PE) of the yolk sac. Al-

though there are several reports about the establishment of XEN cell lines, the cell lines have not been reported yet in the canine. Here, we generated canine XEN-like cells via induced pluripotent stem cells (iPSCs) from canine embryonic fibroblasts by using doxycycline (DOX)-inducible lentiviral vectors carrying four mouse cDNAs (Oct4, Sox2, Klf4, and c-Myc). Five days after transduction, first iPSC colonies emerged in serum-free medium supplemented with DOX. The colonies which showed doom shape were isolated mechanically and transfer onto feeder cells. These iPSC colonies exhibited ALP positive activity and after a few passages, some colonies changed their morphology to a flat shape. They were composed of cuboidal cells and displayed with PrE markers, GATA4, GATA6, and FOXA2, as common characteristics of XEN cells. Moreover, they expressed AFP and TPA, indicating their differentiation potency into VE and PE in differentiation medium. Interestingly, canine XEN-like cells expressed pluripotency marker, OCT3/4, and subset of these cells exhibited ALP positive. The cells were maintained their characteristics stably beyond 20 passages without the expression of transgenes. When the canine XEN-like cells co-cultured with OP9 cells in the medium supplemented with vascular endothelial growth factor they formed unique structure containing individual round cells and produced floating cells into supernatant, which expressed leukocyte antigen CD45. In conclusion, in the present study we have successfully generated canine XEN-like cells which have similar property with XEN cells during the generation of iPSCs. These cells have multi differentiation potencies into both endoderm and blood population. The canine XEN-like cells may help to reveal the mechanism in the canine embryo development in the future.

T2147

EZH2 IMPROVES HEMATOPOIETIC LINEAGE COMMITMENT FROM HUMAN EMBRYONIC STEM CELLS AND INDUCIBLE PLURIPOTENT STEM CELLS

Li, Qi[1], Jiang, Xin-xing[1], Wei, Yun-jian[1], Sun, Fei[2], Long, Ping[1], Li, Ling-li[1], Zhang, Feng-bo[1], Yu, Yan-hong[2] and **Ma, Yanlin**[1], [1]Affiliated Hospital of Hainan Medical University, 31 Longhua Road, Haikou, Hainan, China, [2]Nanfang Hospital, Southern Medical University, Guangzhou, China

Thalassemia is one of most common autosomal recessive genetic disorders, characterized by chronic progressive hemolytic anemia. Clinical management of thalassemia major consists of in regular long-life red blood cell transfusions and iron chelation therapy. The only definitive cure is allogeneic hematopoietic stem cell transplantation (allo – HSCT), but the resource of HSCs is limited. Human induced pluripotent stem cells (hiPSCs) and embryonic stem cells (ESCs), as the unlimited cell resources, bring hope for personalized regeneration cell therapies. To get a broader supply of HSC, a lot of efforts had been done to generate HSC/progenitor cells from human embryonic stem cell (hESC) and human induced pluripotent stem cell (hiPSC), however, the differentiation efficiency is still pretty low, HSC amplification in vitro is so difficult. In order to overcome the two major bottlenecks, clear the gene regulation mechanism which control the early human embryonic hematopoietic generation will be helpful. EZH2 gene, which encodes a member of Polycomb-group (PcG) family, have been proved to be essential to the development of mesoderm and hematopoiesis in mouse model, and many studies have proved that EZH2 have related to oxidative stress and cell apotosis. So we further research whether overexpression or knock out EZH2 gene in human ES/iPS cells could influence hematopoietic differentiation, and then it can provide the basis for the transformation of HSC/HPC clinical treatment.

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T2149

PREDICTING HEPATIC, MESENCHYMAL AND CARDIAC STEM CELL FATE THROUGH GLOBAL PROTEIN EXPRESSION PROFILE IN HUMAN PLURIPOTENT STEM CELLS

Laco, Filip, Woo, Tsung Liang and Oh, Steve, Bioprocessing Technology Institute, Singapore, Singapore

Differentiation of human pluripotent stem cells (hPSCs) can be induced via a small molecule that inhibits GSK (CHIR99021). Dose dependent addition of growth factor BMP-4 showed differentiation into all germ layers including certain endoderm and mesoderm linages such as cardiac, muscle-mesenchymal and hepatic. While reproducibility was demonstrated with embryonic stem cell lines, it has been observed that quantitative differences between induced pluripotent stem cell lines and embryonic stem cells lead to unpredictable and often low differentiation results, as shown with cardiac differentiation. hPSCs genomic variations of germ-layer associated genes were shown to predict their potential to induce neural specification. Therefore, we believe and demonstrate that predispositions of endo-mesoderm consecutive development to cardiac, mesenchymal or hepatic linage is assessable at the proteome level in stem cells for a single step GSK inhibition differentiation protocol. We differentiated over 10 hPSCs from different origins and analysed >100 protein levels and cell populations before and during the differentiation period. Our Data shows that the cell lines can be easily directed with high to low CHIR99021 concentration from cardiac to mesenchymal and hepatic linage. The cell lines have individual dose dependencies; a few

cell lines are restricted in their lineage diversion. Further, our preliminary data shows that variation in several protein markers among them pluripotency marker (Nanog) correlate with the cardiac differentiation results. Our data demonstrates that single GSK inhibition can robustly induce endo- and mesoderm linages differentiation such as cardiac, mesenchymal and hepatic in many platforms. Further, we indicated potential markers for cardiogenic predisposition that can be assessed at the proteome level. Moreover, we hypothesize that the measured differences in protein levels of hPSCs could be an indicator for the small molecule doses driven linage differentiations.

IPS CELLS: EPIGENETICS

T2151

WHOLE GENOME DNA METHYLATION ANALYSIS AIMING FOR SELECTING "SAFE" IPS CELL-DERIVED NEURAL STEM/PROGENITOR CELLS (IPSC-NS/PCS)

Iida, Tsuyoshi[1], Iwanami, Akio[2], Kohyama, MD, Jun[2], Sanosaka, Tsukasa[2], Matsumoto, Morio[2], Nakamura, Masaya[2] and Okano, Hideyuki[2], [1]Keio University, Tokyo, Japan, [2]Keio University School of Medicine, Tokyo, Japan

Recently we have demonstrated the therapeutic potential of transplanting human induced pluripotent stem cell-derived neural stem/progenitor cells (iPSC-NS/PCs) into the injured spinal cord of rodents and primates. However, we also found that some iPSC-NS/PCs lines produce neurogenic tumors after transplantation. The purpose of this study is to investigate the genomic alteration in iPSC-NS/PCs that may be related to cancer pathogenesis through DNA methylation analyses. Samples were prepared as follows: two iPSCs cell lines (253G1 and 201B7), two groups of iPSC-NS/PCs (tumorigenic cell line: 253G1-NS/PC, non-tumorigenic cell lines: 201B7-NS/PC). Infinium HumanMethylation450 BeadChip was used to evaluate genome wide DNA methylation analyses of these cells. DNA samples were also prepared from these iPSC-NS/PCs cell lines of different passage numbers and each methylation status was compared. Significant differences in DNA methylation patterns were appreciated between 253G1- and 201B7-NS/PCs such as some cancer genes, and tumor suppressor genes. Furthermore, DNA methylation patterns of 253G1-NS/PCs became worse in accordance with passage number, especially over 15 passages. Our research group is working vigorously to pursue a clinical trial for patients with spinal cord injury within the next several years. Therefore, it is critical to establish criteria for choosing the "safe" iPSC-NS/PCs. We previously demonstrated differences in the expression levels of specific genes between TC and Non-TC using transcriptome analyses (Nori *et al*, 2015), but detailed mechanism of tumorigenicity still remains unclear. Here we revealed a difference in DNA methylation pattern and difference in some genes' DNA methylation status, which may be associated with the tumorigenicity. These results enable us to establish criteria for quality control of iPSC-NS/PCs in terms of their tumorigenicity.

T2153

Oct4 AND Sox2 ENHANCE Ash2L PROMOTER ACTIVITY AND PROMOTE CELL REPROGRAMMING

Sung, Shih-Yu, Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan

Induced pluripotent stem cells (iPSCs) have a unique genetic program induced by the core pluripotency factors like Oct4, Sox2, and Nanog. iPSCs express upregulated core pluripotency factors and show open chromatin structure around pluripotency gene locus, compared with somatic cells. Given that chromatin-modifying proteins regulate gene expression by manipulating chromatin structure, chromatin-modifying networks are critical regulatory mechanisms for iPSC generation and self-renewal maintenance. Ash2l, a member of trithorax protein family, mediating histone 3 lysine 4 tri-methylation in the mammalian cells, has been proven to be necessary in maintaining self-renewal in mouse embryonic stem cells. However, the regulatory mechanism of Ash2L in iPSCs is still unclear. To understand if the reprogramming transcription factors, including Oct4, Sox2, Klf4 and c-Myc (OSKM), regulate Ash2l expression, we performed a ChIP-sequencing and identified the binding of OSK at ***Ash2l*** locus. Compared to other trithorax protein, the binding enrichment of OSK were particularly found in Ash2l promoter region. To validate the result from ChIP-seq, we used ChIP-qPCR to evaluate the enrichment of OSKM at the promoter of trithorax proteins. To test the putative affinity of OSKM transcription factors to *Ash2L* promoter, we constructed a *Ash2l* promoter-driven luciferase reporter. Transient transduction of Oct4 or Sox2 in HEK-293t cells resulted in a significant increase of luciferase activity, indicating that Oct4 and Sox2 enhanced the transcription of *Ash2l* promoter. Notably, mutated *Ash2l* promoter led to the reduction of luciferase activity resulting from the diminishment of OS binding. Moreover, we identified that both Oct4 and Sox2 were recruited to *Ash2l* promoter during the reprogramming process. To investigate which DNA motif were critical for Oct4 and Sox2 recruitment at Ash2l promoter, we block *Ash2l* promoter with CRISPRi system in mouse embryonic fibroblasts before reprogramming, we found that the OS-binding motif on *Ash2l* promoter is required for efficient reprogramming. Taken together, we demonstrated that Oct4 and Sox2 are recruited to *Ash2l* promoter and responsible for inducing Ash2l expression through enhancing Ash2L promoter activity in reprogramming and iPSCs.

CHROMATIN IN STEM CELLS

T2155

TRANSLATION AND CELLULAR GROWTH IS REQUIRED FOR MAINTENANCE OF OPEN CHROMATIN IN MOUSE EMBRYONIC STEM CELLS

Karslioglu, Aydan, Macrae, Trisha, Diaz, Aaron, McManus, Michael and Ramalho-Santos, Miguel, University of California San Francisco, San Francisco, CA, U.S.

Pluripotent cells that generate the mammalian embryo display an open chromatin pattern characterized by low levels of heterochromatin, high levels of histone modifications associated with gene expression, and a state of global hyper-transcription. We have recently shown that this open chromatin state is essential for the development of rapidly expanding pluripotent cell populations in the mouse embryo. However, the regulation of this chromatin state remains largely unknown. To probe the regulation of open chromatin in stem cells in a systematic manner, we generated a mouse embryonic stem cell line carrying a fluorescent reporter for open chromatin and used it to carry out a genome-wide RNAi screen. In addition to several expected and novel chromatin factors, screen hits are remarkably enriched for ribosomal proteins and other regulators of translation and cellular growth. This suggests that cellular growth pathways, and prominently protein translation, act in a positive feedback loop with chromatin to reinforce a decondensed state. In agreement with this notion, reducing protein translation and cellular growth via small molecule inhibitors leads to a significant reduction of open chromatin levels in as little as 3 hours, as evidenced by reduced reporter activity and histone acetylation. Additionally, transcriptional output is reduced rapidly in response to growth inhibition. Heterochromatin, on the other hand, does not appear to be affected by this treatment. We are following up on these findings using chromatin analyses and proteomics. Our results indicate that a high level protein synthesis is required to maintain the open chromatin of rapidly growing embryonic stem cells, which in turn is essential for hyper-transcription and retention of full developmental potential.

T2157

THE DIFFERENT STATES OF MOUSE IN VITRO PLURIPOTENCY ADOPT DIVERSE EPIGENETIC PROGRAMS TO TIGHTLY CONTROL HETEROCHROMATIN REGIONS.

Tosolini, Matteo, Brochard, Vincent, Chebrout, Martine, Adenot, Pierre, Jouneau, Luc, Beaujean, Nathalie, Bonnet-Garnier, Amélie and Jouneau, Alice, INRA - UMR Biology of Reproduction and Development, Jouy en Josas, France

Embryonic stem cells (ESC) and epiblast stem cells (EpiSC) are in vitro representatives of the different states of pluripotency in mouse, the naïve, early epiblast at blastocyst stage and the primed, late epiblast of a post-implantation embryo, respectively. They are well characterized at the molecular level but few is known about their chromatin features particularly concerning heterochromatin compartments and their regulations. In Eukaryote, constitutive heterochromatin is a gene-poor compartment constituted of repeat DNA sequences, generally silenced and located at telomeres, centromeres and pericentromeric regions. Tight control of these regions is extremely important for chromosomal stability. Beside the telomeric sequences, there are two types of DNA sequences in mouse: major satellite and minor satellite repeats at the pericentromeric and centromeric regions, respectively. In mouse somatic cells, these regions from different chromosomes organize in clusters. The first insight about different epigenetic regulations of these sequences according to the pluripotent state comes from our observation that H3K9me3 forms foci in EpiSC, like differentiated cells, while ESCs cultured in 2i medium present mostly H3K27me3 clusters. Transcription of major and minor satellite repeats is strongly repressed in EpiSCs whereas ESCs transcribe at high level these repeats, even if 2i condition leads to a considerable down-regulation particularly of minor satellites. To further investigate the interplay of these two histone modifications on the transcription of major and minor satellites we studied ESCs and EpiSCs lacking either H3K9me3 or H3K27me3, or both. Interestingly our results reveal that the absence of both marks in EpiSCs impairs the normal silencing of satellite repeats while the same condition in ESCs is transcriptionally repressive. Ongoing work suggests that differential involvement of DNA methylation could play a role in such contrasting phenomena. We show that ESCs and EpiSCs differ in the epigenetic regulation of pericentromeric and centromeric regions. Altogether, our study addresses the question of the functional link between pluripotency status and heterochromatin organization.

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GERMLINE CELLS

T2159

NEURONATIN PROGRESSES CELL DIFFERENTIATION BY PARTICIPATING IN REGULATION OF INTRACELLULAR Ca2+-LEVEL.

Kanno, Naoko[1], Yoshida, Saishu[1,2], Ueharu, Hiroki[1], Kato, Takako[2,3] and Kato, Yukio[1,4], [1]Division of Life Science, Graduate School of Agriculture, Meiji University, Kawasaki-shi, Japan, [2]Organization for the Strategic Coordination of Research and Intellectual Property, Meiji University, Kawasaki-shi, Japan, [3]Institute of Reproduction and Endocrinology, Meiji University, Kawasaki-shi, Japan, [4]Department of Life Science, School of Agriculture, Meiji University, Kawasaki-shi, Japan

Neuronatin (NNAT) was first identified in the neonatal neural tissue and was predicted to be involved in neurogenesis. Due to the characteristic hydrophobic amino terminal and hydrophilic carboxy terminal, NNAT is assumed to be membrane protein in the endoplasmic reticulum (ER), one of the Ca^{2+}-storage organelles. In the pituitary, NNAT was also found and it was reported that the expression of *Nnat* is regulated by PROP1, a pituitary specific transcription factor important for pituitary organogenesis. Recently, we reported that NNAT plays a role in the rat pituitary stem/progenitor cells and supports progression of terminal differentiation presenting in plural subcellular organelles including the ER but not the Golgi. However, the vital role of NNAT in tissue organogenesis is still obscure. In this study, to study a role of NNAT in cell differentiation, we analyzed by immunohistochemistry the adult rat testis progressing perpetual spermatogenesis and the tongue showing high cell turnover rate. Results showed that, in the testis, NNAT is first identified in cytoplasm of the round spermatids and then limited to the acrosome, which is known as a Ca^{2+}-storage organelle, in the elongated spermatids during the final step of spermatogenesis. In the spermiation phase, the intensity of immuno-reactive signals in the acrosome of the spermatozoa decreased during transportation from the caput to cauda epididymis. In the tongue, SOX2-positive basal cells are bipotential progenitor cells to differentiate into keratinized pore cells and taste bud cells. NNAT-positive signals located in basal cells and pore cells but not in taste bud cells. Recent studies reported that differentiation of keratinocytes in the skin is regulated by intracellular Ca^{2+}-level, and that NNAT promotes neurogenesis by co-localization with sarco/endoplasmic reticulum Ca^{2+}-ATPase to up-regulate intercellular Ca^{2+}. These reports together with our results suggest that NNAT progresses cell differentiation by participating in regulation of intracellular Ca^{2+}-level.

T2161

DEFICIENCY OF SMN AFFECTS GERM CELL DEVELOPMENT AND MAINTENANCE IN MICE

Chang, Wei-Fang[1], Xu, Jie[2], Liao, Hung-Fu[1], Chang, Chia-Chun[1], Liu, Ji-Long[3] and **Sung, Li-Ying**[4], [1]National Taiwan University, Taipei, Taiwan, [2]University of Michigan Medical Center, Ann Arbor, MI, U.S., [3]University of Oxford, Oxford, U.K., [4]Institute of Biotechnology, National Taiwan University, Taipei, Taiwan

Survival motor neuron (SMN) is known to be involved in various biological processes, such as transcriptional regulation, assembly of snRNPs and cellular trafficking. *SMN* gene mutations cause spinal muscular atrophy (SMA), an autosomal recessive disease and the most common genetic cause of childhood mortality. In its most severe type, the consequences of SMA include motor neuron loss, muscle degeneration and death. We have previously shown that SMN plays roles in stem cell division, proliferation and differentiation. In this study, we investigated the role of SMN in germ cell development and maintenance in a mouse model of SMA. Immunohistochemical staining and western blot revealed that SMN is enriched in testis, ovary, and spermatogonial stem cells (SSCs) of wild type (WT) mice. In SMA mice, the growth of gonadal tissues decreased in both males and females. The expression of spermatogonia marker, PLZF, was down regulated, evidenced by immunohistochemical staining. Moreover, the expression levels of Plzf, Sall4 and Lin28 in Thy1 sorted SSCs are significantly decreased following SMN knockdown. Consistently, there were more atretic follicles in ovaries of female SMA mice than in their littermate controls. Taken together, our results suggest that SMN plays a role in the development and maintenance of germ cell in mice.

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TOTIPOTENT/EARLY EMBRYO CELLS

T2163

DEFINING THREE LINEAGES OF THE HUMAN BLASTOCYST BY SINGLE CELL RNA-SEQ

Fogarty, Norah[1], Blakeley, Paul[1], del Valle, Ignacio[1], Wamaitha, Sissy E.[1], Hu, Tim X[2,3], Elder, Kay[4], Snell, Philip[4], Christie, Leila[4], Robson, Paul[2,5] and Niakan, Kathy[1], [1]The Francis Crick Institute, London, U.K., [2]Genome Institute of Singapore, Singapore, Singapore, [3]MRC Functional Genomics, Oxford, U.K., [4]Bourn Hall Clinic, Cambridge, U.K., [5]The Jackson Laboratory for Genomic Medicine, Farmington, CT, U.S.

Three lineages comprise the blastocyst: the epiblast (EPI) which gives rise to the embryo proper, the primitive endoderm (PE) which contributes to the yolk sac and the trophectoderm (TE) which contributes to the placenta. The molecular mechanisms regulating the specification of these lineages have been extensively studied in the mouse however, comparatively little is known about lineage specification in the human blastocyst. Here, we provide fundamental insights into early human development by single-cell RNA-seq of human and mouse preimplantation embryos. We integrate our own single-cell dataset with a previously published human dataset and compare this with a published mouse single-cell dataset. We elucidate conserved transcriptional programs along with those that are human specific. Importantly, we validate our RNA-seq findings by immunostaining. For example, we identify several genes exclusively expressed in the human EPI, including the transcription factor KLF17. Key components of the TGF-β signaling pathway, including NODAL, GDF3, TGFBR1/ALK5, LEFTY1, SMAD2, SMAD4 and TDGF1, are also enriched in the human EPI. Intriguingly, we demonstrate that inhibition of TGF-β signaling abrogates NANOG expression in human EPI cells, consistent with a requirement for this pathway in pluripotency. GATA2/Gata2 and GATA3/Gata3 show conserved enrichment in the TE. In contrast although the key TE factors Id2, Elf5, Eomes and Tcfap2c are exclusively localized to this lineage in the mouse, the human orthologues are either absent or expressed in alternative lineages. Interestingly, in the human TE we observe heterogeneous expression of key regulators of cytotrophoblast proliferation and differentiation TP63 and OVOL1 which may suggest further segregation within this lineage. Importantly, we also identify genes with conserved expression dynamics, including Foxa2/FOXA2, which we show is a novel marker of the PE in both human and mouse embryos. Comparison of the human EPI to existing embryonic stem cells (hESCs) reveals conservation of pluripotency but also additional pathways more enriched in hESCs. Our analysis highlights significant differences in human preimplantation development compared with mouse and provides a molecular blueprint to understand human embryogenesis and its relationship to stem cells.

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T2165

SPATIAL TRANSCRIPTOME OF MID-GASTRULA MOUSE EMBRYO: MOLECULAR ANNOTATION OF TRANSCRIPTIONAL ACTIVITY, LINEAGE FATES AND CELL IDENTITY

Peng, Guangdun[1], Suo, Shengbao[2], Chen, Jun[3], Chen, Weiyang[2], Liu, Chang[2], Tam, Patrick P.L.[4], Han, Jingdong[2] and Jing, Naihe[2], [1]SIBCB, SIBS, CAS, Shanghai, China, [2]Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China, [3]National Institute of Biological Sciences, Beijing, Beijing, China, [4]Children's Medical Research Institute, Westmead, Australia

During mouse embryogenesis, pluripotent cells in the epiblast become progressively restricted in developmental potential as they are allocated to different tissue lineages. To characterize the transcriptional activity accompanying lineage specification and regionalization of cell fates, we examined the transcriptome of cell populations at anatomically defined sites in the epiblast of mid-gastrulation mouse embryo. By 3-D rendition, expression of over 20,000 transcripts can be visualized digitally in a quantitative whole mount format. Analysis of the spatial transcriptome data revealed domains of transcriptional and signaling activity and identified functionally related genes, genes in synexpression groups and novel lineage markers. These unique gene sets provide the zipcodes for mapping pluripotent stem cells and their derivatives to the equivalent cell populations in the epiblast, enabling the delineation of their cell states and lineage identity and provide the molecular annotation of lineage propensity and lineage specification during gastrulation and the directed differentiation of stem cells.

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EMBRYONIC STEM CELL DIFFERENTIATION

T2169

RAT PLURIPOTENT STEM CELLS AS SOURCE OF NEURONAL STEM CELLS

Abello, Javier[1,2], Hong, He[2], Frey, Andrew[2], Tague, Sarah[3] and Weiss, Mark[2,4], [1]Kansas State University, Manhattan, KS, U.S., [2]Kansas State, Manhattan, KS, U.S., [3]University of Kansas, Kansas City, KS, U.S., [4]Kansas State Univ, Manhattan, KS, U.S.

Producing neuronal cells in-vitro has been a particular interest because of their potential as therapeutic applications in regenerative medicine. Traditionally, E14 (embryonic day 14) cortical tissues are used as source for differentiation because highly reproducible results can be generated in mouse and rat. Recently, much effort has been made to use ESCs or induced pluripotent stem cells (iPSCs) to differentiate into neuronal cells. Here, we sought to develop a robust and highly reproducible method of neuron differentiation from rat ESCs (lines from 3 different rat strains) and iPSCs (one line from Fischer 344 rat fibroblasts). The hanging drop method was the most highly reproducible method of producing consistent EBs. The EBs early induction with N2/ B27 medium with early exposure to rho-kinase inhibitor (ROCK). Process extension was dependent upon the laminin coating, and SMAD inhibitors were used for neuronal specification. Neural differentiation was induced using BDNF, ascorbic acid, and ROCK. Neural differentiation was verified by RT-PCR and qRT-PCR, cellular morphology, and immunocytochemistry using nestin and beta3 tubulin. Downregulation of the pluripotency markers Oct4 and nanog was found over the 12 day protocol and upregulation of the markers nestin and beta3 tubulin. Our findings demonstrate outstanding reproducibility of neural differentiation across all four cell lines.

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T2171

hESCs UNDERGO INTRINSIC ASYMMETRIC CELL DIVISION WHEN DIFFERENTIATING INTO THE PRIMITIVE STREAK

Brown, Kate, Stanford University, Stanford, CA, U.S. and Nusse, Roeland, Howard Hughes Medical Institute, Department of Developmental Biology, Stanford, CA, U.S.

Stem cells have the potential to undergo symmetric or asymmetric cell division (ACD). As opposed to a symmetric division, daughter cells from an ACD exhibit fates that differ from each other. Various factors determine which type of division occurs. These factors can be extrinsic (niche) factors or intrinsically partitioned components inherited by daughter cells upon cytokinesis. However, the processes involved in ACD in different stem cell populations remain an open question. We have made the unexpected observation that hESCs can undergo intrinsic ACD while differentiating into primitive streak cells. Previous work from our lab demonstrated that Wnt3a anchored to beads promotes ACD in mouse embryonic stem cells (mESCs). Therefore, we evaluated the effect of localized Wnt3a as a niche factor on human embryonic stem cells (hESCs). When grown as single cells, a condition necessary to evaluate the effects of signals, hESCs are prone to undergo apoptosis. However, after introduction of BCL2, hESCs survive and divide as single cells. Pairing live imaging and immunofluorescence allowed us to determine the history of cell division and to track daughter cells. On single cells, we evaluated the effects of localized Wnt3a on hESC division orientation and fate decisions. We found that Wnt3a beads, unlike on mESCs, were not able to orient the division of hESCs. Surprisingly however, the daughter pairs of hESCs do exhibit hallmarks of asymmetric cell fate (i.e. expression of primitive streak markers MIXL1 and BRACHYURY) in a Wnt dependent manner. In most cases, asymmetry becomes detectable 10 hours after division of the mother cell. This asymmetry suggests that although Wnt signaling is required for the expression of primitive streak markers, there is likely to be a parallel mechanism for differences between the two daughter cells.

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T2173

PROMOTION OF THE NOTCH SIGNALLING PATHWAY IN HUMAN RETINAL PROGENITORS INDUCES INCREASED GENE EXPRESSION OF RETINAL GLIA CELL MARKERS

Sook Hyun Chung[1], Weiyong Shen[1], Belinda Yau[1], Kathryn Davidson[2], Alice Pebay[2] and Mark Gillies[1], [1]University of Sydney, Sydney, Australia, [2]Centre for Eye Research Australia, Melbourne, Australia

Müller cells, the principal glial cells in the retina, play an important role in maintaining retinal homeostasis. Dysfunction of Müller cells has been reported in some retinal diseases such as diabetic retinopathy and macular telangiectasia type 2. A cell signalling pathway, has been identified to promote glial cell differentiation. Previous animal studies revealed that misexpression of genes related to this pathway in retinal progenitors promoted gliogenesis. Based on this, we hypothesised that activation of the cell signalling pathway in human retinal progenitors will induce increased expression of genes that are specific to retinal glial cells. We firstly differentiated H9 human embryonic stem (ES) cells (WA09, WiCell) into retinal

progenitors according to published methods with minor modifications. Briefly, ~50 clumps of undifferentiated ES cells were plated on matrigel coated plates and cultured with chemically defined neural induction media containing DKK-1 (peprotech), IGF-1 (peprotech) and Noggin (R&D Systems) for 3 weeks. Once retinal progenitor differentiation was confirmed by qRT-PCR with eye field primers: Six6, Pax6, Chx10, LhX and Rx, promotion of the cell signalling pathway was initiated by treating the cells with two ligands The results revealed that effector genes downstream of the cell signalling were highly upregulated 9 days after treatment with the ligands, whereas glial cell markers, cellular retinaldehyde binding protein (CRALBP), glial fibrillary acidic protein (GFAP) and vimentin, were upregulated after 6 weeks. Interestingly, GFAP exhibited the most marked increase of all when compared with its expression in untreated retinal progenitors. Its Ct value differences from retinal progenitors ranged between 3 and 11. Peak expression of GFAP was recorded at week 4 and maintained until week 6. We acknowledge that the data presenting here is preliminary and limited to genomic analyses, however, the results may contribute to a better understanding of the role of the identified cell signalling pathway in Müller cell differentiation.

Funding Source: The Ophthalmic Research Institute Australia 2016

T2175

HIGHLY EFFICIENT IMMUNOMAGNETIC PURIFICATION OF CARDIOMYOCYTES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Noack, Kristin[1], Bosio, Andreas[1] and **Eckardt, Dominik**[2], [1]Miltenyi Biotec GmbH, Bergisch Gladbach, Germany, [2]Miltenyi Biotec GmbH, Bergisch-Gladbach, Germany

Pure cardiomyocytes derived from human pluripotent stem cells (hPSCs) are of high interest for heart disease modeling, drug safety studies or development of cellular therapies. Although several protocols for cardiac differentiation of hPSCs have been developed, major limitations are high variability in differentiation efficacy e.g. due to clone-to-clone or experiment-to-experiment variations. Therefore, we have developed several tools to improve the workflow starting with CM differentiation, monolayer dissociation, immunomagnetic purification of CMs and downstream analysis using flow cytometry. hPSCs were maintained under xeno-free conditions in StemMACS iPS-Brew XF medium to keep pluripotency for >20 passages and enable for efficient cardiac differentiation. We chose a monolayer differentiation protocol based on the timely regulated activation and inhibition of Wnt signaling by small molecules. In order to identify antibodies suitable for CM enrichment or depletion of non-myocytes, we performed a surface marker screen with >400 antibodies between days 10-20 of differentiation. Besides identification of new surface markers, we confirmed expression of Sirpa and VCAM-1. Interestingly, our data indicate that these markers either label CMs and non-myocytes or only subpopulations of CMs. Based on these data we developed a novel magnetic cell separation procedure consistently delivering CM purities of up to 98%, independent of the differentiation protocol, the hPSC line used or time point and efficacy of differentiation. Magnetically enriched CMs showed a typical morphology, initiated contractions after replating and could be stably maintained in culture. Interestingly, enriched CMs could also be cryopreserved in StemMACS CryoBrew medium. In order to address potential CM subtype heterogeneity, we developed recombinant antibodies against general CM markers such as a-Actinin, Myosin Heavy Chain or Troponin T as well as subtype-specific antibodies against MLC2a and MLC2v distinguishing between atrial and ventricular CMs, respectively. Taken together, we have developed novel tools supporting the workflow for efficient generation, magnetic purification and flow cytometry or immunofluorecence-based characterization of hPSC-derived cardiomyocytes.

T2177

TISSUE SPECIFIC LAMININS GENERATE CLINICALLY COMPLIANT HPSC CULTURES FOR CLINICAL TRIALS

Hagbard, Louise, Xiao, Zhijie, Kallur, Therese, Ericsson, Jesper and Sun, Yi, BioLamina AB, Sundbyberg, Sweden

The lack of defined, xeno-free, robust methods for expansion and specialization of human pluripotent stem cells (hPSC) has hindered both the advancement of basic research and the translation into clinical settings. The expression and composition of the basement membrane proteins are essential for embryonic morphogenesis and adult functions. Laminins are the only basement membrane proteins that are tissue specific and with the use of the specific combination of xeno-free and defined human recombinant laminins, the natural environment for each specific cell type can be created, which in turn generate high quality cell cultures of well defined phenotypes. Laminin-521 (LN-521) is the laminin isoform naturally expressed by hPSCs and is also one of the most commonly expressed laminins after birth. LN-521 maintains high degree of hPSC homogeneity, pluripotency and genetic stability when used in vitro. Due to robust support provided by LN-521, human embryonic stem cell (hESC) lines can even be derived from a single blastomere, circumventing the ethical issues associated with hESCs. Here, we show how culture on LN-521, alone or in combination with other laminin isoforms, can been used to generate specialized cells from hPSC. 1) RPE cells can effectively be derived from LN-521 cultured hESC, exhibiting na-

tive characteristics including morphology, pigmentation, marker expression, polarization and phagocytic activity. Transplanted cells exhibit long-term integration and photoreceptor rescue capacity. 2) A highly pure population of dopaminergic progenitors can effectively be derived from laminin cultured hESC, with a 30-fold increase in cell yield compared to previous protocols. 3) By using heart specific laminins in the natural combination, high ratio of beating cardiomyocytes with characteristic morphology and marker expression can be generated. 4) Improved hepatocyte specification and maturation of hESC cultured on LN-521 and other liver specific laminin isoforms. The hepatic cells are highly organized, similar to primary tissue, and with significantly increase in metabolic functions. In addition, we also show how a laminin-521 coating substrate, fully compatible with GMP requirements, is being developed which facilitates the transition of these pre-clinical research protocols into clinical settings.

T2179

USING CO-CULTURE WITH STROMAL CELLS TO OPTIMIZE THE HEMOPOIETIC DIFFERENTIATION SYSTEM IN VITRO

Jiang, Xin-xing[1], Li, Qi[1], Sun, Fei[2], Long, Ping[1], Wei, Yun-jian[1], Yu, Yan-hong[2], Huang, Yuan-hua[1] and Ma, Yan-lin[1], [1]Affiliated Hospital of Hainan Medical University, 31 Longhua Road, Haikou, Hainan, China, [2]Nanfang Hospital, Southern Medical University, Guangzhou, China

Currently, the only definitive cure to malignant hematological disorders is allogeneic hematopoietic stem cell transplantation (allo – HSCT), but the resource of HSCs is limited. Embryonic stem cells (ESCs), which possess the potential for infinite proliferation and the ability to generate cells of all three germ layers, offer a new opportunity for cell resurces. In the current progress, safety and effectivity methods in the derivation of hematopoietic stem/precursor cells (HSPCs) from embryonic stem cells have been widely investigated. In this study, using classical method, that is coculture with OP9 stromal cells, we established a faster and more efficient way to produce CD34 positive HSPCs in vitro. Compared to traditional co-culture system, we set six different inoculation density for OP9 stromal cells and cultivated 24h, then co-cultured with ESCs. In this induced system, we explored an optimal density, which induced ESCs differentiate into hematopoietic stem/ precursor cells, and provided a more efficient differentiation induction method to in vitro research

Keywords Embryonic stem cells (ESCs); Hematopoietic stem/precursor cells (HSPCs); OP9 cells; Differentiation

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T2181

IMPROVEMENT OF HEPATIC METABOLIZING ACTIVITY IN HUMAN ES CELL-DERIVED HEPATOCYTES USING REPEATED EXPOSURES TO XENOBIOTICS

Kim, Hyojin, Kim, Jong Hyun, Jang, Yu Jin, Han, Jiyou, Park, Ji Young, Lee, GyungGyu, Son, Jeong Sang, Lee, Jaehun and Kim, Jong-Hoon, Korea university, Seoul, Korea, South

As a promising cell source for clinical application and in vitro drug screen, hepatic functions of human stem cell-derived hepatocyte-like cells (HLCs) should be increased comparable to human primary hepatocytes. However, the expression and activity of hepatic metabolizing enzymes of final HLCs are usually much lower than primary human hepatocytes. Here, we showed significantly increased hepatic metabolizing enzymes (Phase I, II, and nuclear receptor) using an optimized hepatic differentiation protocol; three-dimensional (3D) spheroidal culture and repeated exposure to xenobiotics. Prior to generate highly functional HLCs, >90% albumin-positive HLCs were produced by using our hepatic differentiating protocol without purification. Then we generated 3D spheroidal aggregates of hepablast, which have high viability and proliferation ability. Our data showed that the 3D hepatoblast-spheroids expressed significantly increased activity of hepatic enzymes, compared to 2D-cultured HLCs. Furthermore, when the 3D hepablast-spheroids were repeatedly exposed to xenobiotic (acetaminophen, rifampicin, and phenobarbital), the expression of hepatic enzymes were much more increased, compared to non-exposed group. In conclusion, our repeated exposure to xenobiotics for maturing hepatic functions of HLCs would be a new method to produce highly functional HLCs for clinical and in vitro drug screen

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T2183

GENERATING MESENCHYMAL STEM CELLS FROM HUMAN EMBRYONIC STEM CELLS IN SERUM-FREE, XENO-FREE, AND ALL-DEFINED MEDIA

Li, Enqin[1] and Xu, Ren-He[1,2], [1]University of Macau, Taipa, Macao, [2]University of Macau, Taipa, China

Mesenchymal stem cells (MSCs) have been derived from a variety of tissues using either serum-containing (SC) or serum-free (SF) media. Yet, little is known about the differences between MSCs derived and maintained in these two types of media. We have recently derived MSCs from human embryonic stem cells via trophoblast formation (named TMSCs). Trophoblast differentiation is induced by BMP4 in a SF medium, whereas MSC differentiation from trophoblasts is in a SC medium. Since serum contains xenogenic and undefined components, they add complexity and uncertainty for studying TMSCs. We show here that TMSCs can also be derived in two sequentially used SF media, and the cells demonstrate intriguing differences in derivation efficiency, senescence, cytogenetic stability, and response to inflammatory cytokines, compared to TMSCs derived in the SC medium. Based on these, we have developed a new protocol for TMSC generation under SF, xeno-free, and all-defined conditions. Our findings shall facilitate the research and clinical applications of the therapeutically promising cells.

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T2185

PROFILING ANALYSIS OF PROTEIN TYROSINE PHOSPHATASES DURING NEURONAL DIFFERENTIATION

MiHee, Oh, sun Young, Kim, Kyu-Suk, Lee, Won-Kon, Kim, Kyoung-Jin, Oh, Sang Chul, Lee, Kwang-Hee, Bae and Baek-Soo, Han, KRIBB, Daejeon, Korea

During neuronal differentiation, it is generally accepted that many kinases and phosphatases fulfill different roles. In this study, phosphor-tyrosine phosphatases were focused on and their expression profiling was evaluated during neuronal differentiation of mouse J1 embryonic stem cells. Among 83 phosphor-tyrosine phosphatases, expressions of 21 PTPs were increased but mRNA expressions of 10 PTPs decreased depending on the differentiation. We checked the protein expression patterns for the cases where PTPs mRNA expressions changed. Some of them showed consistent results with the mRNA expressions. In particular, it was found that dual-specific phosphatase23 (DUSP23) affected neuronal differentiation. The knock-down of DUSP23 decreased neuronal differentiation in terms of neuronal outgrowth and the expression of neuronal marker proteins and mRNAs. Taken together, the obtained results show that many PTPs play specific roles during neuronal differentiation and manipulating their activities by activators or inhibitors could adjust neuronal differentiation.

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T2187

SPONTANEOUSLY EXTRUDED syntaxin4 DISTURB STEMNESS OF EMBRYONIC STEM CELLS VIA REGULATION OF zscan4 FAMILY.

Natsumi, Hagiwara and Yohei, Hirai, Kwansei Gakuin University, Sanda, Japan

In embryonic stem (ES) cell colonies, a small cell population that displays irregular morphology often appears spontaneously, however, molecular mechanisms accounting for such stem cell plasticity remains largely elusive. In the present study, we show that the spontaneous extracellular translocation of syntaxin4 may be an important element for the appearance of such aberrant cells in the clonal stem cells. The proteins in the syntaxin family are known to mediate fusion of cytoplasmic vesicles to the target membrane, yet subpopulations of certain syntaxins, including syntaxin2 and 4, translocate across the cell membrane in response to external stimuli so as to function as signaling molecules. We found that a small subpopulation of ES and embryonic carcinoma (EC) cells extruded syntaxin4 at the cell surface even in medium for the maintenance of their stemnesss. When cell surface syntaxin4 was artificially expressed using tet-inducible expression system, dramatic changes in the cell morphology was induced in both ES and EC cell lines. In the EC cell line F9 that retains a potential to differentiate into endodermal lineage, the cell surface expression of syntaxin4 led to active pseudopodial protrusions with a dramatic down-regulation of E-cadherin. In contrast, both the pluripotent ES cells and another EC cell line P19CL6 that is capable of differentiation into mesodermal and ectodermal lineages, became flattened with up-regulation of mesodermal markers including T (brachyury) and α-SMA. ES cells up-regulated P-cadherin, instead of diminution of E-cadherin. To further identify downstream elements of the syntaxin4's effect in ES cells, we employed next-generation RNA-seq analyses. Intriguingly, the expression of extracellular syntaxin4 appeared to induce steep decline in the expression of all the zscan4 family members. These zscan4 proteins have SCAN and zinc finger domains and reportedly function as key transcriptional factors for the maintenance of genome integrity and pluripotency in ES cells. These results suggest that stem cells spontaneously extruded syntaxin4 extracellularly, which in turn locally

impacts cell morphology and abrogate subsequent differentiation through the regulation of factors controlling stemness.

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T2189

SINGLE-CELL TRANSCRIPT ANALYSIS OF HUMAN ENDOCRINE CELL DEVELOPMENT OF THE PANCREAS USING AN HESC-BASED MODEL SYSTEM

Petersen, Maja Borup Kjær[1,2], Hess, Katja[2], Hansson, Mattias[1], Grapin-Botton, Anne[2] and Honoré, Christian[1], [1]Novo Nordisk, DK-2760 Måløv, Denmark, [2]The Danish Stem Cell Centre, DanStem, University of Copenhagen, DK-2200 Copenhagen, Denmark

The islets of Langerhans are home to five different endocrine cell types, that all arise from a common progenitor that express the pro-endocrine gene Neurogenin 3. Pancreatic endocrine progenitors derived from human embryonic stem cells (hESCs) represent a promising source for cell-based therapies for diabetes, as well as they offer a unique opportunity to study the development of human pancreatic tissue in vitro. Here we investigate the specification of the endocrine cell types of the pancreas from NEUROG3$^+$ progenitors. Using a NEUROG3-GFP reporter hESC line, NEUROG3$^+$ progenitors of the pancreas are generated using directed differentiation. hESC-derived endocrine progenitors are sorted and analysed at single-cell level using quantitative RT-PCR to assay 88 genes mainly representing pro-endocrine markers and signaling-pathway-associated transcripts. The aim is to determine any potential heterogeneity within the progenitor population and gain insight into the mechanisms governing endocrine cell specification. Such analysis will provide novel scientific information concerning the development of the human endocrine lineages, which in turn can be used for controlling the specification of individual pancreatic islet cell types in vitro.

T2191

DIRECTING DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO SPECIFIC NEURONAL FATES USING A DESIGN OF EXPERIMENTS APPROACH

Sears, Katie[1,2], Bukys, Michael[3], Finney, Krystal[4] and Jensen, Jan[1,3], [1]Cleveland Clinic Lerner Research Institute, Cleveland, OH, U.S., [2]Lerner College of Medicine, Case Western Reserve University, Cleveland, OH, U.S., [3]Cleveland Clinic, Cleveland, OH, U.S., [4]Trailhead Biosystems, Cleveland, OH, U.S.

One of the most significant challenges to the implementation of cell therapy for the treatment of central nervous system (CNS) disease is a lack of accessibility to large numbers of specific human neurons. In vitro differentiation of human pluripotent stem cells (hPSCs) represents the most promising source of these cells. However, the development of differentiation protocols for specific neuronal subtypes has largely been done by varying one factor at a time in culture. While some efficient protocols have been established in this way, we believe a more comprehensive approach will enhance the development of protocols. For that reason, our lab has developed a systematic approach to directed differentiation that allows us to elucidate in detail the signaling pathways that drive cell fate in divergent directions. During development, cell fate decisions are driven by signaling molecules known as morphogens, which induce changes in expression of genes that give cells their cell type-specific characteristics. Our approach utilizes Design of Experiments (DoE) to comprehensively model the effects of morphogens on the expression of fate-defining genes. Using DoE, we determine which experimental conditions will provide the most information about the effects of up to 12 different factors on the expression of 106 genes of interest. In a single experiment, we test 96 different combinations and concentrations of 6 different morphogens and their inhibitors and quantify their effects using qPCR. From that data, we construct a multivariable model for the control of each gene measured and extract the morphogen conditions that will maximize expression of genes that are important for any given cell fate. Using this completely data-driven approach, we have developed a highly robust and rapid neural induction protocol and have described aspects of regional patterning in the neuroectodermal field. For example, we demonstrate that retinoic acid (RA) signaling is important for maximal expression of broad neural markers (SOX1, PAX6) and that RA acts synergistically with components of dual-SMAD inhibition protocols to upregulate those genes. RA signaling during neural induction also impacts expression of FOXA2, MITF, MNX1, and GBX2, suggesting that early patterning with RA is important for control of neuronal regionalization.

T2193

ENHANCED ENDODERMAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS ON ACTIVIN-TETHERED ELECTROSPUN NANOFIBERS

Tahamtani, Yaser, Royan Institute, Teheran, Iran

Human embryonic stem cell (hESC)-derived endodermal cells are of interest for the development of cellular therapies to treat disorders such as liver failure and diabetes. The soluble form of activin A (Act) has been widely used as an in vitro inducer of definitive endoderm (DE). However, the localized delivery of differentiation inducers through covalent immobilization may be advantageous for enhancing stability and efficacy of the delivery. Therefore, we have developed a nanofibrous poly (ε-caprolactone) substrate, biofunctionalized with Act, for directed differentiation of hESCs into DE. Bioconjugation of Act on nanofibrous meshes was confirmed by enzyme-linked immunosorbent assay (ELISA) and immunostaining. In order to investigate the bioactivity of immobilized Act (iAct), hESCs were cultivated on the Act-conjugated nanofibers for five days. The nanofibers with covalent iAct significantly increased expression levels of the endodermal markers SOX17, FOXA2 and CXCR4, compared to physically adsorbed Act (aAct) or without Act (noAct). In addition, iAct retained its bioactivity after storage at 37 °C for five days in the absence of cell seeding. The capability of cultivated cells to generate the DE-derived lineage was evaluated through further differentiation of seeded cells into hepatocyte-like cells (HLCs). Interestingly, the iAct sample showed a higher level of hepatic markers compared to the aAct sample. We also demonstrated that iAct in the presence of soluble Act (sAct) could improve the conventional protocol used to generate HLCs from hESCs. The results of this study can be a step towards creating more stable and defined methods for DE differentiation of hESC.

Funding Source: Reasearch Grant from Royan Institute

T2195

ZEB1-/- HUMAN ESCS UNDERGO ATTENUATED DIFFERENTIATION OF SYNCYTIOTROPHOBLASTS BY WNT PATHWAY

Wei, Yanxing[1], Yu, Yanhong[2], Shan, Yongli[3] and Huang, Wenhao[3], [1]Nanfang Hospital of Southern Medical University, Guangzhou, China, [2]Nan Fang Hospital, Southern Medical University, Guangzhou, China, [3]CAS Key Laboratory of Regenerative Biology, South China Institute for Stem Cell Biology and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China

In human placenta, syncytiotrophoblasts acts as an important component with secreting hormones such as hCG and PGF to maternal circulation for supporting a normal pregnancy. Defect of syncytiotrophoblast differentiation may lead to pregnancy loss or birth defects.Although the regulation of syncytiotrophoblasts had been explained by some factors (growth factors, cAMP and negative feedback from the hormones), however, few reports elucidated the specific gene during this process. Recently, CRISP/Case 9 technique, which precisely manipulate endogenous gene expression, had opened a new chapter in understanding the gene function and regulation. Hence, based on this technique, we generated homozygous ZEB1 knockout human embryonic stem cells (ZEB1$^{-/-}$hESCs), and then performed differentiation into different subtypes of trophoblast under E6 medium with adding 10ng/ml BMP4 and small molecules (A83-01 and PD173074). At day 9, the differentiated cells were harvested to be analyzed by immunostaining for specific markers, Q-PCR for gene expression, and biofunction tests. In order to understand the ZEB1 regulation whether by Wnt pathway, we endeavored to add the Wnt inhibitor (CHIR 99021) and activator(WAY-316606).Both cell lines showed transient expression of CDX2 and ELF5 at day 2 of differentiaton from ESCs to trophoblasts. And at day 9, ZEB1$^{-/-}$hESCs demonstrated positive immunostaining of KRT7 without difference to controls. However, no syncytiotrophoblast formation was observed during the whole process as the H1 did at day 3. Q-PCR revealed obvious neglected expression of CGB, CGA and PGF. On the other hand, the HLA-G expression and invasion ability showed no obvious difference. Furthermore, after adding CHIR 99021 and WAY-316606 , the ZEB1$^{-/-}$hESCs demonstrated no change in forming syncytiotrophoblast, where the hESCs controls showed higher expression of CGA, CGB and PGF as well as higher levels of hCG and PGF in ELISA. Our findings revealed the essential roles of ZEB1 in development of syncytiotrophoblasts through Wnt pathway by novel CRISP/Case 9 technique from ESCs accompanying by a transient trophoblast stem cell state based on newly BMP4 plus A83-01 and PD173074 driven system.

T2197

PROMOTION OF EARLY HEMATOPOIESIS FROM HUMAN PLURIPOTENT STEM CELLS TRIGGERED BY THE TEMPORAL OVEREXPRESSION OF GATA FACTORS

Zhou, Ya[1,2], Cheng, Bo[1], Bian, Guohui[1,2] and Ma, Feng[1], [1]Institute of Blood Transfusion, Chinese Academy of Medical Sciences, Chengdu, China, [2]State Key Laboratory of Experimental Hematology, Tianjin, China

Hematopoiesis is regulated by the spatial and temporal control of transcriptional networks. Two members of GATA family, GATA-1 and GATA-2, have homologous zinc fingers and govern the development of multiple hematopoietic cells. GATA-1 and GATA-2 show dynamic changes in the expression profiles and patterns during erythrocytes and mast cells differentiation. GATA-1 is essential for terminal differentiation of erythrocytes and megakaryocytes while high level of GATA-2 expression is needed in hematopoietic stem/progenitor cells (HSPCs), eosinophils and mast cells. However, temporal overexpression of GATA-1 or GATA-2 in the development of early hematopoietic cells from human embryonic stem cells (hESCs) is still unclear. Here, we report the establishment of inducible overexpression of GATA-1 or GATA-2 in hESCs (H1) transduced with PiggyBac vector by doxycycline (DOX) to investigate their role in early hematopoiesis. We first investigated the endogenous expression profile of GATA-1 and GATA-2 in the hESCs and mAGM-3 coculture from day 0 to14 by qRT-PCR. It was found the expression of GATA-1 increased gradually from day 0 to 10 then kept stably until day 14, while expression of GATA-2 increased from day 0 to 8 and gradully decreased from day 8 to 14, indicating their distinct role in the early hematopoiesis. We analysed the composition and proportion of hematopoietic cells in the PB-GATA-1-hESCs and PB-GATA-2-hESCs in cocultures at day 14 by a time-course triggering of DOX (at day 0,1,2,3,4,6,8 and 10). We found the number of $CD34^+CD43^+$ cells decreased when induced overexpression of GATA-1 at day 0 and 1, however, increased 2.7 fold at day 3, 4 and 6 than that without DOX. The number of $CD34^+CD45^+$ cells increased 1.8 fold when adding DOX at day6than that without DOX. By overexpression of GATA-2, the number of $CD34^+CD43^+$ and CD34+CD45+ cells were all increased from day 0 to 10 (2 to 4.9 fold for $CD34^+CD43^+$ cells and 1.6 to 5.2 fold for $CD34^+CD45^+$ cells). Presently, we are investigating the relationship of exogenous and endogenous expression of GATA-1 and GATA-2 in the hESC-derived hematopoiesis. The property and differentiational potential of the $CD34^+CD43^+$ cells and $CD34^+CD45^+$ cells gained by up-regulating with GATA-1 and GATA-2 are being explored in order to develop possible clinical applications.

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T2199

OPTIMIZATION OF CEREBRAL ORGANOID DIFFERENTIATION FOR DIFFERENT LINES OF HUMAN PLURIPOTENT STEM CELLS

Vishlaghi, Neda[1,2], Watanabe, Momoko[2] and Novitch, Bennett G.[2], [1]CIRM CSUN/UCLA bridges program, Northridge, CA, U.S., [2]UCLA/Broad Stem Cell Research Center, Los Angeles, CA, U.S.

The cerebral cortex is a specialized region of the brain responsible for higher neurological functions such as sensory processing, learning and memory, cognition, and abstract thinking. This region is disproportionally enlarged in humans and other primates, and thought to be one of the factors that allows for our enhanced intellectual capacities compared to lower species such as rodents. Hence, rodent models are not ideal tools for studying the primate-specific features that drive brain growth and complexity. Several studies have shown that human pluripotent stem cells (PSCs) have the ability to form three-dimensional cortex-like structures called cerebral organoids that recapitulate distinct aspects of human brain development. However, our and others results indicate that cortical organoid formation is often variable and inefficient, differing between hPSC lines and maintenance methods. Here, we examine the cerebral organoid differentiation capacities of different hPSC lines, and culture parameters including mouse embryonic feeder (MEF)-dependent and feeder free conditions. Our data indicate that cortical organoids can be reproducibly and efficiently produced from many hPSC lines solely when grown with high quality MEFs and defined media components. Under these conditions, >80% of cells within the organoids express the forebrain markers FOXG1, LHX2, and PAX6 and exhibit apicobasal neuroepithelial morphologies. We lastly present data examining the transcriptome signatures of successful and failed organoid differentiation. A better understanding of these features will help predict experimental outcomes and improve the methods used to create cerebral organoids for modeling normal and diseased human brain development.

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EMBRYONIC STEM CELL PLURIPOTENCY

T2203

LARGE GENETIC SCREEN FOR TUMORIGENESIS-RELATED GENES IN HUMAN PLURIPOTENT STEM CELLS

Weissbein, Uri, Plotnik, Omer and Benvenisty, Nissim, The Azrieli Center for Stem Cell and Genetic Research, The Hebrew University of Jerusalem, Jerusalem, Israel

Human pluripotent stem cells (hPSCs) generate tumors when injected into animals, and these tumors are more aggressive if the PSCs are genetically aberrant. The tumorigenicity of hPSCs is the most problematic issue in their clinical use. In order to identify the key genes that are involved in the tumorigenicity and the culture adaptation of hPSCs, we set up a system for genome wide screen for genes that can give growth selective advantage either in vitro or in vivo. Our screen involves the generation of two large libraries of karyotypically normal hPSCs, with more than $5x10^5$ random integrations of CMV enhancer and promoter sequences using PiggyBac transposons. We then exposed these libraries to multiple treatments that subjected them to stress and growth competition in culture and in animals. First, we grew the libraries for 20 passages and followed the pattern of integrations during culture adaptation. In both libraries we detected specific integrations that rapidly took over the culture, accompanied by increase growth rate. Examination of the integration sites revealed the role of specific genes including transcription factors and receptors, and the advantageous effects of inhibition of the RHO-ROCK pathway during prolong culturing. Importantly, we could show by analysis of expression data that this pathway is also altered during culturing of genetically unmodified hPSCs. Thus, alterations of this pathway can happen naturally by growth selection forces. Next, we treated the cells with PluriSIn#1, which selectively inhibits growth of PSCs by increasing ER stress. We could show that RAS pathway hyper-activation reduces the sensitivity to PluriSIn#1 treatments. Lastly, by producing teratomas from the transposon insertion libraries, we could demonstrate the strong clonality of the tumors, and to point to the genes that support tumor production. Our screen revealed the key genes and pathways relevant for the tumorigenicity and survival of PSCs, and thus should assist in understanding and reducing their tumorigenic potential.

T2205

COMPREHENSIVE CELL SURFACE PROTEIN PROFILING IDENTIFIES NOVEL MARKERS OF HUMAN NAÏVE AND PRIMED PLURIPOTENT STATES

Collier, Amanda Jayne[1,2], Panula, Sarita[3], Lanner, Fredrik[3] and Rugg-Gunn, Peter J.[1,2], [1]Babraham Institute, Cambridge, U.K., [2]Wellcome Trust – Medical Research Council Cambridge Stem Cell Institute, Cambridge, U.K., [3]Karolinska Institute, Stockholm, Sweden

Human pluripotent stem cells (PSCs) exist in multiple states of pluripotency; broadly categorised as naive and primed states. The distinct pluripotent states are characterised by transcriptional, epigenetic and signalling differences, and provide an important system to investigate the early stages of human development. Naïve cells can be obtained through primed-to-naïve resetting using defined transcription factors and ground state culture conditions, however there are no reliable methods to prospectively isolate unmodified naïve cells during this process. To overcome this limitation, PSC state-specific surface protein expression is a desirable tool to identify, isolate and characterise live populations of cells. Here we report the comprehensive profiling of naïve and primed PSC lines, screening over 500 antibodies to human cell surface proteins by flow cytometry. Identified state-specific surface proteins were validated across multiple other naïve PSC lines, notably derived using different methods of primed-to-naïve resetting including induced PSC reprogramming. Furthermore, naïve PSCs were positive by immunofluorescence microscopy for naïve cell-surface proteins, together with transcription factors implicated in ground state pluripotency such as KLF4; whereas primed PSCs were devoid of both naïve associated transcription factors and naïve cell-surface proteins. In addition, isolated populations with high naïve cell-surface protein expression exhibit increased expression of known naïve pluripotency associated genes, relative to unsorted cultures. Naïve-specific cell surface proteins are induced during the primed-to-naïve resetting process and enable the isolation of naïve cells. Interestingly, several of the proteins show differences in the timing of their appearance, suggesting the existence of intermediate cell populations. Similarly, naïve-specific cell surface proteins are extinguished during naïve-to-primed differentiation, concurrent with transcriptional downregulation of naïve pluripotency associated genes. Our state-specific surface proteins will enable intermediate PSC populations to be isolated and characterised during state transitioning experiments, thus informing us about the biology and hallmark characteristics of human pluripotent states.

T2207

COMMON GENETIC VARIATION AT THE NODAL LOCUS SHOWS A STRONG SEX BIAS IN HUMAN EMBRYONIC STEM CELL LINES, IS ASSOCIATED WITH XIST EXPRESSION, AND DIRECTLY CONTROLS THE NOVEL ALTERNATIVE SPLICING OF HUMAN NODAL

Findlay, Scott[1,2] and Postovit, Lynne-Marie[1], [1]University of Alberta, Edmonton, AB, Canada, [2]Western University, London, ON, Canada

The potential use of pluripotent stem cells for personalized regenerative medicine necessitates an improved understanding of how germ-line genetic variation may affect pluripotency. Given previous reports of a female bias in established human embryonic stem cell hES cell lines, sex-specific differences must also be considered. Our work identifies genetic polymorphisms that may have affected the establishment of widely used hESC lines in a sex-specific fashion. We demonstrate that the minor allele of the human single nucleotide polymorphism (SNP) rs2231947 found within the NODAL gene locus is under-represented in male but not female hES cell lines. In established female cell lines, the minor allele for SNP rs2231947 also shows a strong positive correlation with XIST expression. We demonstrate that this SNP is functional in that it directly controls the alternative splicing of a novel NODAL transcript isoform. This NODAL transcript variant is differentially post-translationally modified in that it is N-glycosylated in the mature peptide. Introduction of a similar N-glycosylation site in the constitutive NODAL isoform has been previously shown to enhance its stability and signalling range. However, we demonstrate that the NODAL variant isoform is unable to induce canonical SMAD2-dependent signals in a Zebrafish model of NODAL signalling. Comprehensive characterization of the linkage group marked by SNP rs2231947 reveals other SNPs with potential function in hES cells located within the proximal epiblast enhancer upstream of NODAL. Our work helps detail how genetic heterogeneity in the NODAL gene locus is manifested in human embryonic stem cell biology, and highlights the need to identify how specific genetic variants can explain important differences between pluripotent cell line models both within and between species. Genetic variation at the NODAL locus may play a role in the female bias in established hES cell lines, as well as in variable XIST expression between female hES cell lines—both of which are previously reported yet still unexplained phenomena in hES cells.

T2209

TANKYRASE INHIBITION STABILIZES NONTRANSGENIC CHEMICAL REVERSION OF RHESUS MONKEY PLURIPOTENT STEM CELLS TO A NAÏVE GROUND STATE

He, Yunlong[1,2], Pather, Sarshan[2], Zimmerlin, Ludovic[2], Mitalipov, Shoukhrat M.[3] and Zambidis, Elias[2], [1]Johns Hopkins University, Baltimore, MD, U.S., [2]Johns Hopkins University School of Medicine, Baltimore, MD, U.S., [3]Oregon Health & Science Univ, Beaverton, OR, U.S.

The derivation of human pluripotent stem cells (hPSC) in stable naïve states has wide impact in human developmental biology. However, both human and non-human primate (NHP) PSC exist in similar conventional primed epiblast pluripotent states, and are unstable in classical naïve mouse ESC WNT and MEK/ERK signal inhibition (2i) conditions. We recently established that small molecule inhibition of WNT, ERK and tankyrase signaling (LIF-3i medium) was sufficient for stable naïve reversion of a repertoire of hPSC. LIF-3i-treated human PSC acquired defining characteristics of mouse ESC including naïve-specific epigenetic features, and high clonal self-renewal that was independent of MEK-ERK signalling, but dependent on JAK-STAT3 and BMP4 signaling. Reduced hPSC lineage priming and optimized WNT signalling via tankyrase inhibition were important determinants of stable acquisition of this human naive ground state. Herein, we optimized these LIF-3i conditions to similarly revert several non-transgenic rhesus monkey PSC (rPSC) to stable naïve states. These naïve LIF-3i-reverted rPSC lines, including IVF-derived ORMES-22, parthenogenetic rPES-2 and somatic cell nuclear transfer-derived CRES-2, maintained significantly more stable SSEA4$^+$TRA-1-81$^+$ undifferentiated states than in primed bFGF cultures, and acquired multiple classical characteristic of mouse ESC. These characteristics included formation of small, mESC-like dome-shaped colonies that could be maintained long-term with MEK-ERK inhibition at high clonal proliferation rates, and with increased expression of naïve-specific transcripts (e.g., KLF2, ESRRB). The high similarities between human and NHP PSC and embryonic development potentially permit testing the efficacy of tankyrase inhibition in stabilizing primate pluripotency via use of naïve-reverted rPSC in a chimera assay, following introduction into host rhesus preimplantation embryos.

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T2211

REPRESSION OF THE ARYL HYDROCARBON RECEPTOR IS REQUIRED TO MAINTAIN MITOTIC PROGRESSION AND PREVENT LOSS OF PLURIPOTENCY OF EMBRYONIC STEM CELLS

Ko, Chia-I, Fan, YunXia, Wang, Qin, Xia, Ying and Puga, Alvaro, University of Cincinnati, Cincinnati, OH, U.S.

Lack of cell cycle checkpoints and uninterrupted passage through S-phase continuously renew the embryonic stem (ES) cell population and maintain pluripotency. We have showed that expression of the aryl hydrocarbon receptor (AHR), an environmental sensor and transcriptional regulator, is repressed in ES cells by binding of complex containing pluripotency factors and polycomb group proteins. In this study, we investigated the impact of untimely upregulation of AHR in ES cells by following the expression of eGFP and the Pac gene, encoding the puromycin resistance, under the control of the AHR-responsive Cyp1a1 promoter. We show that to regulate mitotic progression and pluripotency, ES cells must maintain the AHR in a persistent state of repression. Repression however is not absolute, but in fact oscillates between reversible states of expression and repression, with 12-15% of the cells escaping repression at any one time. Cells that escape repression also show reduced expression of pluripotency factors and lost pluripotent state. Additionally, AHR down-regulates MID1 expression and disrupts the MID1-PP2A-CDC25B-CDK1 signaling pathway that regulates mitosis. AHR-expressing ES cells delay the mitotic progression through down-regulating MID1 expression, which subsequently decreasing the PP2A phosphatase activity. Reduction of PP2A complex activity results in elevated CDC25B and lowered Y15-phosphorylated CDK1, leading to accelerated entry and delayed exit of mitosis. Unlike the bulk of the cell population that upon stimulation differentiates into cardiomyocytes, AHR-expressing ES cells restrict cardiogenesis and commit to a neuroglia cell fate. Our observations suggest AHR-regulated ES mitotic progression and pluripotency maintenance, which are related to the commitment of these cells during differentiation. It appears that the untimely expression of the Ahr gene in ES cells needs to be repressed to maintain mitotic progression, prevent premature loss of pluripotency, and ultimately preserve differentiation potential. This work was supported by NIH grants R01ES06273.

T2213

STAT3 PROMOTES MITOCHONDRIAL TRANSCRIPTION AND RESPIRATION DURING MAINTENANCE AND INDUCTION OF NAIVE PLURIPOTENCY

Carbognin, Elena[1], Betto, Riccardo[1], Soriano, Maria[2], Smith, Austin G.[3] and **Martello, Graziano**[1], [1]University of Padova, PADOVA, Italy, [2]University of Padova, Padova, Italy, [3]Wellcome Trust - Medical Research Council Cambridge Stem Cell Institute, Cambridge, U.K.

Transcription factor STAT3 directs self-renewal of pluripotent mouse embryonic stem (ES) cells downstream of the cytokine leukaemia inhibitory factor (LIF). STAT3 up-regulates pivotal transcription factors in the ES cell gene regulatory network to sustain naive identity. STAT3 also contributes to the rapid proliferation of ES cells. Here we show that STAT3 increases expression of mitochondrial-encoded transcripts and enhances mitochondrial respiration. Chromatin immunoprecipitation reveals that STAT3 binds to the mitochondrial genome, consistent with direct transcriptional regulation. An engineered form of STAT3 that localizes predominantly to mitochondria is sufficient to support enhanced proliferation of ES cells, but not to maintain their undifferentiated phenotype. Furthermore, during reprogramming from primed to naïve states of pluripotency STAT3 similarly up-regulates mitochondrial transcripts and facilitates metabolic resetting. These findings suggest that the potent stimulation of naïve pluripotency by LIF/STAT3 is attributable to parallel and synergistic induction of both mitochondrial respiration and nuclear transcription factors.

T2215

STIFFNESS AND MITOCHONDRIA – COMPLEMENTARY PLAYERS IN PLURIPOTENCY

Perestrelo, Tânia[1,2], Wu, Pei-Hsun[1,3], Gilkes, Daniele[3,4], Correia, Marcelo[2,5], Le, Christopher[3], Pereira, Sandro[5], Chen, Weitong[3], Rodrigues, Ana S.[5], Sousa, Maria I.[5,6], Ramalho-Santos, João[5,6] and Wirtz, Denis[1,3], [1]Institute for Nanobiotechnology at Johns Hopkins University, Baltimore, MD, U.S., [2]PhD Program in Experimental Biology and Biomedicine (PDBEB), Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal, [3]Department of Chemical and Biomolecular Engineering, The Johns Hopkins University, Baltimore, MD, U.S., [4]Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, U.S., [5]CNC-Center for Neuroscience and Cell Biology, Coimbra, Portugal, [6]Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Embryonic stem cells have the holotype of pluripotency, with defining characteristics such as self-renewal, low cytoplasm-to-nucleus ratio, and the capacity to differentiate readily in culture. The pluripotent phenotype can involve distinct states (naïve vs. primed) and various approaches have been described in order to control cell fate. Extracellular substrate patterning and mechanical stiffness regulate stem cell pluripotency and differentiation, and we and others have shown that metabolic requirements change during stem cell differentiation, suggesting mitochondria as one of the major players in regulating stem cell fate. Using mouse embryonic stem cells, we hypothesized that pluripotent stem cell fate can be regulated by modulating microenvironment stiffness in tandem with mitochondrial function. To test this hypothesis, we first cultured stem cells on matrices of different stiffness, and confirmed that embryonic stem cells differentiate more readily on stiffer substrates. We observed that stem cell exposure to matrices with distinct stiffness, before differentiation is triggered, induces changes in the differentiation rate. Concomitantly, mitochondrial modulators induce a decrease in the expression of differentiation markers, while maintaining the expression of pluripotent markers. This suggests that substrate compliance and regulated mitochondrial activity can synergistically suppress differentiation. Together, our results demonstrate that the combination of soft substrates and mitochondrial modulators is able to better control embryonic stem cell fate, promoting the maintenance of a naïve pluripotency ground state.

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T2217

THE HUMAN EMBRYONIC STEM CELL GENE HECAT5 STIMULATES THE ROBUST CELL EXPANSION VIA INTERACTION WITH MUSCARINIC ACETYLCHOLINE RECEPTOR M3

Shin, Hee Sun, Ajou University School of Medicine, Suwon, Korea, South

Human embryonic stem cells (hESCs) offer great potential for developmental research and for therapeutic use. Recent studies have identified many transcription factors for maintaining the stemness. However, membrane receptors that maintain stem cell characteristics are largely unknown. Here, we identified a hESC-specific membrane receptor, HECAT5, by using the digital differential display (DDD). This hESC-specific HECAT5 significantly increased cell expansion as well as the population of S phase. To investigate the possibility that HECAT5 mediate this effect through the interaction with an interacting candidate, muscarinic acetylcholine receptor M3 (M3R), we treated M3R modulators. M3R agonists robustly promoted the cell proliferation while antagonists inhibited it in only HECAT5-overexpressed cells. Furthermore, HECAT5 enhanced the cell-type specific tumorigenicity in xenograft models. These results demonstrate that HECAT5 stimulates the cell expansion by interacting with M3R.

T2219

A NOVEL GENE REGULATES HUMAN EMBRYONIC STEM CELL RENEWAL THROUGH GSH HOMEOSTASIS

Wang, Chengkai[1], Yang, Shang-Chih[1,2], Huang, Wei-Kai[3], Yang, Bei-Chia[4], Yu, John[4] and Lu, Jean[1], [1]Genomics research center, Academia sinica, Taipei, Taiwan, [2]Institute of Biochem. & Mol. Biol., Taipei, Taiwan, [3]Johns Hopkins University School of Medicine, Baltimore, MD, U.S., [4]Graduate Institute of Biomedical Sciences, Taoyuan City, Taiwan

Human embryonic stem cells (hESCs) derived from the inner cell mass of the early embryo and is characterized by its pluripotency, unlimited proliferation ability, and oncogenicity. hESCs can differentiate into embryoid body which composed by ectoderm, endoderm, and mesoderm cells. In addition, the oxidation stress can force ESC differentiation, but the direct molecular mechanism is unknown. By a high throughput screen with 517 shRNA, we pinpoint that 21 genes are essential for the ESC renewal. Among them, hESC-A is one of the most promising hits since it can efficiently affect both ES cell expansion and pluripotency to a level comparable to the knockdown of c-Myc. The hESC-A is a novel gene that its function and signals have never been reported. Thus we are the first group to demonstrate hESC-A gene functions. We found (1) hESC-A expression is enriched in undifferen-

tiated hESCs, but not in differentiated ESCs and human fibroblasts. (2) hESC-A is essential for the expression of critical stem cell transcriptional factor such as Sox2 and Nanog, and the downregulation of hESC-A upregulate the expression level of p27. (3)In addition, the expression of shRNA of hESC-A (sh-hESC-A) forced hESC differentiated into endoderm and expressed the master regulator Brachyury. (4)Moreover, hESC-A is crucial for the maintenance of reduction/oxidation status of glutathione (GSH)/ Glutathione disulfide (GSSG) and preventing of oxidative apoptosis.Thus for the first time, we proposed a novel gene, hESC-A, can bridge the pluripotency signal with the oxidation pathway. This will contribute to our understanding in stem cell biology and the reduction pathway.

T2221

TCF7L1 DRIVES EXIT FROM NAÏVE PLURIPOTENCY BY ENHANCER INACTIVATION

Zhang, Jenny Y., Shy, Brian R. and Merrill, Bradley J., University of Illinois at Chicago, Chicago, IL, U.S.

The transition of mouse embryonic stem cells (ESCs) to epiblast-like stem cells (EpiLCs) provides an in vitro system to study the developmental shift from naïve to primed pluripotency. Differences between these two states are exemplified by altered response to Wnt/β-catenin stimulation: the naïve, ground-state of ESCs enforces self-renewal whereas EpiLCs are primed for lineage specification following Wnt/β-catenin activation. β-catenin exerts transcriptional control by binding Tcf/Lefs, a family of DNA-binding proteins previously shown to play various roles in maintenance of and exit from the naïve state. Tcf7l1 limits ESC self-renewal, Tcf7 counteracts Tcf7l1's effects in ESCs, and Lef1 is up-regulated during differentiation. To definitively identify requirements of individual Tcf/Lef proteins in pluripotent cells, we used CRISPR/Cas9 mutagenesis to generate sixteen ESC lines containing all possible combinations of loss-of-function Tcf/Lef mutations. Surprisingly, ESCs lacking all four Tcf/Lefs (4x-/-) did not display significant growth, viability, morphological or transcriptome defects, indicating that Tcf/Lefs are dispensable for naïve pluripotency. Like ESCs deficient in only Tcf7l1, 4x-/- ESCs could not transition to the primed state under EpiLC culture conditions. However, Tcf7l1-containing cells lacking all other Tcf/Lefs underwent a wildtype-like transition to EpiLC. Together, these data show Tcf7l1 is the only Tcf/Lef necessary and sufficient to mediate exit from naïve pluripotency. Combined analyses of nucleosome occupancy, transcription factor binding, and histone modifications shows that Tcf7l1 binds highly active enhancers of the naïve state, which become inactivated in mature lineages. Tcf7l1-/- ESCs subjected to EpiLC conditions are unable to take on wildtype changes in nucleosome occupancy or gene expression at these enhancers, suggesting Tcf7l1 functions by reducing accessibility to naive-specific enhancers during the naïve-to-primed switch. Here in a complementary role to pioneer factors, which provide accessibility to closed regulatory regions, Tcf7l1 is proposed to be a member of "past-burying" factors necessary to inactivate naïve enhancers during differentiation.

EMBRYONIC STEM CELL CLINICAL APPLICATION

T2225

EFFECTIVE XENO-FREE CRYOPRESERVATION USING SLOW FREEZING METHOD FOR HUMAN EMBRYONIC STEM CELLS

Jung, Juwon[1], Seol, Hye-Won[2], Lee, Ji Young[1] and Choi, Young Min[3], [1]IRMP, Seoul National University, Seoul, Korea, South, [2]IRMP, MRC, Seoul National University, Seoul, Korea, South, [3]Seoul Natl Univ, College of Medicine, Department of OB/Gyn, Seoul, Korea, South

Effective cryopreservation and storage of Human embryonic stem cells (hESCs) are important for use regenerative medicine and successful cryopreservation of hESCs requires that effective and simple freezing methods for large scale storage in cell bank for the cell therapeutic application. Freezing and thawing of hESCs depends on optimal conditions could be affect on cell recovery. We performed to the four freezing media combined of cryoprotectant, 10% DMSO/90% KO SR XenoFree, 5% HES/5% DMSO/90% KO SR XenoFree and commercially xeno-free freezing medium, stem-cellbanker and cryo-gold for clinical grade hESC lines, SNUhES42 and SNUhES43, recovery rates using slow freezing and rapid thawing methods. 10% DMSO/90% KO SR XenoFree and stem-cellbanker was the best cell recovery efficiency over 70% both of SNUhES42, 43 and 5% HES/5% DMSO/90% KO SR XenoFree combined freezing media was cell recovery rates 35% in SNUhES42 and 15% in SNUhES43, however, cryo-gold was not recovery of hESCs colonies. We showed an animal products free freezing medium for hESCs the combination of DMSO, HES and KO SR Xenofree using conventional slow freezing method. This approach could be used for basic stem cell research and for clinical application studies. This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIP) (2012M3A9C6049722).

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T2227

INVESTIGATING THE EPIGENETIC MODULATION OF MIRNA BY TRITHORAX FAMILY DURING HEPATIC DIFFERENTIATION AND IN THE PATHOGENESIS OF LIVER DISEASES

Tsai, Pinghsing, Inst of Pharmacology, Taipei, Taiwan and Chiou, Shih-Hwa, National Yang-Ming Univeristy, Taipei, Taiwan

Nonalcoholic steatohepatitis (NASH) potentially threatens people health, and its pathogenesis has been linked to impaired lipid metabolism, tissue inflammation and epithelial-mesenchymal transition, and may have further to develop liver fibrosis, or hepatocellular carcinoma. Recently studies have indicated the positive correlation between the expression of Trithorax family gene and the incidence of NASH. Our preliminary findings revealed that ASH2L, a member of Trithorax family, can epigenetically modify H3K4me3 and subsequently promotes its transcription and cell proliferation. Some studies have demonstrated ASH2L and hepatocellular carcinoma is closely linked, however its involvement in NASH is still unknown. We found that embryonic stem cells and hepatoma cells carry high expression of ASH2L. Using quantitative chromatin immunoprecipitation, we found that ASH2L binds to regulatory element which can positively regulate the transcripts of microRNAs and lipid metabolism related genes in the embryonic stem cells. In addition, we established NASH mouse model by feeding methionine and choline deficient diet in ob/ob obese mice. In the livers from these NASH mouse model, we found the upregulation of ASH2L in the liver homogenates and increased miR155 in the exosomal fraction. Administration f miR155 inhibitor could effectively ameliorate the steatosis in this NASH model. Moreover, miR155 overexpression upregulated several factors related to epithelial-mesenchymal transition, whereas miR155 knockdown suppressed them. We further found that miR155 directly bound to the 3'UTR of SMAD4 and SOCS1, the downstream molecules of miR155. Following our previous study for ASH2L epigenetics and preliminary findings, we attempted to further elucidate the role of ASH2L in the regulation of micro-ribonucleic acid, lipid metabolism and epithelial-mesenchymal transition. Our findings are helpful and beneficial to the development of therapeutic strategies against candidate targets or miRNAs in nonalcoholic steatohepatitis.

CANCER CELLS

T2229

ESTABLISHMENT AND CHARACTERIZATION OF INDUCED OVARIAN CANCER STEM CELLS BY DEFINED FACTORS

Chen, Yu An[1], Lu, Chen Yu[1], Cheng, Wen Fang[2], Ho, Hong Nerng[1,3] and Chen, Hsin Fu[1,3], [1]Graduate Institute of Medical Genomics and Proteomics, College of Medicine and the Hospital National Taiwan University, Taipei, Taiwan, [2]Graduate Institute of Oncology, College of Medicine and the Hospital National Taiwan University, Taipei, Taiwan, [3]Department of Obstetrics and Gynecology, College of Medicine and the Hospital National Taiwan University, Taipei, Taiwan

Drug resistance and metastasis are the major factors leading to tragic prognosis and high mortality rates in cancer patients. Recent evidences suggest that cancer stem cells (CSCs) possess essential property including tolerance to chemotherapy, the ability to self-renew and generate cancer cells by asymmetric cell division. Nevertheless, the currently known CSC biology likely explain only the tip of an iceberg about the property, acquisition, maintenance and function of CSCs. Up to now, the information about CSCs by data mining remains limited due to the rarity of CSCs population in clinical samples. Ovarian cancer is one of the most lethal malignancy of the female reproductive system. CSCs in ovarian cancers, similar to those in other cancer cell types, may acquire the molecular armaments of stem cells, which carry stem cell property and can effectively determine the level of malignant potential of the cancer cells. In this study aiming to reprogram ovarian cancer cells into a putative CSC stage, we introduced a set of defined factors (OCT3/4, SOX2, KLF4 and c-Myc) into human serous ovarian cancer cells (OVCAR-3 cell line) through Sendai virus reprogramming,. We observed that the ovarian cancer cells transduced with the four Yamanaka factors demonstrated dissimilar developmental dynamics to traditional induced pluripotent stem (iPS) cells, and showed significantly enhanced pluripotent stem cell properties and drug resistance. We thus named the cells with CSC properties as induced ovarian CSCs (iOCSCs). These iOCSCs could be maintained and expanded up to at least 40 passages. Consistent with the original ovarian cancer cells, iOCSCs could be differentiated into ovarian lineage cells, displaying markers as CA125 and CK-7. Intriguingly, iOCSCs also acquired the ability to differentiate into cells belonging to the three embryonic germ layers in vitro and in vivo, similar to human iPS cells or ES cells. We also performed 3D culture of iOCSCs to generate organoids displaying ovarian lineage characteristics. This present approach thus makes it feasible to obtain abundant CSCs from ovarian cancer cell line to support our current knowledge and further in-depth research

into ovarian CSCs. Hopefully this cancer cell-to-CSC model can also help in developing new therapeutic strategies to treat ovarian cancers.

T2231

LOSS OF THE TRANSCRIPTIONAL REPRESSOR MUSCLEBLIND-LIKE 3 (MBNL3) CAUSE CANCER STEM CELLS TO SWITCH TO AN EMBRYONIC RNA SPLICING PATTERN ENHANCING THEIR SELF-RENEWING CAPACITY

Holm, Frida L[1], Hellqvist, Eva[1], Mason, Cayla[1], Barrett, Christian[2], Ali, Shawn[1], Chun, Elisabeth[3], Marra, Marco[4], Frazer, Kelly[2], Sadarangani, Anil[1] and Jamieson, Catriona H.M.[5], [1]Division of Regenerative Medicine, La Jolla, CA, U.S., [2]Institute for Genomic Medicine, Department of Pediatrics, University of California, La Jolla, CA, U.S., [3]Canada's Michael Smith Genome Center, Vancouver, British Columbia, BC, Canada, [4]University of British Columbia, Vancouver, BC, Canada, [5]Moores Cancer Center University of California San Diego, La Jolla, CA, U.S.

Since the establishment of human embryonic stem cell (hESC) lines and the discovery of stem cell characteristic induction in somatic cells by transcription factors OCT3/4, SOX2, C-MYC, and KLF4, human pluripotent stem cell research has provided key insights into fundamental facets of human developmental biology. Formative research suggests that a hESC-specific alternative splicing gene regulatory network, which is repressed by Muscleblind-like (MBNL) RNA binding proteins, is involved in cell reprogramming. We have for the first time demonstrated that lentiviral knockdown of MBNL3 resulted in reversion to an embryonic alternative splice isoform program typified by overexpression of CD44 transcript variant 3 (CD44v3) and blast crisis leukemia stem cell proliferation.

The particular roles of alternative splicing of CD44 in human leukemia stem cell generation and the mechanism(s) governing splice isoform usage has not been elucidated. We performed extensive leukemia stem cell whole transcriptome RNA-sequencing (Illumina HiSeq 2000), lentiviral overexpression and knockdown and discovered that decreased expression of MBNL3, a repressor of an embryonic alternative splicing program and reprogramming, activated a pluripotency network and increased expression of a pro-survival isoform of CD44v3, which is more commonly expressed in hESCs. This resulted in malignant reprogramming of progenitors in blast crisis CML endowing them with unbridled survival and self-renewal capacity. This is the first description of MBNL3 downregulation as a mechanism of reversion to an embryonic alternative splicing program, which elicits malignant progenitor reprogramming of progenitors into self-renewing leukemia stem cells. While isoform specific lentiviral CD44v3 overexpression enhanced chronic phase CML progenitor replating capacity, lentiviral shRNA knockdown abrogated these effects. In keeping with activation of a stem cell reprogramming network, CD44v3 upregulation is associated with increased expression of pluripotency transcription factors, including OCT4, SOX2 and b-catenin in addition to the pro-survival long isoforms of MCL1 and BCLX resulting in increased self-renewal and apoptosis resistance

Funding Source: The Swedish Childhood Cancer Foundation

T2233

RHENIUM-188 RADIOPHARMACEUTICAL REPRESSES IN VIVO GROWTH AND STEM CELL PROPERTIES OF HNSCC CANCER STEM CELLS VIA LET-7-ASSOCIATED SIGNALING PATHWAY

Lin, Liang-Ting[1], Chang, Chun-Yuan[2], Chang, Chih-Hsien[3], Wang, Hsin-Ell[2], Lee, Te-Wei[3], Lee, Yi-Jang[2] and Chiou, Shih-Hwa[4], [1]Taipei Veterans General Hospital, Taipei, Taiwan, [2]National Yang-Ming University, Taipei, Taiwan, [3]Institute of Nuclear Energy Research, Taoyuan, Taiwan, [4]National Yang-Ming Univeristy, Taipei, Taiwan

Head and neck squamous cell carcinoma (HNSCC) is generally treated with surgical resection with adjuvant radiotherapy and chemotherapy regarding the possible retained pathogenic tissues. However, severe side effects commonly restrict the therapeutic strategies regarding critical organs surrounded. Moreover, a subset of drug resistant cancer cells, also known as cancer stem cells (CSC), share the similar gene signature with stem cells and are the major cause of setbacks in patients. The current administration of chemodrugs as well as external beam radiotherapy are not effective enough to treat HNSCC in a curative intent. PEGylated liposome is a reliable carrier developed for therapeutic agent delivery, including radiopharmaceuticals, through enhanced permeability and retention (EPR) effect-mediated tumor targeting. In this study, we encapsulated Rhenium-188 (^{188}Re), a theranostic radioisotope emitting not only diagnostic gamma-ray but also therapeutic high energy beta particles, into PEGylated liposomes to demonstrate both the pharmacokinetics and therapeutic efficacy of ^{188}Re-liposome in HNSCC-bearing mice. Non-invasive molecular imaging displayed that the *in vivo* tumor growth was significantly suppressed in ^{188}Re-liposome comparing to free form ^{188}Re-BMEDA. Despite the ionizing radiation has long been linked to the induction of DNA damage, little is known for the molecular profiles and the response of HNSCC cancer stem cells against radiopharmaceutical treatment. Using global gene expression array, we identified a tumor suppressive microRNA subfamily, let-7, was abundant while

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HNSCC treated with ^{188}Re-liposome. Concomitantly, the downstream genes, HMGA2 and ARID3B, were found decreased in the ^{188}Re-liposome treated ex vivo samples, whereas the upstream LIN28B was not changed. Inhibition of let-7 dramatically withdrew the tumor suppressive effect of ^{188}Re-liposome, suggesting the crossfire of ^{188}Re-ionizing radiation not only directly kills cells but also triggers a let-7-associated tumor suppressive signal in a LIN28-independent regulation. Taken together, we unveil that let-7 is involved in the ^{188}Re-liposome-induced tumor suppressive signal in addition to IR-induced cell death, which can be an effective candidate for eliminating HNSCC CSC in future.

Funding Source: This study is funded by Ministry of Science and Technology and Institute of Nuclear Energy Research, Taiwan.

T2235

MODULATION OF IMMUNE CHECKPOINTS AND EFFECTS ON CANCER STEM CELLS IN GLIOMA

Murphy, Nicole Elizabeth[1], Grossauer, Stefan[1], Koeck, Katharina[1], Meyers, Ian[2], McMahon, Martin[3], James, C. David[1] and Petritsch, Claudia[3], [1]UNIVERSITY OF CALIFORNIA, SAN FRANCISCO, San Francisco, CA, U.S., [2]University of California, San Francisco, San Francisco, CA, U.S., [3]University of California San Francisco, San Francisco, CA, U.S.

Cancer stem cells (CSCs) are tumor subpopulations with heightened malignant potential when xenografted. Glioblastoma (GBM) CSCs exhibit stem like features, such as self-renewal through asymmetric cell division and survive standard radiation- and chemotherapy and evade targeted MEK pathway inhibition in tumors with elevated MEK pathway activation (Lerner et al, 2015, Cancer Research, 75: p5355). Interestingly, CSCs are immune suppressive in part due to their expression of immune checkpoint inhibitors like programmed cell death ligand1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Inhibitors of checkpoint inhibitors (anti-PD-L1/anti-CTL4A) stimulate the immune system and show striking clinical responses in many solid cancer types. How these inhibitors affect CSCS is an important but poorly understood question. The objective of our research is to determine whether anti-PD-L1 and/or anti-CTLA4 eliminate CSCs and thereby elicit a more durable anti-tumor response than MEK inhibitors. To reach our objective we will test the hypothesis that anti-PD-L1/anti-CTLA-4 treatment will eliminate CSCs more effectively than MEK pathway inhibition. We test our hypothesis in our novel immune-competent mouse model of glioma, which is constitutively activated for MEK pathway (Grossauer et al, In Preparation). Mice carrying orthotopic tumor allografts will be treated with anti-PD-L1, anti-CTL4 and anti MEK pathway therapy as single agents or in combination. Tumor growth effects will be measured using bioluminescence and symptom-free survival. CSCs frequency, cell proliferation rate, survival and cell division mode will be determined by immunofluorescence staining of tumors from resected brains of all treatment groups. By comparing tumors before treatment to 14- and 30- day-treated tumors, we will be able to assess the effects of novel immune checkpoint inhibitors on CSCs over time. We expect the frequency of CSCs to decrease most, with the combination treatment and if this is combined with a robust reduction in tumor growth and increased survival, we will conclude that this regimen is most effective in part because it effectively reduces CSC growth in addition to non-CSC growth. Our study is expected to provide important insights into the interaction of CSCs with novel immune checkpoint inhibitors

T2237

THE ROLE OF YY1 IN NEURAL CREST DEVELOPMENT AND MELANOMA FORMATION

Varum, Sandra[1], Baggiolini, Arianna[2], Zurkirchen, Luis[1], Levesque, Mitch[1], Dummer, Reinhard[1] and Sommer, Lukas[1], [1]University of Zurich, Zurich, Switzerland, [2]Universität Zürich, Zürich, Switzerland

There is a growing body of evidence suggesting that cancer cells highjack developmental programs to mediate disease initiation and progression. Melanoma is a neural crest-related cancer that originates from atypical melanocytic behavior and is known to be a highly heterogeneous and invasive cancer type. These properties are reminiscent of the broad differentiation potential and high migratory capacity of neural crest (NC) cells. Our study aimed to identify new transcription factors that are required for NC development and to determine whether these factors play a role in melanoma disease formation and progression. To address this question we performed a microarray analysis comparing migratory NC stem cells to NC cells primed to differentiation. To determine key transcription factors that could explain the global gene expression observed during the NC differentiation process, we employed a motif activity response analysis using ISMARA. The analysis revealed that undifferentiated NC cells displayed high Yin Yang (Yy1) motif activity when compared to their primed counterparts. Yy1 is a transcription factor that plays pleiotropic roles, namely in proliferation, differentiation, and survival but in a cell type-dependent manner. Further, it is known that Yy1 is overexpressed in many cancer types. To dissect the *in vivo* role of Yy1 during NC cell development, we conditionally ablated Yy1 in the premigratory NC. Strikingly, loss of Yy1 in the NC resulted in agenesis or hypoplasia of various NC derivatives. However, mutant derivatives contained differentiated cells, suggesting that differentiation as such is not impaired by Yy1 inactivation. We next sought to determine whether Yy1 played a role in melanoma initiation or progression. For that purpose we conditional ablated one copy of Yy1 in the melanocytic lineage of the *TyR:: N-Ras* ^{Q61K} *Ink4a*$^{-/-}$ melanoma mouse

model. Reduction of Yy1 levels was sufficient to prevent melanoma formation in this model. Taken together, our results suggest that Yy1 is not only important for NC development but that it also plays a role in melanoma formation. Thus, understanding NC development might provide new insights in melanoma biology.

T2239

INHIBITION OF SLUG EXPRESSION ENHANCES MULTIDRUG RESISTANCE OF HEPATOCELLULAR CARCINOMA CELLS

Zhao, Kewen[1], Zhao, Xin-Yu[1], Wang, Xiao-Bo[1], Fu, Rong-Jie[1] and Huang, Lei[2], [1]Shanghai Jiao Tong University School of Medicine, Shanghai, China, [2]Key Laboratory of Cell Differentiation and Apoptosis of the Chinese Ministry of Education CShanghai Jiao Tong University School of Medicine, Shanghai, China

Drug resistance is the main challenge of cancer therapy, which results in poor prognosis and low overall survival. Slug was used to be reported as an epithelial-to-mesenchymal transition-inducing transcription factor (EMT-TF) that drives neoplastic epithelial cells into more malignant mesenchymal phenotype. Ectopic expression of Slug can also promote the tumor-initiating ability of human breast cancer cells when collaborated with Sox9. However, recent study showed that physiological level of Slug reduced in the hyperplastic lesions of human breast [2015, nature, Ye X. and Weinberg RA]. So it remains largely unknown the roles of endogenous Slug in sensitivity of chemotherapy drugs to malignant tumor. Herein, we inhibited Slug expression with shRNAs in hepatocellular carcinoma cells (HCCs) and identified that anchorage-independent growth, including the formation of tumor sphere and soft agar colony, was increased accompanied with the decrease of Slug expression. Suppression Slug sufficed to up-regulate several cancer stem genes, including ALDH1A1. Although unrelated with the metastatic abilities as migration and invasion, slug inhibition did increase the efflux of Hoechst 33342 and enhance multidrug resistance of HCCs. Decreasing Slug level could increase several ATP-binding cassette (ABC) transporters genes such as ABCB1 and ABCG2. Moreover, ABC transporters' inhibitor verapamil could rescue the multidrug resistance induced by Slug inhibition. Our study provides evidence that Slug influences drug sensitivity in HCCs, which sheds new insights on mechanisms of multidrug resistance of hepatocellular carcinoma.

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TECHNOLOGIES FOR STEM CELL RESEARCH

T3001

FULLY AUTOMATED EXPANSION, SCHEDULING AND NON-INVASIVE QC OF HUMAN IPSCS ON THE STEMCELLFACTORY

Andreas, Elanzew[1,2], Oliver, Rippel[1], Daniel, Langendoerfer[1], Michael, Kulik[3], Friedrich, Schenk[3], Oliver, Brüstle[1,2] and Simone, Haupt[1,2], [1]LIFE&BRAIN GmbH, Bonn, Germany, [2]Institute of Reconstructive Neurobiology, Bonn, Germany, [3]Fraunhofer Institute for Production Technology IPT, Aachen, Germany

The biomedical prospects of human induced pluripotent stem cells (hiPSCs) have created an urgent need for standardized and automated processes for reprogramming and expansion of hiPSC lines from large patient cohorts. This need can be met by the StemCellFactory (www.stemcellfactory.de), a large system integration that automates and controls all required cell culture steps, ranging from adult human dermal fibroblast (HF) expansion via feeder-free, Sendai virus-based reprogramming to clonal selection and enzyme-free expansion of the obtained hiPSC clones and lines. For the establishment of a fully automated production process the integration of on-line measurement technologies for QC and remote cell culture monitoring are required. Threshold based process logics were implemented that compute and translate the acquired data into computer-directed cell culture workflows. To fully automate the decision making for sub-cultivation and maintenance of hiPSC a plate reader and an automated high-speed microscopy platform were implemented into the StemCellFactory. An image-based detection assay was established that enables dynamic feedback by computing confluence-based splitting ratios. For parallel and fully automated hiPSC generation and cultivation, well-based and plate-based automated splitting protocols were developed, which enable clonal expansion of individual hiPSC clones in 24-well MTPs as well as scaled production of hiPSCs in 6-well MTPs. Eventually, extensive biological validation was performed to confirmed that automatically expanded hiPSCs remain pluripotent upon automated long-term (10 passages) cultivation. In summary our data show that analysis of in-process generated data largely facilitates automation of highly dynamic cell culture processes.

T3003

HIGH-THROUGHPUT ARRAYS OF MULTIPLEXED CELLULAR COMMUNITIES REVEAL DYNAMICS OF ADULT RAT HIPPOCAMPAL NEURAL STEM CELL FATE DECISIONS

Bremer, Andrew W.[1], Scheideler, Olivia[1], Chen, Sisi[2], Gartner, Zev[3], Sohn, Lydia[4] and Schaffer, David V.[4], [1]University of California Berkeley, University of California San Francisco, Berkeley, CA, U.S., [2]University of California, San Francisco, San Francisco, CA, U.S., [3]University of California, San Francisco, San Francisco, CA, U.S., [4]University of California, Berkeley, Berkeley, CA, U.S.

Cell-cell interactions govern many biological processes, requiring single cells to make decisions within a complex milieu of instructive inputs. This is particularly relevant to the stem cell niche, wherein stem cells integrate a multitude of signals from neighboring cells that ultimately guide cell behavior, ranging from quiescence, proliferation, differentiation and migration. Current technologies fall short in supporting investigations of how the spatial variation of instructive cues and arrangement of heterotypic cells within a niche direct stem cell behavior, including how a stem cell resolves the presentation of multiple signals that drive distinct behaviors. To obtain a more complete understanding of how single cells make decisions within a complex microenvironment, we developed a novel method that enables long-term investigation of single-cell behavior within the context of multiplexed cellular communities. Utilizing DNA-programmed adhesion of cells, our platform consists of arrayed cellular communities and allows control over the number and initial position of at least four distinct cell types within each community. We also display the ability to track single-cell migration, proliferation, and differentiation. In one application of this platform, we studied the dynamics of adult hippocampal neural stem cell fate decisions within the context of competing juxtacrine signals. First, we engineered two astrocyte cell lines to constitutively overexpress either Delta-like 1 or Ephrin-B2, two membrane-bound juxtacrine ligands that instruct competing fate decisions on adult neural stem cells. Next, we utilized our platform to generate three-cell communities consisting of one neural stem cell and two astrocytes, one from each engineered astrocyte cell line. In response to these competing cues, we discovered that the neural stem cell prioritizes the stem cell maintenance signal imposed by Delta-like 1 over Ephrin-B2's cue to differentiate, suggesting a potential signaling hierarchy within the adult rat neural stem cell niche. With this platform in-hand, we are well positioned to dissect and model complex signaling networks within a broad range of biological systems.

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T3005

R26R-AR: A CRE-ACTIVABLE REPORTER MOUSE OVEREXPRESSING MYRAKT AND H-RASV12

Fan, Hsiang-Hsuan[1], Su, I-Chang[1,2], Hong, Jin-Bon[1,3], Lin, Chang-Ching[2], Lee, Tung-Lung[3], Yang, Tsung-Lin[3], Yu, I-Shing[1], Lin, Shu-Wha[1] and **Chen, You-Tzung**[1], [1]National Taiwan University College of Medicine, Taipei, Taiwan, [2]Taipei Cathay General Hosp, Taipei, Taiwan, [3]National Taiwan University Hospital, Taipei, Taiwan

Stem cells have a greater potential of tumorigenesis than the ordinary somatic cells in some cancer models, such as the intestinal cancer. Activation of AKT and RAS signaling pathways are often involved in tumorigenesis. Here, we generated a conditional mouse model overexpressing active forms of AKT and RAS in a temporal and tissue-specific control. Crossing with an Alb-Cre transgenic mouse, we found co-expression of active forms of AKT and RAS in liver results in liver cancer formation early in life. We also found co-expression of active forms of AKT and RAS in skin under K15-CrePR induction results in skin tumor formation. Our mouse model may help to uncover the mechanism of AKT-and RAS-related tumorigenesis.

T3007

ENHANCED PROCESSING FOR THE CLINICAL TRANSLATION OF HUMAN COMPACT BONE MESENCHYMAL STEM CELLS

Fernandez-Moure, Joseph[1], Martinez, Rudy[2], Weiner, Bradley[1], Rameshwar, Pranela[3], Coleman, Michael[2] and Tasciotti, Ennio[1], [1]Houston Methodist Research Institute, Houston, TX, U.S., [2]Ingeneron Inc., Houston, TX, U.S., [3]Rutgers New Jersey Medical School, Newark, NJ, U.S.

The key to the translation of cell based therapies in surgical application hinges on adhering to the guidelines of minimal manipulation and the ability to reliably and reproducibly supply a population of cells that can be used in the intraoperative setting. Compact bone fraction mesenchymal stem cells (CBF-MSC) have been shown to have greater osteogenic potential when compared to adipose and bone marrow. A limiting factor in the translation of this cell source in clinical tissue engineering is the long processing time to obtain the cells and the inability to

achieve the standards of minimal manipulation required for clinical use. In this study, we sought to investigate the capabilities of a previously validated system for rapid processing of tissue to process bone for the concentration of MSC. We hypothesized that a population of cells fulfilling minimal criteria for MSC can be processed from bone within a time frame capable of being applied intraoperatively. Bone chips from patients undergoing spinal laminectomy were cleaned of soft tissue debris and rinsed of all marrow componenets. Bone MSCs were isolated using the InGeneron TRT™ Processing Unit and method. Tissue was processed with a proprietary enzyme mixture and purified through filtration and centrifugation. Cell counts and viability were performed with a hemocytometer using a SYTO-13 fluorsecent stain. Freshly isolated cells were frozen for flow cytometry or cultured on T-75 culture flasks.

Results: Processing time was decreased from 3 days to 2 hours. Over one million cells were isolated. Cell isolated showed spindle like morphology and adherence to plastic. Cells isolated also showed 90% viablity post-processing. Flow cytometry of cell surface markers staining strongly positive for CD90 and osteocalcin and negtive or inconclusive for CD34 and CD45.The goal of stem cell processing is to enrich target populations. Intraoperative uses are hindered by long processing times and manipulation of tissues. Here we demonstrate a method of isolation that is compatible with intraoperative use in both timing and setup. With enhanced processing of primary tissues and maintenance of cell viablity this is a translational advancement of this technology for use in the intraoperative setting for clinical orthopedic tissue enginering.

T3009

RE-ENGINEERED RNA-GUIDED FOKI-NUCLEASES FOR IMPROVED GENOME EDITING OF HUMAN PLURIPOTENT STEM CELLS.

Havlicek, Steven[1], Shen, Yang[1], Alpagu, Yunus[2], Fu, Zhiyan[1], Dunn, Norris R.[2] and Stanton, Lawrence[3], [1]Genome Institute of Singapore, Singapore, Singapore, [2]Institute of Medical Biology, A*STAR, Singapore, Singapore, [3]Genome Inst of Singapore, Singapore, Singapore

The CRISPR/Cas9 system enables scientists to perform targeted changes in the genomes of cells and organisms, and therefore holds tremendous promise for healthcare, agriculture, and basic science. However, many groups have reported high-frequency off-target effects of Cas9, a major limitation hampering its utility particularly as a therapeutic tool. Hence, there have been considerable efforts to improve the specificity of gene targeting while maintaining efficiency in the process. Promising approaches take advantage of alternative nucleases derived from Cas9 such as the development of RNA-guided FokI-nucleases (RFNs). This system has been shown to have the highest editing specificity, but this improvement has come with serious losses of cleavage efficiency and problematic restrictions in the number of targetable sites in the genome. We have developed new RFN chimeras that exhibit improved cleavage efficiencies and have a greatly expanded targeting range in the genome. We illustrate these properties both by targeting the transgene GFP in human HEK293T-GFP cells, as well as by modifying endogenous genes in human embryonic stem cells and HEK293T cells. Importantly, these new RFN chimeras retain their high fidelity. The results described here represent significant improvements and substantial advances over existing technology, allowing a larger number of target sites to be edited more efficiently, at greater design flexibility.

T3011

IMPROVED TRANSFECTION EFFICIENCY IN HUMAN EMBRYONIC STEM CELL USING NON-ENZYMATIC METHOD

Hwang, In-Kyu, Konkuk University, Seoul/Gwangjin-gu, Korea, South

Human embryonic stem cells (hESCs) are pluripotent cells used in many basic and regenerative medicine studies due to their ability to self-renew, proliferate, and differentiate. While genetically modified hESCs can be highly valuable for research purposes, low transfection efficiency often limits the generation of modified hESCs. Here, we transiently passaged transfected hESC lines with enzyme based reagents (trypsin and trypLE) and non-enzyme based reagent (EDTA) to improve transfection efficiency. Flow cytometric analysis using an enhanced green fluorescent protein (EGFP) vector showed that EDTA method is more efficient for transfection in comparison to standard enzyme and normal (up to 9.98 ~ 19.34 %). In addition, the EDTA approach maintained stable cell viability and steady recovery rate of hESCs after transfection. Transgene expression was maintained throughout three germ layer differentiation. These results demonstrate that genetic modification of hESCs using EDTA method provides an ideal tool for basic and applied research using pluripotent stem cells.

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T3013

DEVELOPING CELL CULTURE STRATEGIES FOR EFFICIENT GENOME EDITING IN HPSCS

Yu, Zhiyuan, Zhang, Ping and **Kim, Jean Jieun**, Baylor College of Medicine, Houston, TX, U.S.

Human pluripotent stem cells (hPSCs) can be powerful experimental platforms, especially in combination with genome editing techniques. However, this approach is still riddled with bottlenecks, including inefficient delivery of the genome editing tools into the hPSCs, harsh conditions for selecting transfected cells, labor-intensive clonal screening, potential loss of pluripotency, and risk of genomic instability. We tried to address some of these concerns by comparing several methodologies side by side: non-enzymatic cell-cluster passaging versus enzymatic single-cell passaging, different types of media to support feeder-independent cell culture, and different transfection reagents. We used an all-in-one vector that expresses Cas9, a single guide RNA, and a cell-surface marker for genome editing. We show that cells adapted to single-cell passaging are more amenable to transfection, and that they can be magnetically sorted through the use of a cell-surface marker. Following adaptation to three different kinds of media, we quantified expression of key pluripotency markers by qRT-PCR, as well as their ability to be single-cell cloned after magnetic sorting. In sum, we present a workflow for efficient genome editing in hPSCs that minimally impacts pluripotency and genetic stability.

T3015

GMP COMPLIANT STABLE AND EFFICIENT EXPANSION OF PLURIPOTENT STEM CELLS IN A CLOSED CULTIVATION SYSTEM

Kurtz, Annett, Schult, Silke, Bretz, Andrea, Jüngerkes, Frank, Rockel, Thomas D., Bosio, Andreas and Knöbel, Sebastian, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany

Human pluripotent stem cells (hPSCs) hold great promise for clinical use and cell therapy applications. To ensure highest quality and safety of the resulting cellular products, suitable hPSCs lines have to be maintained under standardized cultivation conditions and procedures. To this, we have developed our xeno-free iPS-Brew GMP medium following the recommendations of USP <1043> on ancillary materials, thus enabling expansion of PSC for clinical use. For qualification of the iPS-Brew GMP Medium, iPSC were expanded for 20 passages on recombinant Laminin-521. Resulting cells displayed a normal karyotype and a highly pluripotent immunophenotype as assessed by multi-color flow cytometric analysis. To increase the level of process standardization and product safety we developed a procedure for cultivation of PSCs using the integrated cell processing platform CliniMACS Prodigy®. PSCs could be expanded by a factor of 25 in a single passage using automatic coating, feeding and harvesting procedures in a closed, single use tubing set (TS730) under adherent culture conditions. Subsequently, these cells could be replated and differentiated into neural progenitor cells within the closed system, illustrating the feasibility of an automated cell production for future clinical cell production.

T3017

MODELING HUMAN RISK FOR CORONARY ARTERY DISEASE USING GENOME EDITING AND IPSC DIFFERENTIATION

Lo Sardo, Valentina, Ferguson, William Corbett, Chubukov, Pavel, Duran, Michael and Baldwin, Kristin, The Scripps Research Institute, La Jolla, CA, U.S.

Induced pluripotent stem cells (iPSC) based models of monogenic human diseases show great promise. However, monogenic diseases are relatively rare. Instead, risk for most common human diseases is impacted by genetic variation in non-coding regions of the genome that provide few obvious mechanistic implications for disease. For example, one common human haplotype (~60kb) at the 9p21 locus has been identified in multiple GWAS studies as having a strong impact on coronary artery disease (CAD). However, little is known about the specific 60kb CAD region and its role in CAD pathology of vasculature cell subtypes. No protein-coding genes are present and the region spans more than 10 exons of a long non-coding RNA, CDKN2B-AS (also known as *Anril*). Here, we apply genome editing to delete the 60kb 9p21 locus from iPSCs generated from patients carrying the risk or non risk haplotypes. Despite the large genomic deletion, 9p21 CAD knockout iPSCs are largely unaffected. However, vascular smooth muscle cells produced from the iPSCs exhibit clear risk associated differences in their differentiation and patterns of gene expression. This study provides insight into the previously unknown role of the 9p21 genomic region and offers a method to explore the functional impact of human genomic risk regions by using large scale genome editing approaches.

T3019

TO EACH IT'S OWN: TISSUE-SPECIFIC EXTRACELLULAR MATRIX PROVIDES AN OPTIMAL NICHE FOR STUDYING MESENCHYMAL STEM CELLS DERIVED FROM BONE MARROW AND ADIPOSE TISSUES

Marinkovic, Milos[1], Block, Travis[1,2], Rakian, Rubie[1], Wang, Exing[1], Dean, David[1], Reilly, Matthew[3] and Chen, Xiao-Dong[4], [1]University of Texas Health Science Center at San Antonio, San Antonio, TX, U.S., [2]University of Texas at San Antonio, San Antonio, TX, U.S., [3]Ohio State University, Columbus, OH, U.S., [4]UT Health Science Center at San Antonio, San Antonio, TX, U.S.

Recent research has established the acute sensitivity of stem cells to the chemical and physical cues in their local environment (extracellular matrix, ECM) that affect cell behavior. Current stem cell culture methods employ a "one size fits all" paradigm, utilizing synthetic substrates, such as tissue culture plastic (TCP), that cannot possibly recapitulate the native stem cell niche. In order to more accurately study stem cell behavior and develop effective tissue engineering and therapeutic strategies, culture environments that faithfully reproduce the native tissue-specific cell niche are required. To address this need, we prepared bone marrow (BM)-, adipose (AD)-, and skin (SK)-derived ECM, produced ex vivo by stromal cells or fibroblasts derived from these tissues. After decellularization, washing, and sterilization, the ECMs were used as cell culture substrates in order to evaluate their ability to mimic the BM and AD tissue microenvironments (niches). We assessed the ability of the ECMs to influence BM- and AD-mesenchymal stem cell (MSC) proliferation, cell spreading/morphology, and differentiation relative to standard TCP. All three types of ECM promoted MSC proliferation compared to TCP, but this effect was especially enhanced when the origin of the MSCs (BM or AD) matched the origin of the cells that produced the ECM. Furthermore, BM- and AD-ECM displayed "tissue-specificity" in directing MSC differentiation towards their respective cell lineage. Studies of cell spreading morphology further showed that each ECM uniquely affected MSCs, irrespective of cell origin. Each type of ECM was found to exhibit unique topographical and mechanical properties, such as surface roughness, fiber alignment, surface energy and storage modulus. We postulate that these differences may be attributable to variations in the ratio of individual collagens to small proteoglycans found in each ECM. The present study provides evidence demonstrating that the ECMs recapitulate specific elements of their native stem cell niche and constitute an appropriate model for the development of tissue-specific culture systems for research, tissue engineering and therapeutic applications.

T3021

RECOMBINANT HUMAN LAMININ-521 SUPPORTS PSC SURVIVAL IN ESSENTIAL 8™ MEDIA DURING CRITICAL TRANSITIONS

Sangenario, Lauren, Kuninger, David and **Newman, Rhonda A.**, Thermo Fisher Scientific, Frederick, MD, U.S.

Feeder-free culture of human pluripotent stem cells (PSCs) in the Essential 8™ Medium paired with a truncated form of recombinant human Vitronectin system supports long term maintenance of normal PSC morphology, pluripotency, and karyotype in the absence of rho-associated coiled-coil kinase (ROCK) inhibitors. However, in certain applications requiring single cell dissociation of PSCs and subsequent manipulation required for gene editing and high throughput screening, inclusion of ROCK inhibitors is required to ensure consistent recovery of PSCs. We tested multiple matrices to identify surfaces that provided maximal support of PSCs cultured in the Essential 8™ Medium system in various applications, somatic cell reprogramming, feeder-dependent to feeder-free transitioning, clonal expansion, and routine PSC culture. Here we show that the recombinant human Laminin-521 provided the best overall performance, including improved PSC recovery and survival following single cell passaging in the absence of ROCK inhibitors. In addition Laminin-521 was shown to support long term culture of PSCs, while maintaining normal PSC morphology, pluripotency, karyotype, and differentiation potential. Several aspects of the process workflow for PSC passaging and culture were evaluated for optimization and versatility with the Laminin-521 matrix; these included cell seeding density, passaging reagent, matrix coating concentration, duration and temperature, plate manufacturer, and inclusion of RevitaCell™ Supplement, a cocktail containing a pro-survival small molecule coupled with antioxidants and free radical scavengers. For downstream applications in which recovery of PSCs from low density seeding is required, coupling of Laminin-521 with RevitaCell™ Supplement provided increased cell recovery from cell seeding density of 150 cells/cm^2. Laminin-521 also supported transition of feeder-dependent PSCs to the feeder-free better than other matrices that were tested and showed increased feeder-free cloning efficiency for neonatal human dermal fibroblasts following somatic cell reprogramming using Cytotune™-iPS 2.0 Sendai Reprogramming Kit. Together, these data demonstrate the robustness of the Essential 8™ Medium/recombinant human Laminin-521 in supporting a broad range of PSC applications.

T3023

UNBIASED SCREENING FOR CHEMOTHERAPY RESISTANCE GENES USING HAPLOID HUMAN EMBRYONIC STEM CELLS

Peretz, Mordecai[1], Sagi, Ido[2], Weissbein, Uri[2], Yanuka, Ofra[2], Egli, Dieter[3,4] and Benvenisty, Nissim[2], [1]The Hebrew University, Kfar-Saba, Israel, [2]The Hebrew University, Jerusalem, Israel, [3]Columbia University, New York, NY, U.S., [4]The New York Stem Cell Foundation Research Institute, New York, NY, U.S.

Haploid human embryonic stem (ES) cells have been recently isolated and characterized by us. These cells are very useful for genetic screens as they harbor a single allele for each gene. In order to identify genes and pathways involved in resistance to common chemotherapies, we have screened human ES cells for their sensitivity to a large collection of drugs included in the NCI - Oncology Drugs Set Compound Library. Among 89 tested chemotherapies, 20 induced dramatic elimination of human ES cells, and three were selected for further analysis. These included azacitidine, vorinostat and bleomycin, which elicit DNA demethylation, histone acetylation and DNA strand breaks, respectively. We then established a gene trap library of mutant haploid human ES cells, based on PiggyBac transposon integration. The loss-of-function library of haploid cells consisted of more than 160,000 independent integrations covering over 10,000 genes. The gene trap library was treated for several days with different concentrations of each of the three aforementioned chemotherapies. High throughput sequencing analysis of the extracted DNA demonstrated enrichments for specific insertions for each of the chemotherapies. Thus, we were able to suggest a role for telomere length in resistance to bleomycin, through the disruption of the telomerase-regulatory proteins RIF1 and PINX1. This analysis exemplifies the usefulness of haploid human ES cells for the characterization of pathways required in chemotherapy resistance.

T3025

MEDIA AND MICROCARRIERS MUST BE OPTIMIZED WHEN TRANSITIONING MESENCHYMAL STEM CELL EXPANSION TO STIRRED TANK BIOREACTORS

Schnitzler, Aletta Christina, Verma, Anjali, Punreddy, Sandhya, Aysola, Manjula, Murrell, Julie and Rook, Martha, MilliporeSigma, Bedford, MA, U.S.

The long-term outlook for stem cell therapy predicts an increased need for high quality materials that are animal origin-free and are also compatible with the limited number of downstream processing steps. Human mesenchymal stromal/stem cells (hMSCs) are an attractive target for clinical study as therapeutic agents. Large scale manufacturing of these adherent-dependent cell types necessitates movement away from planar culture and toward technologies such as stirred tank bioreactors where suspension culture using microcarriers is enabled. Both the microcarriers and cell culture medium or supplements may contain animal-derived components. Fetal bovine serum (FBS) in particular is associated with regulatory, supply, and consistency challenges. Eliminating this commonly-used reagent will require thorough evaluation of animal origin-free materials for compatibility with stem cell therapy applications. Here, we evaluated growth of bone marrow derived hMSCs with a variety of microcarriers, cell culture media formulations and serum-free supplements. A wide range of performance was observed between the different types of media and serum-free supplements. Also a positive performance in static culture was not necessarily predictive of that under agitated conditions with microcarriers. Additionally, we use recombinant trypsin and associated animal-free inhibitors for a fully FBS-free system. The combination of serum-free systems and high quality reagents supports the future implementation of large scale manufacturing solutions of hMSCs that will be required following clinical success.

T3027

QUANTITATIVE EVALUATION OF PLURIPOTENT STEM CELL PROCESSES, QUALITY, AND BIOMARKERS USING STEMCELLQC A VIDEO BIOINFORMATICS SOFTWARE TOOLKIT

Zahedi, Atena[1], On, Vincent[2], Bays, Brett[2], Omaiye, Esther[3], Bhanu, Bir[2] and **Talbot, Prue**[3], [1]university of california, riverside, Corona, CA, U.S., [2]University of California, Riverside, CA, U.S., [3]University of California Riverside, Riverside, CA, U.S.

Quality control is a major challenge in basic stem cell research, translational studies, and clinical application of stem cell therapies. During culture, pluripotent stem cells can be stressed and their health compromised. Such changes are not necessarily obvious by visual monitoring. Video bioinformatics (VBI) software provides a quantitative, unbiased, automated, label free, high content profiling technology that extracts information from time-lapse images of live cells in culture. The extracted data can be used in any stem cell laboratory to monitor cell quality, processes, and health and to experimentally evaluate culture conditions or drugs with therapeutic potential. StemCellQC, a VBI tool developed in our laboratory, was used to analyze 24 morphological and dynamic features in healthy, unhealthy, and dying human embryonic stem cell (hESC) colonies and to identify those features that were affected in each group. Multiple features differed in the healthy versus unhealthy/dying groups, and these features were linked to morphology, growth, motility, and death. Biomarkers were also discovered that predicted

cell processes, such as cell death, before they were detectable by manual observation. The classifier in StemCellQC distinguished healthy and unhealthy/dying hESC colonies with 96% accuracy by non-invasively measuring and tracking dynamic and morphological features over 48 hours. StemCellQC reduced the time and resources required to track multiple pluripotent stem cell colonies and eliminated handling errors and false classifications due to human bias. StemCellQC provided both user-specified and classifier-determined analysis in cases where the affected features are not intuitive or anticipated. StemCellQC could be a valuable tool in any laboratory working with pluripotent stem cells, where maintaining stem cell quality and/or monitoring experimental changes in cellular processes are essential.

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T3029

A ROBUST PLATFORM FOR GENERATION AND HIGH THROUGHPUT FUNCTIONAL ANALYSIS OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Willems, Erik[1], Kimler, Kyle[1], Boucher, Shayne E.[2], Asprer, Joanna[1], Sylakowski, Kyle[1], Lakshmipathy, Uma[1], Kuninger, David[2] and Piper, David[1], [1]Thermo Fisher Scientific, Carlsbad, CA, U.S., [2]Thermo Fisher Scientific, Frederick, MD, U.S.

Induced pluripotent stem cells (iPSCs) and their differentiated progeny provide useful tools for safety testing and promise to provide more predictive results from assays that translate to pre-clinical and clinical outcomes with greater fidelity than current heterologous expression systems or non-human cardiomyocytes. Moreover, disease-specific iPSCs can be derived directly from patients with known disease phenotypes or can be mimicked by introducing known mutations into the iPSC genome by the CRISPR/Cas9 or the TALEN systems. The ability to derive human cardiomyocytes from these iPSCs supports the study of cardiac diseases *in vitro*, and facilitates the discovery of drugs that could compensate for cardiac abnormalities. However, these drug screening efforts would be improved by more reliable, reproducible and scalable cardiomyocyte differentiation from human iPSCs.Here, we describe a workflow that facilitates high-throughput functional screening with human iPSC-derived cardiomyocytes. This workflow relies on a robust system to efficiently generate cardiomyocytes from multiple human iPSC lines, yielding cardiomyocyte purities of over 50%, which can be further increased using a metabolic enrichment step. We further show that these iPSC-derived cardiomyocytes can be replated into 384-well plates to support calcium flux and membrane potential assays using Fluo-4 and FluoVolt™ dyes, respectively. We demonstrate that function of the iPSC-derived cardiomyocytes can be measured accurately as shown by the expected pharmacology and physiology of a variety of known and selective ion channel activity modulators. In summary, we provide tools that can be used in high-throughput screening platforms to reliably produce and analyze human cardiomyocytes at a scale sufficient to support functional cardiomyocyte screening assays. These assays can be used for lead identification screens or lead optimization profiling that support drug discovery efforts for cardiac disease as patient-specific or disease-specific cellular contexts may be generated by taking advantage of either patient-derived or CRISPR/TALEN edited iPSCs. Furthermore, the assays can be directly implemented in safety studies to identify compounds that may have cardiac liabilities.

T3031

NEW PARADIGM OF SCALABLE MANUFACTURING FOR ALLOGENEIC CELL THERAPY PRODUCTS

Lee, Brian[1], Giroux, Daniel[1], Hashimura, Yas[1], Starkweather, Nathan[1], Rosella, Francisco[1], Wesselschmidt, Robin[1] and Croughan, Matthew[2], [1]PBS Biotech, Inc., Camarillo, CA, U.S., [2]Keck Graduate Institute, Claremont, CA, U.S.

Two dimensional (2-D) cell culture technologies have proven inadequate for large scale manufacturing to meet the anticipated commercial demands for cell therapy products. Growing cells on microcarriers suspended in bioreactors is considered a viable option for scaling up the manufacturing process. However, the commonly used cylindrical bioreactors with horizontal impellers require relatively high agitation power in order to fully suspend microcarriers, which can cause high shear stress to the cells in the culture fluid. Anchorage dependent cells grown on microcarriers are known to be more sensitive to fluid shear stress than suspended single cell cultures. In addition, the cells respond to their growth environment in ways that may affect product quality, safety, and potency. Combination of these facts makes it more challenging to identify the acceptable range of agitation rates to achieve satisfactory cell growth and quality attributes, and this problem becomes worse as the size of bioreactor increases. A single-use bioreactor system using an innovative Vertical-Wheel technology is recently introduced which promotes efficient, homogenous liquid mixing and uniform microcarrier suspension with low power input. Physical measurement of the minimum power levels required for homogeneous suspension of microcarriers and computational fluid dynamic analysis, indicate that the vertical mixing mechanism not only requires very low power input to fully suspend microcarriers, but also the low shear environment remains constant across the full range of vessel sizes from 0.5 to 500 liters. This unique characteristic of the vertical mixing mechanism offers un-

paralleled scalability to achieve a consistent and robust manufacturing process for shear sensitive cellular therapy products. The Vertical-Wheel single-use bioreactors have been evaluated with several different cell types including human bone marrow-derived mesenchymal stem cells (BM-MSC), embryonic and induced pluripotent stem cells (PSC's). The results of physical measurements of Kolmogorov scales, the growth of various types of cells, and the differentiation kinetics of PSC's in various sizes of Vertical-Wheel bioreactors will be discussed.

TISSUE ENGINEERING

T3033

IMMUNO-COMPATIBLE PLURIPOTENT STEM CELL-DERIVED NEURONAL GRAFT: PUTTING THE HLA PARADIGM TO THE TEST IN NON-HUMAN PRIMATES

Aron Badin, Romina[1,2], Bugi, Aurore[3], Williams, Susannah[4], Vadori, Marta[5,6], Jan, Caroline[1,2], Nassi, Alberto[1,2], Lecourtois, Sophie[1,2], Cozzi, Emanuele[5,6], Hantraye, Philippe[1,2] and Perrier, Anselme[7], [1]CEA, DSV I2BM MIRCEN, Fontenay aux Roses, France, [2]CNRS-CEA-Paris-Sud UMR 9199, Fontenay aux Roses, France, [3]CECS, Corbeil-Essonnes, France, [4]CEA, DSV I2BM MIRCEN, Corbeil-Essonnes, France, [5]Transplantation Immunology Unit, Padova, Italy, [6]CORIT,Ospedale Giustinianeo,, Padova, Italy, [7]INSERM, Evry, France

Human pluripotent stem cell (hPSC)-based therapy is a promising option for the treatment of neurodegenerative disorders caused by loss of specific cell populations. Clinical trials using foetal cell therapy in Parkinson's and Huntington's diseases have already paved the way for the development of hPSC-based replacement strategies in the brain. These pioneering trials have also highlighted the risk of rejection of the allogenic grafts showing frequent alloimmunisation to fetal donor antigens resulting, although not systematically, in neuroinflammation and immune rejection. Despite the associated risk of cancer, infection and cardiovascular disease, chronic immunosuppression is used to protect allogenic grafts from rejection. Availability of induced PSCs derived from the patient himself or from selected donors harboring some degree of HLA matching open up opportunities to secure scalable sources of cell therapy product with enhanced or full immunological compatibility. We performed a comparative assessment of the immunogenicity of autologous, haplotype-matched (i.e one of the two major histocompatibility complex (MHC) haplotypes of the recipient matching the one of the line) and two-haplotypes mismatched neuronal grafts in the excitotoxically-lesioned striatum of non-human primates (NHP). First, blood cells from different NHPs, homozygous for MHC Class I & II, were used to produce several iPSC lines. Next, striatal cell populations were generated and their differentiation potential controlled in nude rats. Finally we assessed their potential immunogenicity at 3 and/or 6 months after intra-striatal grafting in haplotypes mis-matched, matched and autologous NHP recipients. Our results suggest that, unlike autologous neuronal grafts, allogenic grafts elicit a local infiltration of $CD8^+$ T cells and an increase in local HLA-DR staining. Interestingly, haplotype-matched grafts seem to trigger a delayed or attenuated immune response in the host. In the specific context of brain transplantation, our pre-clinical data suggest that, as for solid organ transplantation, a better immunological match is associated with improved survival and maturation of the grafts. HLA matching could be a cost effective compromise to reduce peripheral immunosuppression and its side effects in the clinical setting.

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T3035

ENDOTHELIAL OUTGROWTH CELL FOR TISSUE ENGINEERING

Chan, Jerry, KK Women's and Children's Hospital, Singapore, Singapore and Chong, Mark, NTU, Singapore, Singapore

Endothelial outgrowth cells (EOC) have been the subject of intense research for diagnostic and therapeutic applications. Translation to clinics, however, is limited by inconsistent results and significant differences in experimental observations across research groups. We hypothesized culture conditions to influence harvest efficiency of EOC and sought to optimize the protocol for maximal EOC yield from human umbilical cord blood (UCB). Term UCB (n=86) were obtained and the influence of mononuclear cell (MNC) separation, seeding density, sera and substrates on harvest efficiency were systematically evaluated. In spite of a ten-fold reduction in total nucleated cell counts following density centrifugation, EOC could not be generated from direct culture of whole blood, suggesting the process of red blood cell depletion to be critical. Seeding density was found to be inversely related to EOC harvest yield, with a seven-fold increase in number of EOC colonies obtained following a four-fold reduction in seeding density ($p<0.01$). The use of serum concentrations between 5% to 80% did not influence EOC yield. Strikingly, batch dependence was found to be the major factor, with EOC reliably generated from only one out of five lots of sera tested from four different companies. Finally, substrate coating did not influence number of colonies generated, nor EOC cell surface marker profile, in-vitro Matrigel tube formation and acLDL uptake. In

conclusion, isolation of EOC from UCB can be optimized through red blood cell depletion, optimal seeding density, and rigorous batch testing of sera for reliable generation of EOC.

Funding Source: NMRC, Singapore

T3037

VASCULARIZING ENGINEERED TISSUE CONSTRUCTS BY HARNESSING THE INTERACTIONS BETWEEN MULTIPOTENT STROMAL CELLS (MSC) AND ENDOTHELIAL CELLS

Fitzsimmons, Ross E., Aquilino, Mark A., Tarlan, Farhang and Simmons, Craig A., University of Toronto, Toronto, ON, Canada

The successful formation of viable engineered tissues is contingent on overcoming the challenge of forming blood vessels of varying diameters with the appropriate perivascular cell type (pericytes or smooth muscle cells (SMCs)) for trophic and mechanical support of the endothelium. In order to form perfusable vessels, we are exploring the approach of patterning microchannels within collagen-fibrin hydrogels using a custom-built 3D printer followed by seeding the channels with endothelial cells (ECs) to form hierarchical small artery/vein-like vessels. In conjunction with this, we are investigating the interactions between ECs and multipotent stromal cells (MSCs), as MSC paracrine factors can be used to stimulate angiogenesis from EC-seeded channels in order to form capillaries. Additionally, MSCs are recognized as having a pericyte phenotype in vivo and have the capacity to differentiate to SMCs, making MSCs a particularly favorable mural cell source for engineered tissues. Human bone marrow-derived MSCs were used in this study following preliminary characterization. MSCs had a fibroblastic colony forming unit (CFU-F) frequency of 14.5+/-2.6% (SD) and displayed osteogenic and adipogenic differentiation as indicated by increased Alizarin Red and Oil Red O staining compared to controls. The effect of MSCs on sprouting angiogenesis and EC network formation was assessed by embedding ECs in hydrogels either seeded on microcarrier beads or as a single cell suspension, in order to stimulate sprouting and network formation, respectively. Sprout lengths per bead were significantly longer when co-cultured with MSCs compared to EC monocultures at days 14 and 21 (Bonferroni tests: both $p \leq 0.01$). Encapsulated MSCs stained positive for α-smooth muscle cell actin and migrated to an abluminal postion on these capillary-like tubules, suggestive of a pericyte-like phenotype. In the absence of MSCs, both EC sprouts and networks disassembled, and ECs underwent apoptosis as indicated by cleaved caspase-3 staining. Currently, we are investigating the roles shear stress and pulsatile flow have on sprouting from microchannels and inducing differentiation of MSCs to SMCs, in order to generate muscularized vessels that can anastomose with the MSC-stabilized capillary-like vessels described above.

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T3039

MICRORNA-516 PROMOTES MOUSE BONE MARROW MESENCHMAL STEM CELLS DIFFERENTIATION BY TARGETING DEDICATOR OF CYTOKINESIS 5 (DOCK5)

HAN, LU, West China College of Stomatology, Cheng, Du, China and Li, Shasha, West China College of Stomatology, Cheng du, China

MicroRNAs are short non-coding RNAs which have been suggested to play a pivotal role in a variety of biological processes including stem cell differentiation. MicroRNA-516 (miR-516) has been demonstrated up-regulated in osteoblast-like cell lines (MG-63). But the effect of MicroRNA-516 on osteogenic differentiation of mouse bone marrow mesenchymal stem cells (mBMSCs) and its molecular mechanism remain unclear. In this study, biological function of miR-516 during osteogenic differentiation of mBMSC was investigated by transfection of miR-516 mimics or inhibitors. Over-expression of miR-516 promoted osteogenic differentiation of mBMSCs, while suppression of miR-516 inhibited this process. Bioinformatics and dual-luciferase reporter assay were performed to demonstrate that dedicator of cytokinesis 5 (DOCK5) is a direct target of miR-516. Moreover, Pull-down assays indicated that DOCK5 could activate endogenous Rac1. Taken together, our findings suggest that miR-516 plays a positive role in osteogenic differentiation of mBMSCs though DOCK5/ Rac1 pathway.

T3041

INFLAMMATORY CYTOKINE THROUGH CXCR2 SIGNALING PATHWAY CONTROL SENESCENCE OF MESENCHYMAL STEM CELL

Kwon, Jihye, Bae, Yoon Kyung, Kim, Miyeon, Jeon, Hong Bae and Jin, Hye Jin, Biomedical Res Inst, Medipost Co, Ltd, Seongnam, Korea, South

Development of cellular senescence with passing for expansion which is predetermined to obtain sufficient cells for clinical application is one of the major obstacles and therefore considerable efforts have been directed at evading cellular senescence with long-term culture of mesenchymal stem cells (MSCs). A senescent cells has undergone widespread changes in protein expression and secretion, ultimately develops senescence-associated secretory phenotype (SASP). Further investigation of this phenotype is required to permit MSCs-based cell

therapies with improved therapeutic efficacy. Here, we investigated the molecular nature of SASP using human umbilical cord blood-derived MSCs (UCB-MSCs). Both growth-regulated oncogene α (GROα) and interleukin 8 (IL8) are major SASP in aging human MSCs and induced cellular senescence through its cognate receptor chemokine (C-X-C motif) receptor 2 (CXCR2), which is accompanied by an increase in the protein p53 and p21 through the action on ROS-p38-MAPK signaling. It is intriguing to note that secreted GROα or IL8 also induce paracrine senescence as well as autocrine effects on senescent cells with positive feedback loop. Our results thus provide the evidence supporting the existence of SASP in senescent MSCs and contribute novel insights into the role of SASP signaling, which is a causative contributor to MSCs senescence and positively connects the intrinsic and extrinsic circuitry of the process.

T3043

ELIMINATION OF RESIDUAL IPS CELLS IN BIOENGINEERED CARDIAC CELL SHEETS BY TRPV-1 ACTIVATION

Matsuura, Katsuhisa[1,2], Seta, Hiroyoshi[3], Haraguchi, Yuji[1], Alsayegh, Khaled[1], Sekine, Hidekazu[1], Shimizu, Tatsuya[1], Hagiwara, Nobuhisa[1], Yamazaki, Kenji[3] and Okano, Teruo[1], [1]Tokyo Women's Medical University, Tokyo, Japan, [2]Tokyo Womens Medical University, Tokyo, Japan, [3]Tokyo Womens Medical University, 8-1 Kawada-cho Shinjuku, Japan

Although bioengineering cardiac tissues derived human iPS cells is a promising method for regenerative medicine, the development of strategy to eliminate remaining undifferentiated iPS cells in bioengineered cardiac tissues without affecting the viabilities of some kinds of iPS cell-derived somatic cells is indispensable for clinical application. In the present study, we show that TRPV-1 activation through transient culture at 42 °C or with agonists is a simple and useful strategy to eliminate iPS cells from bioengineered cardiac cell sheet tissues. When feeder free human iPS cells were cultured at 42 °C, almost all cells disappeared by 48 hours through apoptosis. Furthermore when iPS cells were co-cultured with iPS cell-derived cardiac cells at 42 °C, the number of Oct4 expressing iPS cells was decreased with the time dependent manner. Conversely, in spite of cultivation at 42 °C, the number of iPS cell-derived cardiomyocytes and fibroblasts was maintained, and cardiac cell sheets were fabricated after reducing the temperature. TRPV-1 expression in iPS cells was upregulated at 42 °C, and the expression was significantly higher than that in cardiomyocytes. Furthermore, TRPV-1 activation through thermal or agonist treatment significantly decreased the expression of Lin28 in cardiac cell sheet tissues. These findings suggest that the difference in tolerance to TRPV-1 activation between iPS cells and iPS cell-derived cardiac cells could be exploited to eliminate remaining iPS cells in bioengineered cell sheet tissues, which will further reduce the risk of tumour formation.

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T3047

USE OF ENGINEERED CARDIAC TISSUE CONSTRUCTS (ECTS) AS A MODEL FOR STUDYING HUMAN CARDIAC DISEASES

Sanchez-Freire, Veronica[1], J. De Lange, Willem[2], Ralphe, J. Carter[2], Pike, Nirupama K.[1] and Armstrong, Chris[1], [1]Stem Cell Theranostics, Redwood City, CA, U.S., [2]University of Wisconsin-Madison, Madison, WI, U.S.

The optimal functioning of human heart depends not only on individual cardiomyocytes but also on cell-cell interactions and a complex extracellular environment. Monolayer or 2D culture systems do not completely mimic cellular functions of the heart, and are limited in their functional potential. The 3D engineered cardiac tissue constructs (ECTs) generated from human induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs) can serve as better models to mimic cardiac function in health and disease states. In order to evaluate the functional aspects of common heart diseases, we designed ECTs using iPSC-CMs from healthy donors and patients with dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM). To generate ECTs, a mixture of iPSC-CMs and extracellular matrix (ECM) was cast in a trough with uniaxial tension. The mixture of cells and ECM then remodeled to form a spontaneously beating condensed strips. Day 1 ECTs showed Dyssynchronous beating, which transitioned to spontaneous beating at ~1Hz by day 3, suggesting the formation of functional syncytium. ECTs were subjected to functional testing, using simultaneous twitch force and calcium transient measurements after 14 days. ECTs produced active forces responsive to stepwise increases in construct length, analogous to the intact heart, in accordance to the Frank-Starling law. A positive force-frequency-response was observed when ECTs were electrically paced between 0.5Hz and 1.5Hz (R^2=0.903±0.043), indicating that calcium/calmodulin–dependent kinase signaling is intact. Inhibition of phosphodiesterase 3 by administration of 10μM Milrinone increased twitch force by 63.5±25.3% ($p<0.001$) and calcium transient amplitude by 44.6±14.2% ($p<0.001$), while accelerating twitch kinetics and the rate of calcium transient decay. Beta-adrenergic stimulation using 1μM Isoproterenol further increased twitch force by 65.7±32.0% ($p<0.001$) and calcium transient amplitude by 74.9±21.0%

(p<0.001) and further accelerated twitch kinetics and the rate of calcium sequestration, indicating intact adrenergic signaling. These results show that iPSC-CM derived ECTs respond appropriately to physiological and pharmacological cues, making them powerful tools for drug discovery, disease modeling and tissue replacement therapy.

T3049

EFFICACY AND SAFETY EVALUATION OF QQC CULTURED EX VIVO EXPANDED PERIPHERAL BLOOD STEM CELL THERAPY PRIOR TO CLINICAL TRANSLATION

Tanaka, Rica T., Kado, Makiko, Fujimura, Satoshi, Arita, Kayo, Tono-Okada, Kayoko, Takahashi, Michiko, Ito-Hirano, Rie, Hagiwara, Hiroko and Mizuno, Hiroshi, Juntendo University School of Medicine, Bunkyo-ku, Japan

The function and the numbers of endothelial progenitor cells (EPC), i.e., CD34+ cells is known to be impaired in diabetic patients. Therefore, the efficacy of autologous EPC therapy is limited. We have recently disclosed the newly developed a serum free ex vivo expansion system called Quantity and Quality Control Culture System (QQc) using peripheral blood mononuclear cells (PbMNC) to potentiate the vasculogenic property of diabetic EPCs for enhanced vasculogenesis and wound healing from small amount of peripheral blood. The purpose of this study is to validate the safety and efficacy of human MNC-QQc cell therapy for non-healing wounds prior to clinical application.PbMNC were isolated from diabetic patients and healthy volunteers. Then underwent QQc for 7 days. The vascular function of QQ cells pre- or post QQc was evaluated with EPC colony forming assay (EPC-CFA), FACS, EPC culture assay, and qRT-PCR. For in vivo study, $1x10^4$cells of pre and post QQc cells were injected in a murine wound healing model. Wound closure and angio-vasculogenesis by anti CD31 staining and/or human specific mitochondria antigen (HMA), was assessed for wound healing.Diabetic patients demonstrated significantly lower number of total colonies pre QQc compared to healthy volunteers. Post QQc, EPC-CFA disclosed predominant generation of functional total EPC-CFU compared to pre QQc with higher RNA transcripts of VEGF, Ang1, Ang2, MMP9. FACS analysis demonstrated significantly higher percentage of CD34+ cells and CD206 cells post QQc in diabetic patients. Transplantation of post diabetic QQc cells consecutively unveiled the greater closure compared to pre QQc diabetic cell Tx with higher wound vascularity compared at day 14 ($p < 0.05$). Co-staining for HMA and CD31 revealed vasculogenesis of Tx cells. In conclusion, QQc system of autologous peripheral blood MNC overcomes the insufficient efficacy of naïve mononuclear cell therapy for wound healing in diabetic patients. 200ml of peripheral blood will be necessary to replace the existing EPC therapy for diabetic patients for wound healing. With this new technology, we will be able to establish outpatient based simple, safe and effective vascular and regenerative therapy for diabetic patients.

Funding Source: Japan Agency for medical research and develpment

T3051

ENGINEERING HETEROTOPIC CHONDROGENIC CELLS FOR CARTILAGE REGENERATION BY MICROENVIRONMENT MODULATION

Yang, Tsung-Lin, National Taiwan Unviersity, Taipei, Taiwan, Wong, Chin-Chean, National Taiwan University, Taipei, Taiwan, Hsiao, Ya-Chuan, Zhongxing Branch, Taipei City Hospital,, Taipei, Taiwan and Chen, You-Tzung, National Taiwan UniversityCollege of Medicine, Taipei, Taiwan

Insufficient autologous cartilage for transplantation has remained an unresolved clinical challenge for decades. Although using heterotopic non-articular chondrocytes as an alternative autologous cell source may be a promising approach, the workable methodology has not been established, and the feasibility has not confirmed yet. Since there are several types of cartilage, heterotopic chondrocytes harvested from distinct tissue origin may be inborn with specific cellular characteristics, which remains the main obstacle for therapeutic applications. Compared with using genetic tools to change the phenotypes of chondrocytes, the way of engineering chondrogenic cells by microenvironmental modulation is preferable for clinical translation. This study aims to investigate the methodology and confirm the feasibility of engineering heterotopic chondrogenic progenitor cells for cartilage regeneration based on biomaterial approaches. The of the chondrogenic cells harvested from auricular cartilage were harvested first for dedifferentiation induction, and subsequently transferred for transdifferentiation to gain the properties of articular cartilage by biomaterial modulation. Increased gene and protein expression of the specific markers of articular chondrocytes were found in the transdifferentiated cells. When these cells were cultivated in the three-dimensional scaffolds fabricated by biomaterial hydrogel, their histological and biomechanical features were changed and became comparable with that of articular chondrocytes. In an in vivo animal model created with osteochondral defects, the experimental groups treated with biomaterial-engineered tissue constructs showed significant cartilage healing when compared with control and shame groups. Histologically, cartilage repair was achieved in the experimental groups showing abundant type II collagen but few type I collagen and elastin. Our results confirmed the feasibility of applying heterotopic chondrogenic cells for cartilage repair by biomaterial induced cell trans-differentiation. This study proposed a simple and promising working method to engineer heterotopic chondrogenic cells for articular cartilage repair

and regeneration, which pave ways for developing potential clinical therapeutics.

Funding Source: The ministry of science and technology, Taiwan

REGENERATION MECHANISMS

T3055

DISCOVERY OF SATELLITE CELL AND REGENERATION- A PARADIGM SHIFT IN THE MUSCLE DEVELOPMENT-REPAIR OF DROSOPHILA

Gunage, Rajesh Dattaram and Raghavan, K. Vijay, NCBS-TIFR, BANGALORE, India

Myogenesis in vertebrates and invertebrates are remarkably similar. Vertebrate postnatal stages of muscle maintenance and regeneration are regulated by adult specific stem cells namely, satellite cells, however, analogous mechanisms remain unknown in invertebrate muscles. In previous study, we identified the muscle-specific stem cells during early developmental stages of myogenesis, that divide asymmetrically to generate the stem cells and postmitotic myoblasts, later involved in flight muscle formation (Gunage et al., 2014). Fate of stem cells in the adult muscles is a mystery. We hypothesized these cells as possible adult stem cells, involved in damage-induced regeneration similar to vertebrate muscles. In this study, by lineage trace of stem cells, we show retention of stem cell descendants as small, unfused cells in close proximity to the mature muscle fibers. Although normally quiescent, these cells retain the potential to become mitotically active and engage in Notch signaling-dependent proliferative activity following muscle fiber injury. Striking analogies of morphological, anatomical and functional features, strongly point toward evolutionary conservation of adult stem cell approach for muscle maintenance and thus we propose these novel cells as Drosophila equivalent of vertebrate muscle satellite cells. Thus, in flies as in vertebrates, the stem cell lineage that generates the adult-specific muscles during development is also available for myogenesis in mature muscle in response to damage. This is a key finding in describing development programming ensuring retention of regeneration potential for adult life thus providing a new avenue for future research. Due to genetic tractability and availability of variety of genome engineering tool, in contrast to vertebrate models, we firmly believe this system would fasten the process of screening for therapeutics as well probing challenging key stem cell questions.

Funding Source: Department of Science and Technology, Government of India and J. C. Bose Fellowship

T3057

MESENCHYMAL STEM CELLS ENGINEERED TO EXPRESS NOGO RECEPTOR BLOCKER EXERT ENHANCED NEUROREGENERATIVE EFFECT IN A MOTOR NEURON MURINE MODEL

Gabr, Hala[1], Abo Elkheir, Wael[2,3], Kishk, Nermeen[4] and Yosef, Eman[1], [1]Cairo Univ, Cairo, Egypt, [2]Military Medical Academy, Cairo, Egypt, [3]Egyptian society for progenitor stem cell research (ESPCR), Cairo, MA, Egypt, [4]Cairo university, Cairo, Egypt

Mesenchymal stem cells (MSCs) present a promising tool for cell therapy, and are currently being tested in a number of clinical trials especially neurodegenerative diseases.

The membrane protein Nogo-A was initially characterized as a CNS-specific inhibitor of axonal regeneration. Recent studies have uncovered regulatory roles of Nogo proteins and their receptors — in precursor migration, neurite growth and branching in the developing nervous system — as well as a growth-restricting function during CNS maturation. The function of Nogo in the adult CNS is now understood to be that of a negative regulator of neuronal growth, leading to stabilization of the CNS wiring at the expense of extensive plastic rearrangements and regeneration after injury**.** Motor neuron disease (MND) is a genetically determined degenerative disease affecting the neural supply of voluntary muscles. Up till now, there is no definite treatment for MND, which usually leads a fatal path.The objective of this work is to study the role of MSCs in neuro-regeneration and compare it to genetically modified MSCs with NOGO receptor gene block in murine animal model of MND.Transfection of in-vitro propagated bone marrow (BM) derived MSCs with a plasmid containing NOGO receptor gene silencing RNA. Murine model of MND was established, and mice were then divided into 3 groups:Group I: were left as positive control, Group II: received genetically modified MSCs systemically in a dose of 2 million/kg, Group III: received unmodified MSCs systemically in a dose of 2 million/kg.All groups were be subjected to an assessment of neuronal regeneration after 4 weeks. Animals were sacrificed and histological evaluation of neuronal regeneration was done. Cell tracking of injected cells will be done through marker gene in modified MSCs and through iron oxide tagging in unmodified MSCs.Both modified and unmodified MSC therapy groups showed clinical improvement, more pronounced in the modified group. Histological sections of mice spinal cords and brains showed positive GFP and iron oxide-positive cells in modified and unmodified groups respectively. Histological evidence of regeneration was found, more pronounced in the modified group.

T3059

CONVERSION OF HUMAN ADIPOGENIC MESENCHYMAL STEM CELLS INTO HIGHLY CONDUCTIVE MYOCYTES

Islas, Jose Francisco[1], Mohamed, Mohamed A.[2], Dacso, Cliford[3], Potaman, Vladimir N.[1], Abbasgholizadeh, Reza[2], Bond, Richard[2], Navran, Stephen[4], Birla, Ravi[2] and Schwartz, Robert J.[1,2], [1]Texas Heart Institute, Houston, TX, U.S., [2]University of Houston, Houston, TX, U.S., [3]BCM, Houston, TX, U.S., [4]Synthecon, Houston, TX, U.S.

In a unique manner human transcription factors ETS2 and MESP1 were sufficient to convert human adipogenic mesenchymal stem cells (hAdMSC) into cardiac progenitor cells (CPCs). These two factors up regulate a cadre of cardiac regulatory factors, Nkx2.5, Tbx5, Mef2C, dHAND and GATA4. Yet, they are unable to produce the appearance of mature myosin heavy chains and many calcium-handling proteins. Nevertheless, the addition of epinephrine was capable of promoting maturation of the electrophysiological and Ca2+ handling properties of hAdMSC converted CPCs. Adrenergic signaling through Beta Adrenergic 2 receptor repositioned converted CPCs into more mature myocytes cells, along with the appearance of RYR2, CAV2.1, CAV3.1, Nav1.5, SERCA2 and CX45 gene transcripts. Following treatment with epinephrine, action potentials were observed in (Nkx2.5-puromycin) drug selected myocytes. Further improvement was fostered by 3D cardio-spheroids formed in a Synthecon, Inc. rotating bioreactor (RCCS). These cardio-spheroids induced the appearance of hypoxic genes: HIF1a/b, PCG1a/b and iNOS2. Induction of the hypoxic program coincided with the robust activation of adult contractile genes, MYH6, MYH7 and TNNI3, ion channel genes, CACNA1C, SCN8a, KCNQ5, KCNQ3 and t-tubule genes, CASQ, JPH, ASPH PLN, TRDN, BIN1 and CALR. These myocytes were found to be electrically coupled and conduct at high rates. Additional in-depth studies demonstrated the appearance of hyperpolarization-activated and cyclic nucleotide-gated channels (HCN1-4). Our experimental paradigm contributes to novel regenerative strategies that enhanced maturation of converted hAdMSC's to electrical active myocytes.

T3061

SPECIFIED Thy1+ FIBROBLASTS DIFFERENTIATES TO CARDIOMYOCYTES DURING HEART REGENERATION IN VIVO/IN VITRO

Morita, Yuika[1], Hotta, Akitsu[2], Qian, Li[3] and Takeuchi, Jun K.[1,4], [1]The University of Tokyo, Bunkyo, Japan, [2]Department of Life Science Frontier, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [3]University of North Carolina at Chapel Hill, Chapel Hill, NC, U.S., [4]The University of Tokyo, Bunkyo, Japan

Recently, it has been reported that mammal heart also has a potential for its regeneration as well as fishes and amphibians. During its regeneration, it is thought that a plasticity of cardiomyocytes is one of the most important things for whether heart can regenerate or not. Here we show that Thy^+; $Vimentin^+$; non-myocyte cells contribute to cardiomyocytes within 3~5 days during its regenerative stages. Interestingly, in these Thy+ cells, one of cardiac progenitors, Sall1 (Morita et al., JMCC 2016) was transiently expressed near resected heart area within 12h after apical resection. These $Thy1^+$;$Sall1^+$ cells differentiated smoothly into cardiomyocytes, and it expression was maintained for 2-5 days during early cardiac regeneration in vitro. In vivo lineage analysis also indicated that cells derived from Sall1+ cells differentiated to cardiomyocytes after its resection. In addition, Thy1+;$Sall^+$;non-$Mesp1^+$ lineage cell positively contributed into cardiomyocytes in $Sall1^{GFP/+}$;$Mesp1^{cre/+}$; ROSA-RFP mice model, suggesting that Sall1 plays as endogenous reprogramming factor from fibroblasts to cardiomyocytes, and $Sall^+$ cells will be an effective source for heart repair in human in the future. In this conference, we will show an origin of $Sall^+$ cells with using several transgenic mice of myocyte-specific/endothelial cell-specific/fibroblast-specific cre lines during cardiac regeneration, and cytokines accelerate its potential for cardiomyocyte renewal.

Funding Source: AMED, JSPS, and JST PRESTO

T3063

ACTIVATION OF THE EPICARDIUM BY Tβ4 RELEASED FROM SELF-ASSEMBLING PEPTIDE AFTER MYOCARDIAL INFARCTION

Tan, Yu-zhen, Shanghai Medical School of Fudan University, Shanghai, China

Myocardial infarction (MI) is a leading cause of death of the cardiovascular diseases. However, there is no desirable therapy for MI. Recent studies suggest that the epicardium plays an important role in cardiomyogenesis during development, while it becomes quiescent in adult heart. Moreover, thymosin beta 4 (Tβ4) has an effect on

activating epicardium. This study investigates enhancement of repair of the infarcted myocardium with Tβ4 released from self-assembling peptide. A new functioned self-assembling peptide which can bind Tβ4 and adhere mesenchymal stem cells (MSCs) was designed and synthesized. Tβ4 released from the self-assembling peptide was quantified by an Acquity ultra-performance liquid chromatography system. Epicardium-derived cells (EPDCs) were isolated from MI models of $Wt1^{CreERT2/+}$,$R26^{mT-mG}$ mice. The effects of the self-assembling peptide and released Tβ4 on survival, proliferation and differentiation of EPDCs and MSCs isolated from rat or mouse bone marrow were examined. The effectiveness of the self-assembling peptide, released Tβ4 and the carried MSCs on activating epicardium and promoting repair of the infarcted myocardium is evaluated in vivo. The self-assembling peptide protected the cells from apoptosis and necrosis in the conditions of hypoxia and serum deprivation. Tβ4 released from the self-assembling peptide stimulated EPDCs or MSCs to proliferate and differentiate towards endothelial cells, smooth muscle cells and cardiomyocytes. At 4 weeks after subepicardial transplantation of self-assembling peptide-loaded Tβ4 and MSCs, cardiac function was improved significantly. In MI models of rats and transgenic mice, $Wt1^{+}$ EPDCs and lineage traced-EPDCs along with GFP increased. These cells were located beneath the epicardium and within the myocardium of the infarct region. Some of the cells expressed CD31, α-SMA and cTnT. In addition, the self-assembling peptide and released Tβ4 promoted survival of the transplanted cells and recruited endogenous stem/progenitor cells into the infarct region to participate in angiogenesis and cardiomyogenesis. These results demonstrate that Tβ4 released from the self-assembling peptide can activate the epicardium effectively. The self-assembling peptide and carried MSCs promote endogenous cardiac repair.

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ETHICS AND PUBLIC POLICY; HISTORY OF STEM CELL RESEARCH; SOCIETY ISSUES; EDUCATION AND OUTREACH

T3067

FIRST-IN-HUMAN STEM CELL TRIALS IN HUNTINGTONS DISEASE: A BIOETHICS SURVEY

Duffy, Alexandra[1,2], Martin, Amanda[1], O'Keefe, Meaghan[3], Michie, Marsha[4], Yarborough, Mark[5] and Wheelock, Vicki[1], [1]University of California Davis Department of Neurology, Sacramento, CA, U.S., [2]University of California Davis Medical Center, California, CA, U.S., [3]University of California Davis, Davis, CA, U.S., [4]University of California, San Francisco Institute for Health and Aging, San Francisco, CA, U.S., [5]University of California Davis Department of Bioethics, Sacramento, CA, U.S.

Experimental treatment approaches and first-in-human Phase 1 trials are ethically complex. These studies create challenges for informed consent, impose burdens and risks to subjects and may offer little or no prospect of clinical benefit. Similarly, these studies create bioethical concerns for investigators, coordinators and study staff. These issues are understudied in the field of regenerative medicine generally and in the Huntington's disease (HD) population specifically. An anonymous survey of HD patients and family members regarding attitudes and concerns about participation in a study that involved stem cells, gene therapy and neurosurgical implantation was approved by the Institutional Review Board at UC Davis offered on the HDSA website from September – December 2014. There were a total of 268 respondents from two groups: 1) Individuals at risk or diagnosed with HD or 2) Family members or caregivers. Respondents were mostly Caucasian (96%) and female (69%) with mean age of 52 (range 18 – 79 years) and drawn from throughout the US. Regarding participation in a first-in-human trial, 67% of individuals with or at risk for HD responded positively compared to 92% in family members responding to having a loved one participate. Ethical concerns regarding experimental approaches in HD included none, source of cells, safety/risks, and informed consent. Reasons for volunteering or supporting this type of study included finding a cure or treatment, helping others, sustaining hope or sense of purpose, and creating new knowledge/advancing science. Expressed concerns about involvement included risks of adverse effects or death, lack of efficacy, need for surgery, and fear of pain. . Burdens of participation included access to participation, risks, uncertainty of the unknown, psychological impact, disclosure of gene status, impact on family, and post-trial support. Additional thoughts from respondents included gratitude, support, remarks on urgency, and sharing of personal stories. Fur-

ther statistical and qualitative analyses will be presented. It remains the bioethical and moral obligation of researchers to understand the motivations and beliefs of potential study participants in order to avoid therapeutic misconceptions, mitigate unrealistic expectations, and navigate a well informed consent process.

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T3069

NATIONAL STRATEGY OF STEM CELL RESEARCH AND INDUSTRY IN KOREA

Kim, Moo Woong, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea, South

Not more than 10 years ago, the use of stem cells was merely a hypothesis, but recently clinical trials have been actively conducted on them as treatment for a variety of diseases. In the near future, they are expected to be used not only for regenerative medicine but for stem cell-based human disease modeling, the regeneration of human organs that uses three-dimensional printing, etc. The Korean government has established policies and systems to keep a balance between the promotion and regulation of stem cell research and industry. With the government's active policies, Korea's stem cell application technology has been at a comparatively higher level and has gotten the world's first item licensed as stem cell treatment. Add to that, Korea is forming a 10-year plan, considering rapidly changing environments, in order to take the technical lead one stage further. In addition, a prediction is made about Korea's stem cells in 2025, as well as a debate is ongoing about plans for an ecological system for stem cell research and industry. This is to introduce the mid and long-term (2016 to 2025) plan for the promotion of stem cell research and industry on which debates are underway in Korea.

T3071

INTERNATIONAL COLLABORATION FOR DEVELOPING THE STANDARDIZED REPORTING GUIDELINE ON CELLULAR ASSAYS AND GLOBAL CELLULAR DATA RETRIEVAL SYSTEM FOR REGENERATIVE MEDICINE

Sakurai, Kunie, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, Kurtz, Andreas, Berlin Center for Regenerative Therapies, Berlin, Germany, Stacy, Glyn, National Institute for Biological Standards and Control an operating centre of the MHRA, London, U.K., Sheldon, Michael H., RUCDR Infinite Biologics, Piscataway, NJ, U.S. and Fujibuchi, Wataru, Kyoto University, Kyoto, Japan

A wide range of stem cell research towards regenerative medicine has been conducted in a large number of domains in the world over the years, and there are vast amounts of assay data which have been produced to elucidate the mechanisms for generation of diverse cell types from a single zygote based on various cellular properties. However, those produced data and information are not fully utilized due to a lack of standardization of cellular assay reporting formats. Here we present reporting guidelines for describing cellular assay data pursuing the facilitation of practical regenerative medicine named 'Minimum Information About a Cellular Assay for Regenerative Medicine (MIACARM)'(Sakurai et al, submitted), which has been developed by international collaboration among Japan, U.K., U.S., and EU. MIACARM was developed on the basis of existing guideline called MIACA, with defined taxonomy of human cell types, which would allow for the description of advanced experimental techniques. Furthermore, MIACARM incorporates necessary items for quality and safety of stem cells that are essential in regenerative medicine, as proposed by the existing literature, and regulations in Japan, U.S. and EU. MIACARM now consists of 253 items that are required to report cellular assays in terms of reproducibility, quality, safety, and efficacy of product cells, and among those, 144 items are about stem cell production and characterization. Using MIACARM, we are currently working on developing an information retrieval system by efforts made through cooperation with RUCDR Infinite Biologics, UK Stem Cell Bank, and European human Pluripotent Stem Cell Registry. We further plan to create a global data exchange network to enhance information flow among various cell banks or registries that have been established at more than 20 sites in the world. Once this global network has been built, it will allow researchers to omnibus-search for the query, for example, "where are the stem cell banks or registries which own iPS cells derived from Alzheimer's disease patients?" It will greatly reduce time and costs caused by unintended experimental duplications that of-

ten happen in distinct organizations. The latest version of MIACARM is available from: http://stemcellinformatics.org/Standards/minimum_info.

LATE BREAKING ABSTRACT

T4001

AIMP2 CONTROLS INTESTINAL STEM CELL COMPARTMENTS BY MODULATING WNT/BETA-CATENIN SIGNALING

Yum, Min Kyu and Kong, Young yun, Seoul National University, Seoul, Korea, South

Wnt/β-catenin signaling is crucial for the proliferation and maintenance of intestinal stem cells (ISCs). However, excessive activation of Wnt/β-catenin signaling leads to the ISC expansion and eventually colorectal cancer. Thus, negative regulators are required to maintain optimal level of Wnt/β-catenin signaling. Here, we investigated the role of Aminoacyl-tRNA synthetase interacting multifunctional protein 2 (AIMP2) as a critical negative regulator of Wnt/β-catenin signaling in intestinal homeostasis and tumorigenesis. In mice, hemizygous deletion of *Aimp2* resulted in enhanced Wnt/β-catenin signaling, increased proliferation of cryptic epithelial cells, and expansion of ISC compartments. Consistently, *Aimp2*$^{+/-}$ mice had increased adenoma formation on an *Apc*$^{Min/+}$ background, suggesting that AIMP2 acts as haploinsufficient tumor suppressor in intestinal tumorigenesis. Mechanistically, AIMP2 bound to Dishevelled-1 (DVL1), which disrupted Dishevelled-AXIN interaction and eventually inhibited Wnt/β-catenin signaling. Furthermore, intestinal organoid culture revealed that AIMP2 inhibited organoid formation and growth by suppressing Wnt/β-catenin signaling in *Aimp2* gene dosage-dependent manner. Collectively, our results show that AIMP2 acts as a haploinsufficient modulator of intestinal tumorigenesis and homeostasis by fine-tuning of Wnt/β-catenin signaling.

Funding Source: This work was supported by Basic Science Research Program (NRF-2014R1A2A1A10052675), Bio & Medical Technology Development Program (NRF-2011-0019269) and a Korea Mouse Phenotyping Project (NRF-2014M3A9D5A01073930) <

T4003

ETANERCEPT-SYNTHESIZING MESENCHYMAL STEM CELLS EFFICIENTLY AMELIORATE COLLAGEN-INDUCED ARTHRITIS

Jung, Seung Min[1], Park, Narae[2], Rim, Yeri Alice[2], Kim, Juryun[2], Jang, Yeonsue[2] and Ju, Ji Hyeon[2], [1]Yonsei University College of Medicine, Seoul, Korea, South, [2]Seoul St. Mary's Hospital, Seoul, Korea, South

Mesenchymal stem cells (MSCs) have multiple favorable properties, including anti-inflammatory and immune-modulatory effects. Scientists have attempted to improve MSC functions by engineering them. In this study, we aimed to invent a novel method to produce the synthetic biological drug TNFR2-Fc (etanercept, Enbrel) from engineered MSCs (eMSCs). Biologics are made of protein components, and thus can be theoretically produced from cells including MSCs. We previously invented a minicircle vector system for expressing biologics in vivo and in cultured cells in vitro. We generated eMSCs by transfecting MSCs with a TNFR2 minicircle plasmid (mcTNFR2). We investigated the anti-arthritic effect of eMSCs in a collagen-induced arthritis model. MSCs were successfully transfected with mcTNFR2. Synthetic TNFR2-Fc derived from eMSCs revealed biologically active properties in vitro. Arthritis was more efficiently ameliorated by eMSCs than by MSCs. eMSCs mainly migrated to the spleen and decreased the CD4+RORγt+ population of splenocytes. eMSCs suppressed osteogenesis with a high efficiency. Etanercept-synthesizing eMSCs efficiently ameliorated arthritis in an experimental mouse model. eMSCs had a superior anti-arthritic activity in comparison to MSCs. Although this strategy is at the proof-of-concept stage, it represents a potential alternative method for the delivery of biologics through eMSCs and cell-based therapy.

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T4005

PROLONGED MITOSIS OF NEURAL PROGENITORS ALTERS CELL FATE IN THE DEVELOPING BRAIN

Pilaz, Louis-Jan[1], McMahon, John J.[1], Miller, Emily E.[1], Lennox, Ashley L.[1], Suzuki, Aussie[2], Salmon, Edward[2] and Silver, Debra L.[1,3], [1]Duke University Medical center, Durham, NC, U.S., [2]University of North Carolina, Chapel Hill, NC, U.S., [3]Duke University Medical Center, Durham, NC, U.S.

Embryonic neocortical development depends on balanced production of progenitors and neurons. Genetic mutations disrupting progenitor mitosis frequently impair neurogenesis; however, the link between altered mitosis and cell fate remains poorly understood. Here we demonstrate that prolonged mitosis of radial glial progenitors directly alters neuronal fate specification and progeny viability. Live imaging of progenitors from a neurogenesis mutant, *Magoh*$^{+/-}$, reveals that mitotic delay significantly correlates with preferential production of neurons instead of progenitors, as well as apoptotic progeny. Independently, two pharmacological ap- proaches reveal a causal relationship between mitotic delay and progeny fate. As mitotic duration increases, progenitors produce substantially more apoptotic progeny or neurons. We show that apoptosis, but not differentiation, is p53 dependent, demonstrating that these are distinct outcomes of mitotic delay. Together our findings reveal that prolonged mitosis is sufficient to alter fates of radial glia progeny and define a new paradigm to understand how mitosis perturbations underlie brain size disorders such as microcephaly.

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T4007

STROKE SERUM PRIMING METHOD INCREASES PROLIFERATION RATES OF MESENCHYMAL STEM CELLS VIA THE REGULATION OF miRNA-20a

Kim, Eun Hee[1], Kim, Dong Hee[2], Kim, Hye Ree[2], Kim, Soo Yoon[3], Sung, Ji Hee[3], Moon, Gyeong Joon[4], Cho, Yeon Hee[3], Son, Jeong Pyo[2], Oh, Mi Jeong[3], Shin, Eun Kyoung[3], Kim, Hyeon Ho[2] and Bang, Oh Young[5], [1]Medical Research Institute, Sungkyunkwan University School of Medicine, Seoul, Korea, [2]Department of Health Sciences and Technology, Samsung Advanced Institute for Health Sciences and Technology (SAIHST), Sungkyunkwan University, Seoul, Korea, [3]Translational and Stem Cell Research Laboratory on Stroke, Samsung Medical Center, Seoul, Korea, [4]Stem Cell and Regenerative Medicine Institute, Samsung Medical Center, Seoul, Korea, [5]Department of Neurology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Transplantation of mesenchymal stem cells (MSCs), expanded with fetal bovine serum (FBS), has some limitations, including a long culture period required to obtain sufficient stem cells. The differences in property bone-marrow derived mesenchymal stem cells cultured from normal and ischemic rats have been reported. It is important to identify factors that are involved in MSCs proliferation. MiRNAs have been appreciated in various cellular functions, including the regulation of proliferation. We investigated miRNAs profiling in stroke serum primed MSCs and tested whether regulation of a certain miRNAs may regulated proliferation rate of rat MSCs.Using MTT assay and Ki-67 immunocytochemistry, we identified that the proliferation rate of MSCs cultured with stroke serum derived from a stroke model was higher than that of MSCs cultured with normal serum or FBS. To study the potential mechanism of MSCs cultured with stroke serum, we profiled their miRNA expression pattern by miRNA microarray analysis. Gene ontology analysis revealed that many differentially expressed genes were involved in biological processes relevant to the regulation of metabolic process, cell proliferation, signaling, etc. Among miRNAs associated with cell proliferation, miR-20a was markedly correlated with the number of MSCs cultured in rat's serum (normal serum or stroke serum). Similarly, miR-20a was increased in MSCs obtained from bone marrow of stroke rats than in MSCs from normal rats. Furthermore, the deregulated miR-20a by transfection of MSCs with pre-miR-20a or anti-miR-20a was significantly correlated with proliferation rate of MSCs. In addition, dual-luciferase reporter assay showed that miR-20a promoted proliferation by suppressing the expression of p21 cyclin-dependent kinase inhibitor 1 (CDKN1A). We conclude that stroke serum priming up-regulated the expression of miR-20a,

which promoted MSC proliferation by regulating the cell cycle inhibitor p21 CDKN1A, and suggest the possible roles of priming methods in modulating characteristics of MSCs by controlling the expression of miRNA in MSCs.

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T4009

PRECONDITIONING OF MESENCHYMAL STEM CELLS USING SERUM OBTAINED DURING ACUTE STROKE

Moon, Gyeong Joon[1], **Cho, Yeon Hee**[2], Kim, Dong Hee[3], Sung, Ji Hee[2], Kim, Sooyoon[2], Son, Jeong Pyo[3], Kim, Eun Hee[4], Oh, Mi Jeong[2], Kim, Hyeon Ho[3], Kim, Suk Jae[5] and Bang, Oh Young[5], [1]Stem Cell and Regenerative Medicine Institute, Samsung Medical Center, Seoul, Korea, [2]Translational and Stem Cell Research Laboratory on Stroke, Samsung Medical Center, Seoul, Korea, [3]Department of Health Sciences and Technology, Samsung Advanced Institute for Health Sciences and Technology (SAIHST), Sungkyunkwan University, Seoul, Korea, [4]Medical Research Institute, Sungkyunkwan University School of Medicine, Seoul, Korea, [5]Department of Neurology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Several clinical trials of stem cell therapies have recently been performed in stroke patients, leading to efforts to enhance the therapeutic effects of stem cells. Aging impairs the function of mesenchymal stem cells (MSCs), and rejuvenation of MSCs may be particularly important for MSCs obtained through long-term *ex vivo* culture expansion and in elderly patients. We investigated the characteristics of MSCs preconditioned by serum obtained from a stroke rat model and in patients with acute ischemic stroke. MSCs were grown in fetal bovine serum (FBS), normal serum (NS), or stroke serum (SS). MSCs cultured with SS or NS exhibited higher proliferation indices than with FBS ($p<0.05$). FBS-MSCs, NS- and SS-MSC showed differential trophic factor expression: *VEGF, GDNF,* and *FGF* were densely expressed in samples cultured with SS ($p<0.05$). In addition, SS-MSCs displayed superior survival under ischemic brain conditions, as well as delayed senescence. Neurogenesis and angiogenesis were markedly increased in rats that received SS-MSCs ($p<0.05$), and these rats showed significant behavioral improvement ($p<0.05$). Similarly, SS-MSCs revealed different cell cycle- or aging-associated mRNA expression at a later passage, and β-galactosidase staining showed a significant senescence decrease after culture with SS ($p<0.05$). Various serum proteins are considered to contribute to preconditioning of MSCs. Culture expansion using SS obtained during the acute phase of stroke could constitute a novel preconditioning method that is feasible and effective for neuro-restoration of stroke. Based on the results of this study, the STARTING-2 trial is currently ongoing.

Funding Source: This study was supported by a grant from the Korea Health Technology R&D Project, the Ministry of Health & Welfare (HI14C14390000).

T4011

CROSS-TALK BETWEEN HUMAN PLACENTA-DERIVED STROMAL CELLS AND RADIATION-INDUCED IMMUNE ENVIRONMENT INDUCES BONE MARROW AND PERIPHERAL BLOOD LINEAGE RECOVERY IN H-ARS MURINE MODEL

Sher, Noa[1], Orschell, Christie[2], Pinzur, Lena[1], Rekhes, Shiran[1], Akyuz, Levent[3], Gaberman, Elena[4], Volk, Hans Dieter[3], Gorodetsky, Raphael[4] and Ofir, Racheli[1], [1]Pluristem Therapeutics Inc, Haifa, Israel, [2]Indiana University School of Medicine, Indianapolis, IN, U.S., [3]Charité - Universitätsmedizin Berlin, Berlin, Germany, [4]Sharett Institute of Oncology, Hadassah - Hebrew University Medical Center, Jerusalem, Israel

Acute Radiation Syndrome (ARS) is a syndrome involving damage to multiple organs caused by exposure to a high dose of ionizing radiation over a short period of time; even low doses of radiation damage the radiosensitive hematopoietic system (causing H-ARS). We tested the ability of a 3D-expanded placenta-derived stromal cell product designated for the treatment of hematological disorders to alleviate symptoms in the H-ARS mouse model. These cells have previously been shown in vitro to secrete hematopoietic proteins, to stimulate colony formation, and to induce bone marrow (BM) migration. Cells were administered intramuscularly to C3H/HeN and C57BL/6 mice, 1 and 5 days after (LD70/30) total body irradiation. Weight, survival, and peripheral blood and BM cellularity were monitored at several time points up to 23 days. Treatment significantly increased survival after irradiation and rescued radio-induced weight loss relative to vehicle-injected controls. In addition, cell treatment significantly increased the number of colony forming hematopoietic progenitors in the BM and raised peripheral blood cellularity to values near those of un-irradiated control values. The injected cells responded to radiation-induced hematopoietic failure by transiently secreting haematopoiesis related proteins to enhance reconstitution of the hematopoietic system. CyTOF analysis of peripheral blood and BM indicated rescue of the blood lineages to levels near those of naïve mice in cell-treated irradiated mice. Taken together, placenta-derived stromal cells have the capacity to alleviate bone marrow failure symptoms in the H-ARS model.

T4013

ZFP281 COORDINATES OPPOSITE FUNCTIONS OF TET1 AND TET2 FOR ALTERNATIVE PLURIPOTENT STATES

Fidalgo, Miguel[1], Huang, Xin[1], Guallar, Diana[1], Sanchez-Priego, Carlos[1], Valdes, V. Julian[1], Saunders, Arven[1], Wu, Wen-Shu[2] and **Wang, Jianlong**[1], [1]Icahn School of Medicine at Mount Sinai, NEW YORK, NY, U.S., [2]University of Illinois at Chicago, Chicago, IL, U.S.

Embryonic stem cells (ESCs) provide a powerful system for dissecting the molecules and mechanisms that regulate mammalian cell state. Pluripotency spans a spectrum encompassing two distinct pluripotent states, naive and primed, which are represented by mouse ESCs and epiblast stem cells (EpiSCs), respectively. Conventional human ESCs share defining features of primed pluripotency with mouse EpiSCs, both of which can be reverted to naive pluripotency by an epigenetic reprogramming process involving DNA demethylation that is incompletely understood, although naive hESCs were established with multiple versions among laboratories, possibly reflecting alternative pluripotent states. It is well established that a set of transcription factors centered on the triumvirate of Oct4, Sox2 and Nanog and a number of epigenetic regulators including DNA and histone modification enzymes play significant roles in controlling stem cell pluripotency. Here we define a pluripotent cell fate gene signature associated with naive and primed pluripotency acquisition, and identify Zfp281 as a key transcriptional regulator for primed pluripotency and also as a barrier to achieve the naive pluripotency of both mouse and human ESCs. Mechanistically, we find that Zfp281 interacts with Tet1, but not Tet2, and that Zfp281 and its direct transcriptional target miR-302/367 negatively regulate *Tet2* expression in establishing and maintaining primed pluripotency. Conversely, ectopic Tet2 alone, but not Tet1, efficiently reprograms primed EpiSCs towards naive pluripotency. Our study reveals a previously uncharacterized molecular circuitry encompassing Zfp281, miRNAs, and the opposing functions of Tet1 and Tet2 in controlling alternative pluripotent states. Studying the molecular mechanisms underlying naive/primed pluripotency and their inter-conversion by developmental transition or molecular reprogramming will yield important insights into early development and mammalian cell fate determination. Ultimately, it will facilitate the application of human pluripotent stem cells in disease therapeutics and regenerative medicine.

Funding Source: NIH R01 (1R01-GM095942), NYSTEM (C028103, C028121)

T4015

STEMPEUCEL® (POOLED ALLOGENEIC BMMSCS) – FIRST STEM CELL PRODUCT APPROVED FOR LIMITED MARKETING IN INDIA FOR CRITICAL LIMB ISCHEMIA DUE TO BUERGER'S DISEASE

Gupta, Pawan Kr[1], CH, Anoop[2], Krishna, Murali[3], Rajkumar, R[4], Dutta, Santanu[5], Sarkar, Uday[6], Desai, Sanjay[7], Radhakrishnan, R[8], Dhar, Anitha[9], Kv, Prasanth[2], Mathiazhagan, R[2], Balasubramanian, Sudha[2] and Majumdar, Anish Sen[2], [1]Stempeutics Research, Bangalore, India, [2]Stempeutics Research Private Ltd., Bangalore, Karnataka, India, [3]Sri Jaydeva Institute of Cardiovascular Sciences, Bangalore, India, [4]SRM Medical College & Hospital, Chennai, India, [5]Nightingale Hospital, Kolkata, India, [6]Health Point Hospital, Kolkata, India, [7]M S Ramaiah Medical College & Hospitals, Bangalore, India, [8]Sri Ramachandra Medical College & Hospital, Chennai, India, [9]All India Institute of Medical Sciences, New Delhi, India

Critical limb ischemia (CLI) due to Buerger's disease (non – atherosclerotic condition) is a major unmet medical need having high incidence of morbidity and is strongly associated with heavy tobacco use. We had conducted a phase 2, dose finding study (NCT01484574) to assess the efficacy and safety of IM injection of stempeucel® (adult human bone marrow derived, cultured pooled, allogeneic mesenchymal stromal cells) in "no – option" patients in Rutherford classification - III-5 and Rutherford- III 6 (with gangrene limited to the toes). Stempeucel® is manufactured from BMMSC obtained from healthy volunteers and the product is comprised of pooled allogeneic BMMSCs. Stempeucel® express MSC-associated surface markers, possess potent immunosuppressive activity and secrete various angiogenic factors including VEGF, angiopoietin, IL8 and HGF. Preclinical studies have demonstrated that the pooled product ameliorates limb necrosis, promote blood flow and prevent limb loss in a mouse model of hind limb ischemia.

The efficacy in the clinical study was measured by primary end points - relief of rest pain and healing of ulcer. Stempeucel® was administered in two doses - 1 and 2 million/kg (36 patients each) and was compared with standard of care arm (SOC) (18 patients). Reduction of rest pain: 2 million/kg group had 0.3 units (SE=0.13) reduction per month compared to SOC group ($p = 0.0193$), CI= (-0.55,-0.05). Ulcer healing: As compared to the SOC group, 2 million/kg group had 11% (SE=0.05) decrease in ulcer size per month ($p=0.0253$) CI = (0.80, 0.99). Secondary end points were also significant in 2 million/kg group like Ankle Brachial Pressure Index ($p=0.0132$), total walking distance ($p=0.0577$), QOL domains: activity ($p=0.0529$), symptom ($p=0.0329$) and pain ($p=0.0422$). Patients in the 2 million/kg dose group showed increased

collaterals (45%) compared to the other groups (1 million/kg {31.58%}; SOC {33.33%}). Total of 47 AEs were reported in 28 patients in all groups which were remotely related or unrelated to stempeucel®. Hence, IM administration of stempeucel® at a dose of 2 million/kg group has shown clinical benefit and is considered to be effective in treating patients of CLI due to Buerger's disease. With this data of safety and efficacy, the Indian FDA has granted limited marketing approval for 200 patients.

T4017

CHARACTERISATION OF IPSC-DERIVED CD34+ CELLS AS PROGENITORS OF ENDOTHELIAL CELLS (PECS) AND THEIR COMPARISON WITH CORD BLOOD, PERIPHERAL AND HUVECS CD34+ CELLS

Jalilian, Elmira[1], Thompson, Hannah[2], Coffey, Peter[2] and Fruttiger, Marcus[1], [1]UCL institute of Ophthalmology, London, U.K., [2]University College London, London, U.K.

There are a number of different stem cell sources that have the potential to be used as therapeutics in vascular degenerative diseases such as diabetic retinopathy. On the one hand, there are so called endothelial progenitor cells (EPCs), which are typically derived from adult blood. They carry the marker CD34, but the true nature and definition of EPCs is still controversial. On the other hand, there are embryonic precursors of endothelial cells (PECs), which also express CD34, and which can be differentiated from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) in vitro. Furthermore, a subpopulation of human umbilical cord endothelial cells (HUVECs) has also been shown to express CD34. Here, we aimed to compare these three different CD34 positive cell populations by full genome transcriptional profiling (RNAseq). hiPSCs were grown on Matrigel coated plates in mTeSR-1 medium. Then, a differentiation protocol optimised in our lab was used to derive CD34+ cells. At day 5 of differentiation treated CD34+ and CD34- cells were isolated by flow cytometry and their transcriptional profile was established by RNASeq. Other CD34+ cells isolated from HUVECs, cord blood and peripheral blood was also processed for RNA-seq.Firstly, we optimised a PEC differentiation protocol and found that VEGF is critical for the transition from mesodermal precursors to PECs. Secondly, principal component analysis (PCA) of our RNA-seq data showed that PECs, HUVECs and blood derived CD34+ cells are fundamentally different cell populations. Thirdly, RNAseq analyses could show that genes that are highly expressed in PECs and low in progenitors of PECs (PPEC) are significantly involved in angiogenesis and blood vessel development. In addition, we also identified potentially novel markers for progenitors of Endothelial Cells (PECs). This study showed that CD34+ cells from different sources are fundamentally different cell populations. Identification of novel markers and better characterisation of these populations will facilitate the translation of regenerative approaches in this field as well as providing potentially novel diagnostic tools.

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T4019

RETINOCURE; ESSENTIAL PREMIXED ORGAN SPECIFIC PEPTIDES DERIVED FROM MULTIPLE ORGANS TO HEAL RETINAL DEGENERATIVE DISEASES.

Abdelkarim, Alaa M, Stellar Biomolecular Research, Frankfurt am Main, Malaysia
Stellar Biomolecular Research UG, Frankfurt, Malaysia

Retinal Degeneration is one of the most complicated incurable health problems. The causes for the retinal degeneration is numerous; start from the genetic defects (more than 179 genes are responsible for retinal degeneration) in what we call it Retinitis pigmentosa, passing by the diabetic and hypertensive retinopathy and topped up by the Age related maculopathy. In one of the statistics, Maculopathy due to all causes in the age group between 45 and 85 represent 8.01% of the population globally. Many approaches tried to use the different types of peptides either synthetic or natural. One of the major challenges that these approached faced and limited its success is the complex embryological origin of the retina, which makes the general principal in cell therapy (organ for organ) fails, is that giving the patient retinal cell or peptides only is not sufficient to reach satisfactory results with the patient. Retinocure, as the most recent patented 100% natural peptides; chosen from highly specific regions and mixed with the percent that suits the different embryological origins of the retina and suits its complexity of structure.I inject 3 ml IM three times wekly of Retinocure for 10 weeks. Perform safety investigations before the injection and every two weeks. Perform visual acuity test, visual field test, ERag and VPG before and after the injection. Result: 3 patient were chosen, all of them can not see except hand movement. The three patient restored their vision with remarkable change in the ERG and VEP

Funding Source: Stellar Biomolecular Research UG

T4021

COMPARATIVE ANALYSES OF NETWORK ACTIVITIES IN NEURONAL POPULATIONS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS

Alshawaf, Abdullah[1], Qiu, Wanzhi[2,3], Viventi, Serena[1], Ivanusic, Jason[1], Kaplan, David[4,5], Petrou, Steven[5], Erlichster, Michael[1], D'Abaco, Giovanna[1], Chana, Gursharan[1], Everall, Ian[1], Skafidas, Efstratios[1] and **Dottori, Mirella**[1,3], [1]University of Melbourne, Carlton, Australia, [2]The university of Melbourne, North Melbourne, Australia, [3]University of Melbourne, Parkville, Australia, [4]Florey Institute of Neuroscience and Mental Health, Parkville, Australia, [5]Florey Institute, Melbourne, Australia

Neurons derived from human pluripotent stem cells (hPSC) are a valuable resource for establishing in vitro models of neurological function and dysfunction. An imperative step for modelling the nervous system in vitro is to characterize functional neuronal network activity. Here, we use microelectrode arrays (MEAs) to measure network activity of hPSC-derived neurons, thereby characterizing the timeline for their maturation and ability to form functional networks. These measurements were conducted over 8 weeks in culture and also in neurons derived from hPSCs by utilising different induction protocols in order to derive various neuronal subtypes. Our data show differences in firing activity between various hPSC-derived neuronal populations, including cortical glutamaterigic, GABAergic neurons and sensory neurons. These studies provide key data for modelling neural circuitry systems in vitro using hPSC-derived neurons.

Funding Source: Australian Research Council, Friedreich Ataxia Research Association U.S. and Australasia

T4023

SUSPENSION SURVIVAL MEDIATED BY S727STAT3-COL XVII-LAMININ 5 DETERMINES TUMOUR INITIATION AND METASTASIS IN CANCER STEM CELLS

Hung, Shih-Chieh, China Medical University & Hospital, Taichung, Taiwan

Targeting tumour initiating cells (TICs) would lead to new therapies to cure cancer. We previously demonstrated that TICs have the capacity to survive under suspension conditions while other cells undergo anoikis. Here we show that TICs exhibit increased phosphorylation levels of S727STAT3 by PP2A inactivation, and in a STAT3-dependent manner, collagen 17 gene expression is upregulated, which also stabilizes laminin 5 and engages cells that form hemidesmosome-like junctions in response. Blocking the PP2A-S727STAT3-collagen 17 pathway inhibits the suspension survival of TICs and their ability to form tumors in mice, while activation of the same pathway increases the suspension survival and tumour initiation capacities of bulk cancer cells. The S727STAT3 phosphorylation levels correlate with collagen 17 expression in colon tumor samples, and correlate inversely with survival. Finally, this signaling axis enhances the ability of TIC to form tumors in mouse models of malignant lung cancer pleural effusion and spontaneous colon cancer metastasis.

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T4025

ALKP2 IS NECESSARY FOR HEART DEVELOPMENT THROUGH ATTENUATION OF WNT/β-CATENIN SIGNALING

Hofsteen, Peter[1], Robitaille, Aaron[2], Palpant, Nathan[3], Pabon, Lil[2], Moon, Randall T.[4] and Murry, Charles[2], [1]University of Washingotn, Seattle, WI, U.S., [2]University of Washington, Seattle, WA, U.S., [3]The University of Queensland, St Lucia, QLD, Australia, [4]Universityof Washington, Seattle, WA, United States

Proper biphasic regulation of the Wnt/β-catenin signaling pathway is imperative for normal cardiomyocyte differentiation from human pluripotent stem cells (hPSC). Wnt/β-catenin signaling needs to be activated to initiate mesoderm specification while progression to cardiac progenitor cells (CPC) and cardiomyocytes requires inhibition of the pathway. Identifying novel regulators of the Wnt/β-catenin signaling pathway provides new insights into normal heart development and how it is mis-regulated in diseased states. We describe a combinatorial screen analyzing chromatin dynamics and gene expression from three distinct mesodermal lineages: cardiomyocyte, endocardial-like endothelium and hemogenic endothelium. Comparison of genes specific to the cardiomyocyte lineage with an independent siRNA screen using mouse hippocampal cells (HT22) stably transduced with a β-catenin activated reporter (BAR) yielded Alpha-protein kinase 2 (ALKP2) as a novel regulator of this pathway. ALPK2 is specifically expressed in CPCs and cardiomyocytes and increased BAR expression following siRNA knockdown (KD). Depletion of ALPK2 results in significant defects in cardiomyocyte differentiation in both hPSCs and zebrafish as demonstrated by both CRISPR/Cas9 mediated deletion and antisense-mediated KD. Additionally, ALPK2 KD increased BAR expression in CPCs confirming ALPK2 negatively regulates Wnt/β-catenin signaling during cardiomyocyte differentiation. Quantitative proteomics of CPCs lacking ALPK2 identified numerous ALPK2 regulated proteins involved in cardiac development and Wnt/β-catenin signaling including GATA4 and β-catenin. Importantly, pharmacologically inhibiting Wnt/β-catenin signaling through stabilization of its destruction complex

partially rescued the ALPK2-mediated defects during hPSC-cardiomyocyte differentiation. Taken together, our work identifies a novel alpha-protein kinase with a key role in human heart development through regulation of Wnt/β-catenin signaling.

T4027

TARGETED RNAi SCREEN REVEALS NOVEL POST-TRANSCRIPTIONAL REGULATORS OF PLURIPOTENCY AND DIFFERENTIATION IN MOUSE EMBRYONIC STEM CELLS

You, Kwontae[1,2], Park, Joha[1,2], Kim, Haedong[1,2], Ha, Minju[1,2] and Kim, V. Narry[1,2], [1]Institute for Basic Science, Seoul, Korea, [2]Seoul Natil University, Seoul, Korea

Embryonic stem cells (ESCs) are tractable model system for the cell fate commitment in early development, and have a great potential for medical application. Significant improvement has been made in gene regulatory networks including signaling molecules, transcriptional factors, and epigenetic regulators. However, only a small number of studies addressed the contribution of post-transcriptional gene regulation in ESCs. To gain more comprehensive insight into the role of post-transcriptional gene regulations in ESCs, we applied RNAi screen for RNA-binding proteins (RBPs), which are important players responsible for all the processes related with RNA transcripts, in mouse ESCs. We found dozens of RBPs required for maintenance or exit from pluripotency, which include splicing regulators, noncoding RNA interacting proteins, decay factors, and ribosome biogenesis factors. Furthermore, we discovered that upregulation of translational capacity is required for ESC maintenance, while suppression of gene expression via RNA decay factors or microRNAs is important for ESC differentiation. Our study uncovers the importance of post-transcriptional mechanisms in the regulation of cellular identity.

Funding Source: This work was supported by IBS-R008-D1 of the Institute for Basic Science from the Ministry of Science, ICT, and Future Planning of Korea.

T4029

HUMAN IPS CELL-DERIVED MOTOR NEURONS FOR MODELING NEUROLOGICAL DISEASES AND DRUG SCREENING

Lin, Nianwei, iXCells Biotechnologies U.S., LLC, San Diego, CA, U.S.

In this study, we developed a robust method to produce highly pure, functional validated motor neurons from human induced pluripotent stem cells (iPSC), which can be used in neurological disease modeling studies. Using xeno-free differentiation conditions, we were able to produce motor neurons from human iPSC, at greater than 85% purity as measured by immunostaining of ISL1, HB9, ChAT and Tuj1. iXCells™ hiPSC-derived motor neurons are functionally verified by neuromuscular junction assay, and electrophysiological assay using a multi-electrode array (MEA) system. These cells can be cryopreserved, thawed, and cultured in defined maintenance media without glia cells. They can also be co-cultured with primary astrocytes for extended periods (>30 days) without losing featured motor neuron markers. Using iPSC lines from multiple donors, including Amyotrophic Lateral Sclerosis (ALS) patients, we have demonstrated the protocol is robust, and independent of the donor iPSC lines. We developed ALS isogenic models carrying multiple mutations in TDP-43 gene, which were used for transcriptome analysis with RNA-seq. Taken together, these data show that our protocol is reproducible in human iPSCs, and is applicable in modeling MN-degenerative diseases and in proof-of-principle drug-screening assays.

T4031

ADVANCES IN DOWN SYNDROME DISEASE MODELING USING HUMAN IPSC

Venkatesh, Aparna[1], Loh, Lijun[1], Chia, Lionel[1], Vu, Anthony[2], Hoon, Shawn[1], Yeo, Gene[3], Choolani, Mahesh[4], Chan, Jerry[5] and Brenner, Sydney[1], [1]Agency for Science, Technology and Research, Singapore, Singapore, [2]University of California, San Diego, San Diego, CA, U.S., [3]University of California San Diego, La Jolla, CA, U.S., [4]National University of Singapore, Singapore, Singapore, [5]KK Women's and Children's Hospital, Singapore, Singapore

Down Syndrome (DS) is one of the most frequent genetic causes of intellectual disability characterized by several pathological phenotypes; among which neurodegeneration, muscular dystrophy and cardiac defects are key features.

In an effort to better understand the pathogenesis of DS, we have analysed amniotic fluid (AF) from woman carrying DS pregnancy compared with that from women carrying healthy fetuses. We have currently optimized the protocol of deriving DS iPSC from fresh DS AF samples through delivery of the Yamanaka transcription factors via non-integrating episomal vectors and compared them to iPSC derived from gestation-matched fetuses, which are karyotypically normal. These DS iPSC have in turn been characterized and differentiated to form motor neurons and cardiomyocytes. In parallel, we have carried out a targeted removal of the trisomy via clustered regularly interspaced short palindromic repeats (CRISPR) technology to create isogenic, disomic lines of the iPSC derived from DS AF cells. These DS iPSCs derived from the AF would further allow for us to study the role of DS specific biomarker genes, by comparing it with healthy closely matched controls. The use of CRISPR on these DS iPSC lines have allowed us to create targeted knockouts of DS specific candidate genes and subsequently, we hope

to study the effect of inhibiting these genes on cellular phenotypes derived from DS iPSC; using the reverted disomic lines as control to provide futher novel mechanistic insights into AD pathogenesis. This study will lay the groundwork for understanding the effect of aneuploidy in the early development of DS and establishing DS cellular phenotypes in culture. Furthermore, it would establish the generation of efficient techniques in deriving iPSC from fetal stem cells as well as identifying possible drug targets for DS therapeutics.

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T4033

THERAPEUTIC POTENTIAL OF HUMAN TONSIL-DERIVED MESENCHYMAL STEM CELLS IN OSTEOPOROSIS ANIMAL MODEL

Kim, Gyungah[1], Park, Yoon Shin[2], Lee, Yunki[3], Jin, Yoon Mi[1], Do, Yuri[1], Ryu, Kyung-Ha[1], Park, Ki Dong[3] and Jo, Inho[4], [1]Ewha Womans University School of Medicine, Seoul, Korea, South, [2]Chungbuk National University, Cheongju, Korea, [3]Ajou University, Suwon, Korea, [4]Ewha Womans University, Seoul, Korea, South

Previously, we have successfully differentiated tonsil-derived mesenchymal stem cells (TMSC) into parathyroid hormone (PTH)-releasing cells. These cells improved the survival rate of rats with hypoparathyroidism. Here, we investigated the therapeutic efficacy of these PTH-releasing TMSC for osteoporosis, as recombinant human PTH (1–34; Teriparatide) is currently the only approved bone-forming agent for osteoporosis treatment. TMSC were isolated from discarded human tonsillar tissues, and control TMSC (cTMSC) and differentiated TMSC (dTMSC) were prepared without or with differentiating chemicals (activin A and sonic hedgehog) respectively. After the differentiation period, TMSC were embedded in hydrogels firstly, and then subcutaneously delivered to animals. Osteoporotic animal model was prepared using ovariectomized (OVX) female mice. During experimental periods (3 months), serum levels of osteocalcin (OC), alkaline phosphatase (ALP), total calcium, and total phosphorus were measured. After 3 months, the femurs were isolated and imaged with micro computed tomography, and bone parameters were analyzed. Three-dimensionally constructed images showed improved and more filled femoral heads in OVX mice treated with TMSC-embedded hydrogels. Serum level of ALP, known to be a bone formation marker, also increased in OVX mice treated with TMSC-embedded hydrogels. Serum OC concentration, which is elevated in osteoporosis, showed a lowering trend upon treatment. Furthermore, hypercalcemia and hypophosphatemia, side effects of PTH treatment, were not observed with TMSC treatment. TMSC therapy was generally well-tolerated, without severe macroscopic changes in livers and kidneys. Moreover, TMSC-embedded hydrogels were effective in subcutaneous fat reduction, showing therapeutic effects against another postmenopausal symptom. In conclusion, TMSC demonstrate a high potential for a stem cell therapy targeting osteoporosis, and using hydrogels as scaffolds increases the therapeutic efficacy.

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T4035

INTERMITTENT HYDROSTATIC PRESSURE IMPROVES CHONDROGENESIS, BUT NOT ADIPOGENESIS, IN HUMAN TONSIL-DERIVED MESENCHYMAL STEM CELLS

Yu, Yeonsil[1], Kim, Ha Yeong[2] and Jo, Inho[1], [1]Ewha Womans University, Seoul, Korea, South, [2]Ewha Womans University School of Medicine, Seoul, Korea

Chondrocytes is influenced by numerous microenvironmental factors and show different responses in these circumstance such as mechanical stresses. Previously, it has been already demonstrated that human tonsil-derived mesenchymal stem cells (TMSC) were differentiated into chondrocyte. TMSC isolated from tonsillar tissue are becoming increasingly recognized for their potential to generate different cell types. The objective of this study was to test the effects of intermittent hydrostatic pressure (IHP) on the chondrogenic differentiation of TMSC. Cells were treated with at 200kPa for 2h/day. The stimulation and resting pattern was 2 and 15 min cycles respectively. MTT assay, flow activated cells sorting (FACS) assay, and western blotting assay were performed. The results from the analyses showed that stimulation with IHP had inhibitory effect on proliferation. There were no differences on mesenchymal stem cell markers. IHP enhanced the chondrogenic differentiation of TMSC, while adipogenic differentiation was inhibited under this condition. Taken altogether, we suggest that IHP is beneficial particularly in condrogenesis of TMSC, but not, adipogenesis.

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T4037

CLONAL COMPENSATION BETWEEN HEMATOPOIETIC STEM CELLS UPON DIFFERENTIATION DEFICIENCY

Nguyen, Lisa, University of Southern California, Glendale, CA, U.S.

In most organ systems, regeneration is a coordinated effort that involves many stem cells, but little is known whether and how individual stem cells compensate for the functional deficiencies of other stem cells. Functional compensation between stem cells is critically important during disease progression and treatment. For example, many hematopoietic disorders are characterized by an unbalanced blood supply where certain cell types are either abnormally abundant or abnormally deficient. Currently, the primary treatment for these diseases requires bone marrow transplantation from healthy donors. Post transplantation, donor hematopoietic stem cells (HSCs) must adapt their differentiation programs to the presences of other HSCs. Few studies have tried to address the coordination between stem cell during this process. Recent work from our group and others suggest that HSCs heterogeneously supply blood. We hypothesize that HSCs also heterogeneously compensate for blood deficiency. To test this hypothesis, we tracked mouse HSCs *in vivo* using a single-cell tracking technology that we had previously developed. We found that in mice, individual HSCs heterogeneously compensate for the lymphopoiesis deficiencies of other HSCs by increasing their clonal expansion and altering their lineage bias. This compensation rescues the overall blood supply and influences blood cell types outside of the deficient lineages in distinct patterns. To further investigate the dynamics of HSC coordination, we precisely perturbed blood production in specific lineages and assayed the temporal responses of individual HSCs. We also identified the molecular regulators and signaling pathways involved in this process. Together, these data show that stem cells interact with each other to form a coordinated network that is robust enough to withstand minor functional disruptions. Exploiting the innate compensation capacity of stem cell networks may improve the diagnosis and treatment of many diseases.

Funding Source: NIH T32 Pre-doctoral Training in Developmental Biology, Stem Cells, and Regeneration (DSCR) Traineeship NIH-K99/R00-HL-1131-4 Grant PI: Rong Lu

T4039

HEMATOPOIETIC STEM CELL HETEROGENEITY IS DETERMINED AT THE CLONAL LEVEL

Jiang, Du and Lu, Rong, University of Southern California, Los Angeles, CA, U.S.

Recent studies suggest that hematopoietic stem cells (HSCs) heterogeneously supply blood cells after transplantation. Little is known about how this heterogeneity is regulated. Here, we used a genetic barcode tracking system to track the blood production of individual HSC clones during serial transplantation in mice. We marked HSC clones with unique genetic barcodes prior to the primary transplantation. Four months later, we recovered HSCs from each primary recipient and transplanted them into multiple secondary recipient mice. Blood cells were sampled and analyzed four months after each transplantation. We found that fewer HSC clones supplied blood cells in the secondary recipients and that their clonal expansion had significantly increased. Surprisingly, many clones found in the peripheral blood of the secondary recipients were not present in the peripheral blood of the primary recipients. Most of these clones were activated in multiple secondary recipients and were biased towards myeloid lineages. Strikingly, HSCs derived from the same clone exhibited consistent clonal expansion and lineage bias characteristics across different secondary recipients. However, these differentiation characteristics changed between the primary and secondary recipients. For example, lineage-balanced HSC clones in the primary recipients mostly converted to lymphoid bias in the secondary recipients, and myeloid-biased clones in the primary recipients were more likely to become lineage balanced in the secondary recipients. Overall, our data suggest that HSCs derived from the same clone exhibit similar blood production characteristics, which are altered in a predictable way upon transplantation. These findings suggest that the clonal-level characteristics of HSC blood production may be exploited in bone marrow transplantation therapy. For example, select HSC clones matching patient specific requirements can be transplanted to improve the efficacy of this treatment.

T4041

WNT/Β-CATENIN SIGNALING PATHWAY ENHANCES HUMAN UCB DERIVED ENDOTHELIAL PROGENITOR CELL ANGIOGENIC POTENTIAL VIA SDF1-CXCR4 AXIS

Kim, Yeon Ju, Pusan National University, Yangsan, Korea, South

Endothelial Progenitor Cells(EPCs) haves been considered potential therapeutic agents for promoting vascular regeneration in ischemic tissues. However, therapeutic uses of autologous EPCs are hindered due to attenuated cellular yields and reduced biological activities in patients with ischemic disease. Wnt/β-catenin signaling pathway is known as a powerful regulator of various cellular mechanisms, including proliferation and differentiation. In this study, we investigated the role of Wnt/β-catenin signaling in the overall function of EPCs. First, we explored the effect of Wnt/β-catenin signaling on endothelial commitment of CD34+ HSCs. Using EPC colony forming assays,

we determined that treatment with CHIR99021, a glycogen synthase kinase-3β (GSK-3β) inhibitor, could augment EPC colony formation. Functional analyses also revealed that activation of Wnt/β-catenin signaling could significantly enhances the proliferation, migration, and invasion capacities of EPCs, as compared with controls. Our examination of potential targets of Wnt/β-catenin signaling indicated that expression of CXCR4 drastically increased in cells with an activated Wnt/β-catenin signaling pathway. Subsequently, experiments using luciferase reporter assays demonstrated that CXCR4 may be a direct target of Wnt/β-catenin signaling. Consequently, our results suggest that Wn/β-catenint signaling plays an important role in EPC commitment and its angiogenic potential and that these effects may be attributed to enhancement of CXCR4 directly regulated by Wnt signaling. Thus, regulation of Wnt/β-catenin signaling in EPCs can be an effective tool for angiogenic therapy.

Funding Source: This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government NRF-2015M3A9B4066493

T4043

ACUTE MYELOID LEUKEMIA (AML) CELL-OF-ORIGIN IDENTIFIED BY CHROMATIN LANDSCAPE OF BULK TUMOR CELLS

George, Joshy[1], Uyar, Asli[1], Young, Kira[2], Kuffler, Lauren[2], Waldron-Francis, Kaiden[2], Marquez, Eladio[1], Ucar, Duygu[1] and **Trowbridge, Jennifer J.**[2], [1]The Jackson Laboratory for Genomic Medicine, Farmington, CT, U.S., [2]The Jackson Laboratory, Bar Harbor, ME, U.S.

Precise identity of tumor cell-of-origin can influence disease prognosis and outcome. A strategy to reliably define cell-of-origin from bulk acute myeloid leukemia (AML) cells remains an unmet need. Here, using a model of *MLL-AF9*-induced AML, we demonstrate that transforming hematopoietic stem cells (HSCs) or multipotent progenitors (MPPs) results in more rapid AML development than transforming lineage-committed common myeloid progenitors (CMPs) or granulocyte-macrophage progenitors (GMPs) (Log-rank test P < 0.0001). Other than *MLL-AF9,* no other recurrent somatic mutations were found that could explain this differential rate of tumor development. RNA-seq of bulk tumor cells revealed a gene expression signature distinguishing HSC-derived versus progenitor-derived AML, including genes involved in immune escape, extravasation, and small GTPase signal transduction. This transcriptional signature was enriched for prognostic genes in human AML data from TCGA (P = 0.00018). However, the transcriptome data alone did not robustly distinguish progenitor-derived (MPP, CMP, GMP) leukemias. As lymphoid-primed multipotent progenitors (L-MPP) and GMPs are thought to be the most common cell types-of-origin in human AML, molecularly distinguishing these tumors would represent an advance beyond current knowledge. We utilized ATAC-seq to compare open chromatin regions of bulk leukemia cells from each cell-of-origin to their respective normal cell types. While the overall open chromatin landscape was highly similar between tumors regardless of their cell-of-origin, the highest correlation was observed between HSC and MPP-derived tumors (r = 0.914), followed by CMP-derived tumors (r = 0.906), with GMP-derived tumors being the most distinct (r = 0.862), consistent with the hierarchical relationship between the cells-of-origin. We identified 182 cell-of-origin-specific open chromatin regions in bulk AML cells. As most of these regions overlap with the enhancer priming mark H3K4me1 and/or the enhancer activation mark H3K27ac, we hypothesize that these regions represent enhancer elements that become activated upon transformation in a cell-of-origin-specific manner. Our work suggests that open chromatin patterns may be leveraged as prognostic signatures in human AML to predict tumor cell-of-origin.

T4045

HEMATOPOIETIC REPROGRAMMING OF ADULT SOMATIC TISSUES

Choi, Kyung-Dal, Kyba, Michael, Richard, Jay and Ismailoglu, Ismail, University of Minnesota, Minneapolis, MN, U.S.

Direct reprogramming of somatic tissue to hematopoietic cell fate opens new opportunities to generate isogenic HSC for cell therapy of hematologic disease. In this study, we generated a mouse model with conditional expression of Scl, Lmo2, and Gata2 (SLG). Upon expression of SLG, mouse embryonic fibroblasts (MEFs) initiate expression of CD41 and are able to form various types of myeloid colonies including multi-potent mixed erythroid colonies, with secondary colony-forming potential. To compare reprogramming efficiencies between embryonic and adult somatic tissues, we isolated fibroblasts from various adult tissues (heart, kidney, lung, skeletal muscle, tail tip, and testis). These tissues showed varied efficiencies in reprogramming. Kidney-derived fibroblasts generated erythroid, myeloid and mixed types of hematopoietic colonies. Testis-derived fibroblasts showed myeloid-restricted potential. However, Heart-, Lung-, skeletal muscle- and tail tip-derived fibroblasts did not show significant hematopoietic conversion. To determine whether fibroblasts had been reprogrammed into progenitor cells with *in vivo* differentiation potential, we transplanted reprogrammed cells and observed that they supported short-term and myeloid-restricted engraftment. Taken together, these data suggest that Scl, Lmo2, and Gata2 are sufficient to convert fibroblasts into a hematopoietic but myeloid-restricted cell fate.

Funding Source: This work was supported by Regenerative Medicine Minnesota (MRM2015 PDSCH 001).

T4047

INDUCTION OF BROWN FAT CELLS FROM iPSCs WITH mRNA FOR DRUG DEVELOPMENT

Ni, Yuhui[1], Ji, Chenbo[1,2], Zhao, Yuanyuan[1], Chammas, Andrew[1] and Wang, Jiwu[1], [1]Allele Biotech, San Diego, CA, U.S., [2]Nanjing Medical University, Nanjing, China

iPSCs from donors with diverse background afford unlimited supplies of primary tissue-resembling cells for disease modeling when efficiently differentiated, CRISPR-engineered if so desired, and genetically analyzed. By using mRNA cocktails, we can create footprint-free iPSCs with ease and under GMP-level control. Efficient procedures have also been created to generate sizable populations of neurons, astrocytes, muscle satellite cells, lung epithelia cells, mesenchymal stem cells, and adipocytes. Among these, brown fat cells are of particular interest because of their unique potential in treating diabetes in cell therapy and as a platform for small molecule screening. Utilizing our newly developed differentiation media and mRNA combination, we enabled iPSC differentiation towards white or brown adipocytes. White adipocytes can be created via the iPSC-MSC-adipocyte route, or from iPSC to adipocyte directly. Brown fat cells can be obtained in as short as 4 days with specific factors introduced into iPSCs as in vitro transcribed mRNAs. All cells have been characterized by oil Red-O staining and RT-PCR analysis of adipocyte-specific genes. Insulin sensitivity, mitochondrial function tests, and unfolded protein response (UPR) will be further performed as functional assays. This set of protocols do not involve the use expensive growth factors, hence are at low cost and suitable for large-scale production for high throughput screening of chemicals that are activators in the brown fat pathway. With a whole cell imaging-based kinetic high throughput platform, pilot assays are under way at Allele Biotechnology.

Funding Source: Induction of Brown Fat Cells from iPSCs with mRNA for Drug Development Yuhui Ni[1], Chenbo Ji[1, 2], Yuanyuan Zhao[1], Andrew Chammas[1], Jiwu Wang[1, 2, 3] [1] Allele Biotechnology & Pharmaceutica

T4049

DELINEATING THE TUMOR-HSPC NICHE CROSSTALK: PERTURBED STROMA AND COMPROMISED HSPC FATE

Dhawan, Abhishek[1], Bonin, Malte von[1,2], Bray, Laura[3,4], Freudenberg, Uwe[3], Bejestani, Elham Pishali[2,5], Werner, Carsten[3], Hofbauer, Lorenz C.[1,5], Wobus, Manja[6] and Bornhäuser, Martin[1,5], [1]University Clinic, Dresden, Dresden, Germany, [2]DKFZ, Dresden, Germany, [3]Max Bergmann Center, Dresden, Germany, [4]Faculty of Health, Queensland University of Technology, Brisbane, Australia, [5]DKTK, Dresden, Germany, [6]Medical Clinic and Policlinic I, University Hospital Carl Gustav Carus, Dresden, Germany

The bone marrow microenvironment, including mesenchymal stromal cells (MSC) as major cellular component, regulates self-renewal and differentiation of hematopoietic stem and progenitor cells (HSPC). Due to the stemness properties, the niche might become an unwitting target of neoplastic transformation. Dissemination of breast cancer cells into the marrow has been described during the early onset of primary disease. Employing advanced co-culture assays, proteome studies, organoid and *in vivo* xenotransplant models, we deciphered the pathophysiological relevance of early bone marrow invasion by disseminated tumor cells (DTC) and its consequences on the HSPC niche. The results showed a basic fibroblast growth factor (bFGF)-mediated, synergistic increase in proliferation of breast cancer cells and MSCs in co-culture. The stromal induction was associated with elevated phosphoinositide-3 kinase (PI3K) signaling in the stroma, which coupled with elevated bFGF levels resulted in increased migration of breast cancer cells towards MSCs, along with regulating the activated stroma mediated induction in proliferation of breast cancer cells. The perturbed cytokine profile in the stroma led to reduction in osteogenic differentiation of MSCs via downregulation of platelet-derived growth factor-BB (PDGF-BB). Organotypic co-cultures of breast cancer cells and MSCs further validated PDGF-BB-mediated block in osteoblast commitment of MSCs. Long term co-cultures of breast cancer cells-HSPCs-MSCs and the *in vivo* studies in NSG mice showed reduction in HSPC number and engraftment in the murine bone marrow along with compromised HSPC support in the altered niche. Our findings delineate the network of paracrine interactions pivotal to DTC-HSPC niche dialogue. Treatment paradigms targeting these interactions might offer novel therapeutic avenues for blocking DTC refuge in the HSPC niche.

T4051

HIPPO AND MECHANICAL SIGNALING COOPERATIVELY DETERMINE EPICARDIAL PROGENITOR DIVERSIFICATION

Xiao, Yang[1,2], Zhang, Min[2], Morikawa, Yuka[3] and Martin, James F.[2,3], [1]Texas A&M Health Science Center, Houston, TX, U.S., [2]Baylor College of Medicine, Houston, TX, U.S., [3]Texas Heart Institute, Houston, TX, U.S.

Epicardium is the outmost layer of heart and serves as essential progenitors of non-cardiomyocytes lineage cardiac fibroblasts, smooth muscle cells and to a lesser extent endothelial cells. Although the lineage contribution of epicardium is known, the mechanisms controlling epicardial lineage choices are poorly understood. The Hippo signaling pathway, a conserved kinase cascade linking extracellular cues, such as mechanical tension, to its nuclear activity.We inactivated Lats kinases, core Hippo pathway components, in the Wt1 epicardial lineage which has little contribution to endothelium. Lats mutant epicardium preferentially generated highly proliferative endothelial cells rather than fibroblasts and smooth muscle cells. Genes characteristic of endothelium and genes encoding proteins that promote vasculogenesis and F-actin polymerization were upregulated in Lats mutant epicardium. Super-resolution microscopy and atomic force microscopy indicated that Lats mutant epicardium was stiffer with more intracellular F-actin bundles. Explant studies revealed that epicardial progenitors cultured on stiff matrices acquired an endothelial phenotype independently of Lats while on softer, more physiologic matrices Lats inhibited the endothelial phenotype. Taken together, Lats inhibits Wt1 derived endothelial lineage expansion in cooperation with cytoskeletal state revealing a requirement for mechanical signaling in epicardial diversification.

T4053

MULTIELECTRODE ARRAY PLATFORM TO STUDY LONG-TERM POTENTIATION IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONAL NETWORKS

Biesmans, Steven, Hinckley, Sandy and Bang, Anne, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, U.S.

Numerous neurological conditions including neurodegenerative and psychiatric disorders such as Alzheimer's disease and clinical depression are characterized by synaptic dysfunction and cognitive impairment. Long-term potentiation (LTP) is a persistent strengthening of synapses between neurons that is considered to be one of the major cellular mechanisms underlying memory and learning. Disease modeling and drug discovery approaches would benefit tremendously from human cell-based platforms to study LTP in disease. Human induced pluripotent stem cells (hiPSC) offer such a system in that they circumvent issues of species specificity, are scalable, and can carry complex genetic backgrounds, which is especially important for modeling diseases with high heritability. Here we present a platform to study LTP in neuronal networks comprised of hiPSC-derived neurons. Using either iCell® DopaNeurons or in-house generated hiPSC-derived cortical neurons co-cultured with human primary astrocytes, we developed a procedure that supports neuronal network formation on 48-well multielectrode arrays. Analysis of spontaneous electrical activity shows that an initial phase of primarily single spike firing is followed by synchronized bursting indicating development of functional neuronal networks. At 4 weeks after plating, 84% of the electrodes recorded spontaneous synchronized bursting events wherein most of the neurons in the network spiked together. At this point, the cultures were exposed to forskolin and rolipram to trigger LTP by increasing intracellular cyclic adenosine monophosphate levels, resulting in a significant increase in the number of network bursts that lasted up to 7 days after washing off the drugs. Long-lasting maintenance of LTP requires de novo gene expression and protein synthesis. Ongoing studies include expression analyses of transcription factors and signaling molecules that regulate enduring LTP. In conclusion, we have developed a robust, medium-throughput platform to study LTP in hiPSC-derived neuronal networks. This technology will allow for disease modeling and drug screening of disease-relevant cell types on patient-specific genetic backgrounds.

T4055

SPHEROID CULTURE CONDITION OPTIMALLY EXPANDS ANGIOGENIC CELLS OF ADIPOSE TISSUE-DERIVED STROMAL VASCULAR FRACTION

Takada, Hitomi[1], Kida, Yasuyuki[2] and Kurisaki, Akira[2], [1]National Institute of Advanced Industrial Science and Technology, Japan, [2]National Institute of Advanced Industrial Science &Technology, Tsukuba, Japan

Cell-based therapy using adipose tissue-derived stromal vascular fraction cells (AT-SVF) is a promising therapeutic strategy for the treatment of ischemic disease. AT-SVF is a heterogeneous cell population including adipose-derived stem cells, pericytes, endothelial cells, fibroblasts, and macrophages. Recent studies suggest that culture conditions greatly affect its heterogeneity, which may contribute to the discrepancies in the results of clinical trials. However, there are few comprehensive studies on how culture conditions affect the composition of cultured AT-SVF cells and their therapeutic potentials. Here, we characterized the AT-SVF cells cultured under the 2D adherent conditions and 3D spheroid condition, and compared their angiogenic ability. Quantitative PCR

analysis showed that AT-SVF spheroids expressed greatly elevated levels of the endothelial cell marker CD31, and the pericyte marker Nestin and NG-2 compared to adherent AT-SVF cells. Interestingly, immunocytochemistry revealed that AT-SVF spheroids formed a double-layered structure: CD31 positive cells are localized at the center of spheroids and Nestin positive cells surround them. AT-SVF spheroids have the ability to form capillary like structures on Matrigel in vitro, while adherent AT-SVF cells do not. When AT-SVF spheroids were transplanted into kidney capsule, they formed functional arteriole. These data suggest that the 3D spheroid culture condition optimally expands angiogenic cells from AT-SVF cells, which can be useful for the treatment of ischemic diseases.

T4057

ANALYZING TECHNOLOGY AND INDUSTRIAL DEVELOPMENT TRENDS OF MESENCHYMAL STEM CELLS FROM PATENT POINT OF VIEW

Wang, Yue[1], Xu, LI[2] and Xu, Ping[2], [1]Shanghai Institutes for Biological Sciences,CAS, Shanghai, China, [2]Shanghai Information Center for Life Sciences,CAS, Shanghai, China

Unlike most adult stem cells, the mesenchymal stem cells (MSCs) are widely distributed in various tissues and have multi-directional differentiation capacity. These features make them of great value in development of stem cell therapy and tissue engineering therapy, thus pointing to a promising future. Using intelligence science theory and methodology, this paper describes the analysis of related international patents of MSCs. It is expected to reflect the current situation of technology R&D and industrial development of MSCs, thereby providing a reference point for scientific decisions in relevant fields. The overall analysis of world patent application indicate that technology R&D in this field started in 1980s and rapidly developed after 2000. After nearly 30 years of development, there are currently more than 4,000 MSCs patents (family) in the world. There are 38 countries worldwide carrying out technology R&D in this field, among which the United States, China, South Korea and Japan rank the highest in patents application number. Based on the overall trend analysis, utilizing methods such as 'ThemeScape Map' analysis, and cluster analysis, hotspot fields in MSCs patent applications could be identified, reflecting the focus of future industrial development in this field. Results show that in earlier patent applications, there is little focus on specific disease, with only a few applications filed in development of cartilage disease related therapy; however during the past 10 years, such focus has become increasingly obvious. Except for cartilage disease, MSCs have also played an important role in development of therapies for spinal cord injury, autoimmune arthritis, myocardial infarction, cancer and other diseases. Finally, from view of patent citation, patent family size, and patent litigation, core patents in each hotspot field are found out. Technology evolution models of these core patents are created to sort out development trends in specified technology fields, predict future technology development trends, and provide references for future industry development in related fields.

POSTER SESSION II EVEN

PRE-CLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL CELLS

T1002

THE EFFECT OF ACUTE LUNG INJURY ON THE IMMUNOMODULATORY POTENCY OF BONE-MARROW-DERIVED MESENCHYMAL STEM CELLS

Antebi, Ben, Walker, Kerfoot P, Moahmmadipoor, Arezoo, Choi, Jae-Hyek, Montgomery, Robbie K, Cancio, Leopoldo C and Batchinsky, Andriy I, U.S.ISR, San Antonio, TX, U.S.

Mesenchymal stem cells (MSCs) play a vital role in the regulation of the immune system following injury, and home to the site with unclear immunomodulatory effects. We harvested bone-marrow-derived MSCs (BMSCs) from 3 groups of anesthetized and mechanically ventilated swine (n=3 in each, 1-2 bone marrow sites per animal; n= 5 sites per group): Group A, uninjured; Group B, Acute Respiratory Distress Syndrome (ARDS, induced by smoke inhalation and 40% burn) treated with inhaled epinephrine; and Group C, ARDS but without epinephrine treatment. Comparative evaluation of the 3 groups was performed in MSCs only (P2-P3); and in MSCs co-cultured with fluorescently-stained (CFSE; 5µM) peripheral blood mononuclear cells (pBMNCs) stimulated with PHA (10µg/ml). Cellular assays included cell counts; flow cytometry for surface markers ($CD45^-$, $CD73^+$, $CD90^+$, and $CD105^+$); Milliplex assay (Luminex) for the inflammatory mediators IL-1β, IL-6, IL-8, IL-10, and TNF-α; RT-PCR for damage associated molecular pattern (DAMP) receptor genes TLR-2, TLR-4, and HMGB1 as well as PDGFr2, VEGF, and CD73; and colony forming unit-fibroblasts (CFU-Fs). All experiments were performed in triplicates. Results showed highest expression of MSC surface markers in the injured untreated (C) group followed by injured treated (B) and uninjured (A). Similarly, gene expression was significantly higher for HMGB1, TLR4, and VEGF in MSCs from group C compared to A, while expression of other tested genes was similar between groups. Evaluation of the co-cultures using flow cytometry suggested that MSCs indeed suppressed the proliferation of stimulated pBMNCs; however, no significant differences were found between groups.

Evaluation of inflammatory mediators in the media after 3 days in culture revealed an increased expression of IL-6, IL-8 and IL-1β in the MSCs co-cultured with stimulated pBMNCs when compared to stimulated pBMNCs alone. These results suggest that ARDS has significant effects on the potency of BMSCs, as cells derived from injured subjects may have different immunomodulatory properties and so could be used to target specific conditions, such as ARDS.

Funding Source: The Geneva Foundation

T1004

A STANDARDIZED AND CHARACTERIZED GMP-COMPLIANT HUMAN PLATELET LYSATE FOR EFFICIENT EXPANSION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS

Viau, Sabrina[1], Chabrand, Lucie[1], Bouilliez, Julien[1], **Eap, Sandy**[1], Bertholet, Pauline[1], Bouckenooghe, Thomas[1], Goudaliez, Francis[2] and Delorme, Bruno[1], [1]Macopharma, Mouvaux, France, [2]Macopharma, Tourcoing, France

Human platelet lysate (hPL) is rich in growth factors (GF) and nutritive elements and represents an advantageous xeno-free alternative to fetal bovine serum for mesenchymal stem cell (hMSC) proliferation. However, there is a great variability in hPL sources and production protocols, resulting in discrepancies in product quality, low management of product safety and poor batch-to-batch standardization. We describe here the development and the characterization of a standardized hPL prepared from outdated screened normal human donor platelet concentrates (PCs), manufactured on an industrial scale (batch sizes of 10 L; 240 donors) and under GMP standards (clean room, trained operators and qualified industrial process). PCs were frozen at -80°C and thawed at +4°C to lyse platelets. The number of freeze-thaw cycles was optimized. Cell debris were removed by centrifugation and the supernatant (hPL) was recovered. In order to ensure final aseptic filtration of hPL, we tested 5 different filter sequences and selected a 0.22µm size pore membrane allowing optimal GF recovery (EGF, VEGF, bFGF, TGF-beta1, PDGF-AB and IGF-1). GMP-compliant 10L batches of aseptic filtered hPL were characterized. We showed a robust standardization between batches in terms of reproducible GF contents, biochemical analyses (such as contents of total proteins, fibrinogen, glucose and iron) and efficacy on BM-hMSC proliferation. Finally, we compared expansion and functional characteristics of BM-hMSCs grown in clinical grade hPL *versus* MSC-screened FBS batch. We showed 1) a reproducible increase in cell growth kinetics using hPL, 2) a maintenance of BM-hMSC membrane marker expression and clonogenic potential, 3) a similar adipogenic and osteogenic differentiation potential and finally 4) that immunosuppressive properties of BM-hMSCs (on PHA-activated allogenic mononuclear cells) cultivated in parallel in both conditions remained also identical. In conclusion, we demonstrated the feasibility to use a standardized, efficient and GMP-compliant hPL for research and cell therapy applications.

T1006

FURTHER CONVERGENT OBSERVATIONS ON THE ANTIHYPERALGESIC EFFECTS OF BONE MARROW STROMAL CELLS IN RODENTS

Guo, Wei, Chu, Yu-Xia, Imai, Satoshi, Yang, Jia-Le, Zou, Shiping, Mohammad, Zaid, Wei, Feng, Dubner, Ronald and Ren, Ke, University of Maryland, Baltimore, MD, U.S.

Bone marrow stromal cells (BMSC) have shown potential to treat chronic pain, although much still needs to be learned about their efficacy and mechanisms of action under different pain conditions. Here we provide further evidence on the effect of BMSC in rodent pain models. While women exhibit higher prevalence of orofacial pain than men, it is unclear whether BMSC produce pain relief in females as that in males. In a model of orofacial pain involving injury of a tendon of the masseter muscle, we show in female rats that rat BMSC (1.5 M cells, i.v.) attenuated behavioral hyperalgesia assessed by von Frey filaments and a conditioned place avoidance test. The antihyperalgesia of BMSC in females lasted for 28-56 d, which is shorter than that seen in males. To coincide preclinical findings with clinical conditions, we extended our observation to human BMSC in rats after L5 spinal nerve ligation (SNL). Human BMSC (1.5 M cells, i.v.) attenuated mechanical and thermal hyperalgesia of the hind paw and suppressed SNL-induced aversive behavior, and the effect persisted through the 8-week observation period. In an *ex vivo* trigeminal slice preparation, BMSC treated-animals showed reduced amplitude and frequency of spontaneous miniature excitatory postsynaptic currents. Electrical stimulation-evoked synaptic current was also reduced in BMSC-treated animals. These results suggest inhibition of trigeminal neuronal hyperexcitability and nociceptive transmission by BMSC. Finally, we observed that GluN2A tyrosine phosphorylation and PKCg immunoreactivity in the rostral ventromedial medulla (RVM), a key site for descending pain modulation, was suppressed at 8-week after BMSC in tendon-injured rats. As PKCg activity related to NMDA receptor activation is critical in opioid tolerance, these results help to understand long-term antihyperalgesia by BMSC, which requires opioid receptors in RVM and apparently lacks the development of tolerance. The present work adds convergent evidence to the growing preclinical literature that support the use of BMSC in pain control.

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T1008

MYOCARDIAL INFARCTION RATS MODEL SET UP AND EVALUATE THE CURATIVE EFFECT ABOUT MESENCHYMAL STEM CELLS FROM HUMAN UMBILICAL CORD OF WHARTON'S JELLY

Hsiao, Chen-Yuan, National Defense Medical Center, Taipei City, Taiwan

Myocardial infarction (MI) is a fatal disease that is increasing in incidence. The worst sequelae of MI include myocardial fibrosis and deterioration of pumping function that can lead to irreversible heart failure. We try to set the MI rats model and examine the effect of transplantation Wharton's jelly mesenchymal stem cells (WJ-MSCs). The rats were intubated and ventilated with a mechanical ventilator. A 2-cm para-sternal incision was made over the chest wall. The heart was explored and the LAD was ligated with 8-O Prolene. ST elevation was thought to indicate a successful model MI. Normal saline, undifferentiated human WJ-MSCs (1.6×10^6) or TGF-β2–stimulated human WJ-MSCs (1.6×10^6) were injected directly into the myocardium around the site of ligation. Electrocardiograph, echocardiogram, serum cardiac Troponin I level, Masson's Trichrome staining, immunohistochemistry were used to analyze the therapeutic effects. Cardiac troponin I (c-TnI) was significant increasing in the LAD ligated animals after 6 hour later, indicating that the rats experienced acute myocardial infarction with severe myocardial damage. In addition, the serum c-TnI level was significantly lower in both the MSC and TGF-β2 groups than in controls. Thus, the transplanted MSCs appear to reverse cardiac damage after LAD ligation. Examined the cardiac function by cardiac echography every two weeks. The LV end-systolic dimension and LV end-distolic dimension were also examined to calculate the ejection fraction (EF) and fractional shortening (FS). The EF in the MI group showed 72.6 ± 5% at day 14 after operation and 51.9 ± 6.6% at day 28 after operation. In the sham groups, the EF showed 92.4 ± 0.7% and 91.7 ± 0.9% respectively. In the undifferentiated WJ-MSCs transplanted group, the EF showed 89 ± 1.2% and 88.2 ± 2.3% respectively; and in the transplanted TGF-β2 group, the EF showed 93.5 ± 1% and 94.1 ± 1.2% respectively. Transplantation of either undifferentiated or TGF-β2 stimulated WJ-MSCs improved left ventricular function after MI. The effects were most marked using undifferentiated WJ-MSCs. These results indicate WJ-MSCs as a potential stem cell source for use in myocardial infarct therapy.

Funding Source: This study was partially supported by research grants from Taiwan Association of Cardiovascular Surgery Research

T1010

OSTEOGENIC POTENTIALS OF BONE MORPHOGENETIC PROTEIN 7 GENE OVEREXPRESSED ADIPOSE DERIVED MESENCHYMAL STEM CELL SHEET IN CANINE BONE DEFECT MODEL

Kim, Yongsun[1], Lee, Seung Hoon[1], Kim, Ahyoung[1], Jo, Kwang Rae[1], Yoon, Yongseok[1], Kim, Wan Hee[1], Yun, Hui-suk[2] and **Kweon, Oh-kyeong**[1], [1]Department of Veterinary Surgery, College of Veterinary Medicine, Seoul National University, Seoul, Korea, South, [2]Functional Materials Division, Korea Institute of Materials Science, Changwon, Korea

Successful repair of bone defects injuries are a major issue in reconstructive surgery. Multipotent mesenchymal stem cells (MSCs) have effective potentials of bone regeneration. Bone morphogenetic protein 7 (BMP7) has been shown to possess strong osteoinductive properties. The aim of this study was to investigate the in vitro osteogenic capacity of BMP7 gene overexpressed adipose derived (Ad-) MSCs sheet. In addition, BMP7 overexpressed Ad-MSC sheet (BMP7-CS) transplanted into critical-sized bone defects in dog and assessed their osteogenesis.

BMP7 gene expressing lentivirus particles were transduced into Ad-MSCs. BMP7 overexpressed Ad-MSCs (BMP7-MSCs) were analyzed by quantitative polymerase chain reaction (qPCR) and western blot technique. Ad-MSCs and BMP7-MSCs were cultured in medium containing ascorbic acid phosphate to create a cell sheet. After 10 days of in vitro culture, osteogenic capacities were evaluated by using the alkaline phosphatase (ALP) activity assay and qPCR. The BMP7-CS and BMP7-CS combined with DBM transplanted into critical-size bone defects and wrapped with composite poly ε-caprolactone/β-tricalcium phosphate. The samples were harvested at 12 weeks after transplantation, newly formed bone mass was measured by micro-computed tomography and histopathological analysis was performed.BMP7 mRNA and protein levels of the BMP7-MSCs up-regulated compared to Ad-MSCs. The ALP activity in Ad-MSCs cell sheet and BMP7-CS were significantly higher than Ad-MSCs. The ALP, runt related transcription factor 2, osteopontin, BMP7, transforming growth factor-β, and platelet-derived growth factor mRNA levels of BMP7-CS up-regulated compared to Ad-MSCs and Ad-MSCs cell sheet. In a segmental bone defect model, the amount of newly formed bone and neovascularization were greater in BMP7-CS, combination of BMP7-CS and DBM than in control groups.BMP7-CS reveals higher osteogenic capacities than Ad-MSCs and Ad-MSCs cell sheet. In addition, BMP7-CS stimulated new bone and vessels formation in a canine critical-size bone defect. These results suggest that the BMP7-CS have potential clinical applications in bone regeneration and can

be used as an alternative treatment modality in bone tissue engineering.

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T1012

PRELIMINARY SAFETY OF INTRACRANIAL IMPLANTATION OF MODIFIED BONE MARROW-DERIVED MESENCHYMAL STEM CELLS (SB623) IN ISCHEMIC STROKE PATIENTS WITH CHRONIC MOTOR DEFICITS: A PHASE 1/2A STUDY

Liu, Wenzhong Jerry[1], Steinberg, Gary[2], Kondziolka, Douglas[3], Wechsler, Lawrence[3], Lunsford, L. Dade[3], Kim, Anthony S.[4], Johnson, Jeremiah[2], Bates, Damien[5], McGrogan, Michael[5], Yankee, Ernest W.[5] and Schwartz, Neil E.[2], [1]SanBio, Mountain View, CA, U.S., [2]Stanford School of Medicine, Stanford, CA, U.S., [3]University of Pittsburgh Medical Center, Pittsburgh, PA, U.S., [4]University of California, San Francisco, San Francisco, CA, U.S., [5]SanBio, Inc., Mountain View, CA, U.S

Currently, there is a lack of clinically effective therapies available to improve sustained chronic motor deficits caused by stroke. We evaluated 12-month pooled preliminary safety data from an ongoing two-year, Phase 1/2A, single ascending dose (2.5, 5, and 10 million cells), open label study (NCT01287936) (N=18), in which modified bone marrow-derived mesenchymal stem cells (SB623) were implanted via stereotactic intracranial injection into patients with stable chronic stroke symptoms (6-60 months post-stroke). The study was approved by an institutional review board, and patients provided written informed consent. Treatment emergent adverse events (TEAEs) were experienced by all 18 patients in the safety population. 2 patients were lost to follow-up. There were no dose-limiting toxicities or deaths, and no patients withdrew from the study due to adverse events. The most frequent TEAEs were post-operative headache (77.8%), nausea (33.3%), vomiting (22.2%), depression (22.2%), muscle spasticity (22.2%), fatigue (16.7%), blood glucose increase (16.7%), and C-reactive protein increase (16.7%). No TEAEs were probably or definitely related to SB623. 6 serious adverse events (SAEs) were reported in 6 patients. While all SAEs were assessed as unrelated or unlikely to be related to SB623, 3 SAEs were assessed as being at least possibly related to surgical procedure (pneumonia, seizure, and subdural hematoma). All SAEs resolved without sequelae. After 12 months there were no significant changes or trends in patients' biochemistry or hematology parameters, vital signs, lipids, or cytokines (TNF-α, IL-6, and INF-γ). In addition, no antibody-related sensitization to SB623 cells was observed. Preliminary pooled safety data may indicate that intracranial injection of SB623 cells in chronic stroke patients was generally safe and well tolerated after 12 months.

Funding Source: Funding for this research was provided by SanBio, Inc.

T1014

HUMAN AMNIOTIC EPITHELIAL CELLS IMPROVE ENZYMATIC ACTIVITY AND BEHAVIORAL PHENOTYPE IN MICE WITH ORNITHINE TRANSCARBAMYLASE DEFICIENCY

Parducho, Kevin Murphy R.[1], Garcia, Irving[1], Nguyen, Samantha[1], Ching, McMillan[1], Grubbs, Brendan[1], Srinivasan, Raghuraman[2], Strom, Stephen[2] and Miki, Toshio[1], [1]University of Southern California, Los Angeles, CA, U.S., [2]Karolinska Institutet, Stockholm, Sweden

Ornithine Transcarbamylase (OTC) is an enzyme expressed in the liver and plays a key role in the breakdown of ammonia. OTC deficiency results in hyperammonemia, which can cause severe brain damage and death. Current treatments for OTC deficiency are liver organ or hepatocyte transplantation. However, there is a dire shortage of livers available for such procedures. In contrast, placentae from childbirth are abundant and contain human amniotic epithelial cells (hAECs) that can differentiate into hepatocytes. To determine if hAECs have a therapeutic effect against OTC deficiency, we transplanted hAECs into the liver pulp of wild type (WT) and spf/ash mice and measured the following parameters: engraftment potential, OTC enzymatic activity, levels of urine metabolite markers, and behavioral response to excess ammonia. We present our findings that hAECs can engraft in immunocompetent mouse livers and provide a therapeutic effect. While OTC enzyme activity from untreated spf/ash controls produced 12.75 ± 0.91 μM citrulline/mg protein/h, spf/ash mice treated with hAECs (spf/ash-hAEC) produced increased activity at 19.08 ± 1.33 μM citrulline/mg protein/h ($p < 0.001$). In addition, spf/ash-hAEC mice possessed levels of urine metabolites more closely to levels found in WT controls ($p < 0.05$), confirming the improvement in OTC enzyme activity. Lastly, spf/ash-hAEC mice better tolerate ammonia challenge, exhibiting improved behavioral phenotypes compared to untreated controls ($p < 0.01$). This growing body of evidence better suggests that hAECs can be used for cell replacement therapy to treat OTC deficiency.

Funding Source: This work was supported by California Institute for Regenerative Medicine (CIRM) grant TR3-05488 and CIRM Bridges to Stem Cell Research Intern program (TB1-01176)

T1016

HEPATIC ARTERIAL INFUSION OF CULTURED AUTOLOGOUS BONE MARROW STEM CELLS HAS A STRONGER THERAPEUTIC EFFECT ON LIVER CIRRHOSIS IN OUR ESTABLISHED CANINE MODEL

Takami, Taro[1], Aibe, Yuki[2], Nishimura, Tatsuro[2], Matsuda, Takashi[2], Matsumoto, Toshihiko[2], Fujisawa, Koichi[3], Yamamoto, Naoki[4], Tani, Kenji[5], Taura, Yasuho[5] and Sakaida, , Isao[2,3], [1]Yamaguchi University Graduate School of Medicine, Ube, Japan, [2]Department of Gastroenterology and Hepatology, Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan, [3]Center for Reparative Medicine, Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan, [4]Yamaguchi University/Gastroenterology & Hepatology·Health Administration Center, Ube Yamaguchi, Japan, [5]Clinical Veterinary Science, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yamaguchi, Japan

We developed a liver regeneration therapy for decompensated liver cirrhosis by the peripheral infusion of cultured autologous bone marrow (BM)-derived mesenchymal stem cells (BMSCs). This clinical study was initiated in March 2015 to evaluate safety (ClinicalTrials.gov; No. NCT02327832). Here, to develop liver regeneration therapy with a stronger therapeutic effect, we established a canine liver cirrhosis model, and then evaluated the efficacy and the safety of hepatic artery infusion of cultured autologous BMSCs than those in peripheral infusion. At first, BM fluid was aspirated from the canine humerus and BMSCs were cultured. And a human central venous catheter was inserted into the canine stomach and repeated injection of carbon tetrachloride (CCl_4) was performed for 10 weeks to induce liver cirrhosis. After 10 weeks of CCl_4 injection, BMSCs were infused back into the same subject (4.0×10^5/kg) via a peripheral vein or a hepatic artery using angiography, in addition to continuing CCl_4 injections, serial liver biopsies and indocyanine green (ICG) tests were performed. Liver fibrosis and ICG half-life in the peripheral vein infusion (Vein) group and the hepatic artery infusion (Artery) group were then compared with those in the cell non-infusion (only CCl_4 injections; Control) group. At first, four weeks after the infusion of BMSCs, liver fibrosis had improved significantly more in the Vein group (n=4) than in the Control group (n=4) (Δfibrotic area (%): −1.9 ± 1.5 vs. +1.4 ± 2.0, $p<0.05$). Shortening of ICG half-life (Δhalf-life of ICG (min): −0.6 ± 0.8 vs. +1.2 ± 1.7, $p<0.05$) was also seen. At the same time point, the Artery group (n=3) showed significantly shorter ICG half-life (Δhalf-life of ICG (min): −2.9 ± 1.1 vs. −0.6 ± 0.8, $p<0.05$) than in the Vein group (n=4). Significant therapeutic effects in the Artery group were also maintained even at 8 weeks after the infusion than in the Vein group. Moreover, no hepatic infarction associated with the hepatic arterial infusion was seen on contrast-enhanced computed tomography. Therefore, our findings indicated that the infusion of cultured autologous BMSCs improved liver fibrosis and liver function in our established canine liver cirrhosis model, and that the infusion via the hepatic artery was a more effective route for cell administration.

Funding Source: Highway Program for Realization of Regenerative Medicine, the Japan Agency for Medical Research and Development (AMED).

T1018

PRE-CELL: FINDINGS FROM A LEAD-IN STUDY FOR A PLANNED PHASE 1 TRIAL OF MESENCHYMAL STEM CELLS GENETICALLY MODIFIED TO PRODUCE BDNF FOR HUNTINGTON'S DISEASE

Wheelock, Vicki[1], Tempkin, Teresa[1], Martin, Amanda[1], Lisa, Mooney[1], Swadell, David[1], Scher, Lorin[1], Farias, Sarah[1], Duffy, Alexandra[2], DeCarli, Charles[1], Brunberg, James[1], Yarborough, Mark[1], Li, Chin-Shang[1], Liu, Yu[1], Dayananthan, Ashok[1], Stout, Julie[3], Moscovitch-Lopatin, Miriam[4], Hersch, Steven[4], Fink, Kyle[5], Annett, Geralyn[1] and Nolta, Jan A.[1], [1]University of California Davis School of Medicine, Sacramento, CA, U.S., [2]University of California Davis Medical Center, California, CA, U.S., [3]Monash University, Clayton, Australia, [4]Massachusetts General Hospital, Boston, MA, U.S., [5]University of California, Davis, Sacramento, CA, U.S.

PRE-CELL is a lead-in observational study that seeks to describe baseline characteristics and longitudinal rate of change in multiple clinical, biomarker and structural MRI measures for a cohort of subjects with early-stage Huntington's disease (HD) who will be candidates for a future planned Phase 1 trial of allogeneic mesenchymal stem cells (MSC) engineered to produce brain-derived neurotrophic factor (BDNF) as a proposed therapeutic for neuroprotection in HD. Adult subjects with genetically-confirmed early-stage HD, without dementia, unstable psychiatric symptoms, or contraindications to MRI or neurosurgery were assessed every 6 months for 12 – 24 months. Forty-two subjects were screened and 32 have been enrolled (38% females, mean age 52 years, range, 23 – 74). A linear mixed effects model was used to analyze the repeated measures of selected clinical outcome findings in the cohort. The estimated change rates for the clinical measures include: Unified Huntington's Disease Rating Scale Total Functional Capacity score, 0.8294 per year, (p-value < 0.0001); Independence Score, -6.499 per year, (p-value < 0.0001); Total Motor Score, 8.3362 per year, (p-value < 0.0001); HD-Quality of Life score, 17.5924 per year, (p-value = 0.0034), and Everyday Cognition composite score,

11.4496 per year, (p-value = 0.0015). Rates of change in the HD Cognitive Assessment Battery will be presented. Preliminary biomarker analysis indicates a strong linear relationship between serum and CSF mutant Huntingtin protein levels (correlation of 0.87774, p-value <0.0001). Analysis of serum and CSF BDNF levels will be presented. Structural MRI analysis shows significant rates of volumetric loss in caudate, putamen-region, and white matter volumes at 6, 12, and 18 months. Bioethics interviews of study subjects and care partners are being collected and will be analyzed. The PRE-CELL study has established rates of disease progression for subjects in multiple clinical, imaging and biomarker measures that are statistically significant and sensitive to change over 6 - 18 months. Results will be utilized as a baseline for subjects who enroll in the future planned Phase 1 safety and tolerability trial of MSC/BDNF once regulatory approval has been obtained.

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T1020

THERAPEUTIC FEASIBILITY AND POTENTIAL OF HUMAN UMBILICAL CORD MSCS AND DERIVATIVES FOR REGENERATION OF INTERVERTEBRAL DISC

Beeravolu, Naimisha Reddy[1,2], McKee, Christina[2,3], Perez-Cruet, Mick[2,4] and **Chaudhry, Rasul**[2,3], [1]Naimisha Reddy Beeravolu, Rochester, MI, U.S., [2]Oakland University- William Beaumont Institute for Stem Cell and Regenerative Medicine, Rochester, MI, U.S., [3]Oakland University, Rochester, MI, U.S., [4]Beaumont Hospital, Royal Oak, MI, U.S.

Intervertebral disc (IVD) degeneration is a common spinal disorder and may manifest with low back pain or sciatica. The degeneration is characterized by the loss of extracellular matrix integrity and dehydration in the nucleus pulposus (NP) of the IVD. Currently, there is no treatment to cure this degenerative disc disease (DDD) and most therapies only alleviate pain symptoms. Recently stem cell based therapies have increasing being tested using bone marrow (BM) mesenchymal stem cells (MSCs) and even embryonic stem cells (ESCs). While use of ESCs is controversial due to ethical reasons, BM-MSCs can cause graft verse host disease (GVHD). However, umbilical cord (UC) MSCs can not only be obtained non-invasively but also have fewer HLA mismatches, thus displaying lower risk of GVHD, which make them more promising for therapeutic applications. In this study, we investigated the potential of UC-MSCs and their derivatives to restore the damaged IVD in the rabbit model. An in vivo rabbit model of DDD was created by fluoroscopic guided percutaneous needle puncture of IVD. After 2 weeks, degeneration was confirmed by MRI and then IVDs were injected with PKH26-labeled UC-MSCs or chondrogenic progenitor cell (CPC) derivatives. Eight weeks after implantation of the labeled cells, rabbits (n=20) were sacrificed and IVDs were harvested. Analysis of harvested IVDs showed co-localization of the transplanted cells in the target region, nucleus pulposus (NP), of the IVD as determined by tracking the labeled cells using confocal microscopy. Further analysis of the harvested IVDs using biochemical, immunohistological and molecular techniques showed significant improvement in the histology, cellularity, extracellular matrix proteins, water and glycosaminoglycan contents as well as enhanced expression of NP specific markers such as SOX9, COL2, ACAN, and FOXF1 in the IVDs transplanted with CPCs compared to MSCs. This study demonstrates that UC-MSCs are a promising source and their chondrogenic derivatives are more effective option for treatment of DDD. Results of this study should also provide impetus to explore wider therapeutic use of UC-MSCs.

MESENCHYMAL STEM CELL DIFFERENTIATION

T1022

IP6K1 REGULATES AGEING IN MARROW-DERIVED MESENCHYMAL STEM CELLS BY BALANCING OSTEOGENESIS AND ADIPOGENESIS

Boregowda, Siddaraju V., Ghoshal, Sarabani, Chakraborty, Anutosh and Phinney, Donald G., Scripps Florida, Jupiter, FL, U.S.

The inositol pyrophosphate (IP7) is synthesized from inositol hexakisphosphates (IP6) by a family of three IP6 kinases (IP6Ks) of which IP6K1 is the major isoform. Deletion of IP6K1 protects mice from high fat diet induced obesity and insulin resistance. IP6K1 promotes insulin resistance by inhibiting the insulin sensitizing protein kinase AKT. Moreover, IP6K2 generated IP7 promotes cell death. Bone marrow-derived mesenchymal stem cells differentiate into chondrogenic, osteogenic, or adipogenic lineages and support hematopoiesis by forming a unique bone marrow niche in an age-depended manner. Whereas MSCs undergo chondrogenic and osteogenic differentiation during prenatal and postnatal development, during organismal aging their potential shifts toward adipogenesis. Therefore, the balance between adipogenic vs. osteogenic differentiation provides a measure of aging of MSCs. Based on the role of IP6Ks in regulating cell metabolism and survival we hypothesized that this kinase family may also contribute to stem cell homeostasis. Here we demonstrate that primary mouse MSCs derived from IP6K1 knockout mice display increased growth and survival as evidenced by lower expression levels of mitochondrial reactive oxygen species (ROS) and Trp53

protein. Moreover, IP6K1$^{-/-}$ MSCs exhibit enhanced osteogenic differentiation and reduced adipogenic potential. To identify IP6K1-interacting proteins responsible for these effects, we employed immune-precipitation coupled with mass spectrometry. This analysis identified adipocyte enhancer-binding protein 1 (AEBP1) as a major IP6K1-interactor in MSCs. Levels of AEBP1, a transcriptional regulator that enhances adipocyte proliferation and inhibits differentiation, were dramatically increased in IP6K1$^{-/-}$ MSCs. These studies identify IP6K1 as a novel regulator of MSC ageing and suggest that targeting the IP6K1-AEBP1 signaling network may offer new strategies to ameliorate age-related diseases including osteoporosis.

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T1024

TRANS-DIFFERENTIATION OF HUMAN WHARTON JELLY DERIVED MESENCHYMAL STEM CELLS INTO NEUROECTODERMAL CELLS

Equbal, Zaffar, Srivastava, Madhulika and Mukhopadhyay, Asok, National Institute of Immunology, New Delhi, India

Owing to the constrains associated with the isolation and long term maintenance of human neuroectodermal cells, scientists have started investigating for the alternate source of cells that can be used to generate neuroectodermal cells. Of all the other possible adult source cells, mesenchymal Stem Cells (MSCs) are found to be of great hope because of hypoimmunogenic, immunomodulatory, and transdifferentiation property. Here we have successfully generated neuroectodermal cells from Human Wharton jelly derived MSCs. These MSCs were extensively characterized based on its morphology, various gene expression profiles (RT-PCR, Immunocytochemistry, FACS and Western blot) and their multi lineage differentiation potential. These cells were found to express ACTA2, vimentin and fibronectin 1, the common genes associated with mesenchymal lineage. These cells also express SOX2 and POU5F1, the pluripotency genes. Apart from CD73, CD90, CD105 and CD146, they express SSEA-4. Unlike BM-MSCs, these cells were negative for CD271. As canonical Wnt is highly active in these MSCs, treatment with FGF2 and EGF laone led to the formation of neuro-spheres like 3-dimentional bodies of sizes ranging from 200 to 600 micron. Further analysis of these cells confirmed drastic decrease in the expression of MSCs related genes with concomitant rise in the expression of SOX1, the important transcription factor involved in neural fate determination. Expression of PAX6, musashi RNA binding protein 1 and nestin was also increased manifold in these differentiated cells as compared with MSCs. We also observed that these spheroid bodies express neural receptor NTRK1 and NTRK3 along with neuronal transcription factor like Neurod1, Neurod3 and Neurog2. The expression of SOX1, SOX2, PAX6, Nestin & Musashi were also confirmed by Immunocytochemistry.

Funding Source: Department of Biotechnology, Ministry of Science and Technology, India.

T1026

HOMING OF FRESHLY ISOLATED MESENCHYMAL STEM CELLS IN THE CORNEA.

Inagaki, Emi[1,2], Ogawa, Yoko[1], Matsuzaki, Yumi[3], Sato, Yukio[2], Okano, Hideyuki[2], Tsubota, Kazuo[1] and Shimmura, Shigeto[1], [1]Keio University School of Medicine, Tokyo, Japan, [2]Department of Physiology, Keio University, School of Medicine, Tokyo, Japan, [3]Shimane University, Faculty of Medicine, Izumo, Japan

Mesenchymal stem cells (MSCs) are considered a useful source for various cellular therapies.Since most clinical protocols are based on intravenous injection of MSCs, it is important to understand the homing mechanisms and differentiation in tissues. The purpose of this study was to test the hypothesis that bone marrow-derived mesenchymal stem cells home into cornea, and if so, to study the characterization of these cells.MSCs were isolated from green fluorescent protein (GFP) transgenic mice based on our previous protocol for prospectively isolating MSCs based on the expression of PDGFRα and SCA-1 (PαS MSCs) (Morikawa et al, JEM 2005). Briefly, crushed bones were chopped and incubated for 1 h at 37 °C in 20 ml of DMEM containing 0.2% (wt/vol) collagenase. The cell suspension was filtered after red blood cell lysis and incubated with antibodies against PDGFR-α, Sca-1, CD45 and Ter119. Appropriate gates are constructed on a cell sorter to exclude dead cells and lineage (CD45(+)Ter-119(+))-positive cells. Approximately 10,000 PαS MSCs were isolated per mouse. MSCs were then transplanted intravenously in WT radiated mice. Weekly examination of the cornea through fluorescent microscopy was performed. GFP labelled PαS MSCs emerged in bilateral corneas 4 weeks after intravenous transplantation. GFP+ cells diminished dramatically towards 5 weeks. We characterized the phenotype of these cells in the cornea and lacrimal gland by immunohistochemistry. Transplanted CD45(-)Ter119(-)Sca1(+) PDGFRα(+) did not express keratocan, a corneal stromal specific marker, as reported in previous studies. However, these cells turned into CD45 (+), CD68 (+), type I collagen (+) and F4/80(+) cells.We successfully visualizing BM-MSCs homing into the cornea. BM-MSCs underwent a phenotype change in cornea and lacriminal gland. Our findings may increase our understandings of the roles of BM-MSCs in peripheral organs.

T1028

OXIDATIVE STRESS SUPPRESSION OF OSTEOGENIC DIFFERENTIATION CAPACITY IN MESENCHYMAL STEM CELLS INVOLVES SIRT1 DEACETYLASE

Lin, Chia-Hua, Li, Nan-Ting, Cheng, Hui-shan and Yen, Men-Luh, Department of Obstetrics/Gynecology, National Taiwan University (Hospital & College of Medicine, National Taiwan University Taipei, Taiwan

With rapidly aging populations worldwide, the incidence of osteoporosis has reached epidemic proportions. A disease of bone fragility, osteoporosis results from an imbalance in the activities of osteoblasts, the bone-forming cells, and osteoclasts, the bone-resorbing cells. Current research has been focused on the osteoblast for discovery of anabolic treatments, which can increase bone formation more significantly than suppressing osteoclastic activity. The progenitor of the osteoblast is the mesenchymal stem cells (MSCs) and was first discovered in the bone marrow. Increasingly, MSC aging is thought to contribute to declining osteoblastic numbers and increasing marrow adiposity. A possible mediator of this process includes reactive oxygen species (ROS), a by-product of oxidative stress and aging. However, ROS effects on MSC differentiation capacity and mechanisms involved have been inconsistently reported. We found that H_2O_2, an ROS, induced MSC lineage commitment toward adipogenesis and away from osteogenesis, increasing PPARγ2-driven but decreased RUNX2-driven transcription activity. In addition, H_2O_2 decreased the activities of SIRT1, a histone deacteylase and longevity gene. In MSCs, we have previously shown that SIRT1 is a co-modulator of RUNX2, and we now found that silencing of SIRT1 activity in MSCs enhanced adipogenic capacity at the expense of osteogenic capacity. Moreover, SIRT1 regulation of RUNX2-driven transcriptional activity is through deacetylation of the ROS-sensitive transcription factor FOXO3a. Taken together, our data implicates that ROS modulates osteogenic capacity of MSCs through the deacetylase activity of SIRT1. We hope our findings can help delineate the molecular mechanisms involved in ROS/age-related perturbations during MSC osteogenesis and lead to discovery of druggable anabolic targets for use towards osteoporosis.

T1030

HUMAN SKIN STEM CELL-DERIVED HEPATIC CELLS FOR IN VITRO TESTING OF HEPATOTOXICITY

Rodrigues, Robim M[1], De Kock, Joery[1], Rogiers, Vera[1] and Vanhaecke, Tamara[2], [1]Vrije Universiteit Brussel, B-1090 Brussels, Belgium, [2]Vrije Universiteit Brussel, Brussels, Belgium

Hepatic toxicity induced by pharmaceutical compounds or drug-induced liver injury (DILI) is one of the major causes of acute liver failure. Yet, no adequate human hepatic screening method exists for safety evaluation of compounds that induce hepatic injury. The current models are based on scarcely available primary hepatocytes or cell lines derived from cancerous liver tissue. New developments in stem cell research might create new possibilities as stem cells represent virtually inexhaustible cell sources that have the ability to differentiate into multiple cell types, including hepatic cells. In this study, we evaluate post natal human skin-derived precursors (hSKP), and more specific their hepatic progeny (hSKP-HPC), as an alternative cell model for screening hepatotoxic compounds. Using a toxicogenomics approach we observed hSKP-HPC respond in a similar way as primary human hepatocytes in culture when exposed to several drugs (acetaminophen, valproic acid, tetracycline, amiodarone,...) known to cause hepatotoxicity. Furthermore, exposure to steatotic and phospholipidotic compounds lead to the intracellular accumulation of microvesicular lipid droplets, typical of these disorders. A quantitative method has been developed to quantify the degree of induced steatosis in hSKP-HPC. Based on the comparison to liver samples of patients suffering from DILI, we could in addition observe that the predictive capacity of hSKP-HPC was as good as that of primary human hepatocytes and substantially better than the DILI predictive capacity of HepG2 cells. In conclusion, our data gives a first indication that hSKP-HPC could represent a suitable human-relevant preclinical model for in vitro testing of hepatotoxic compounds, mitigating the necessity of using primary human hepatocytes.

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T1032

INFUSED BONE MARROW DERIVED CELLS HAVE TWO CAPABILITIES THAT REPAIR OF FIBROSIS AND PHAGOCYTOSIS OF DAMAGED CELLS

Yamamoto, Naoki[1,2], Takami, Taro[3], Matsumoto Toshihiko[2], Fujisawa Koichi[2,4], Uchida Koichi[2,5], Terai, Shuji[2,6], Nishina Hiroshi[7] and Sakaida Isao[2,4], [1]Yamaguchi University/Gastroenterology & Hepatology·Health Administration Center, Ube Yamaguchi, Japan, [2]Yamaguchi University Graduate School of Medicine, Yamaguchi, Japan, [3]Yamaguchi University Graduate School of Medicine, Ube, Japan, [4]Yamaguchi University Graduate School of Medicine, Ube, Japan, [5]Yamaguchi Prefectural University, Yamaguchi, Japan, [6]Niigata University, Graduated School of Medical and Dental Sciences, Niigata, Japan, [7]Tokyo Medical and Dental University, Tokyo, Japan

Recently, Autologous bone marrow cells were useful for the repair therapy in liver cirrhosis and many kind of diseases. We developed the GFP/CCl4 model which monitor the GFP-positive bone marrow cell (BMC) repopulated under liver cirrhosis mice (Hepatology 2004). In this study, we estimated characterization of infused BMC in liver cirrhosis using Electron Microscopy (EM) and the effect of environment in recipient liver. C57BL/6 mice were injected with CCl4 twice a week for 4 weeks to make the liver cirrhosis. GFP-positive BMC were infused from tail vein and sacrificed at 4 weeks after BMC infusion. The liver sample was fixed using both paraformaldehyde+glutaraldehyde and made epon section. We analyzed the characterization of the infused GFP-positive BMC using both EM and Immune EM (IEM). We analyzed the image of IEM, comparing with the character of positive cells by immunohistochemistry and double fluorescent staining(Antibody:GFP,MMP9, hepatoblast-marker-Liv2,Liv8-CD44,A-6,EpCAM,CXCR4,transcription regulator-maternal of inhibitor of differentiation -Maid). We analyzed some kind of gene by Real-Time PCR(-Gene:p16,p21,Sirt1,6,AK4,Hmox,Ncam,SMP30 etc) and DNA array. We found two kinds of GFP positive BMCs in recipient cirrhosis liver using IEM method. One group of GFP positive BMCs was similar to hepatocyte in size(15-30um) and located around fiber. MMP9 positive cells, Maid positive cells, CXCR4 positive cells,Liv8 positive cells were same.These cells were round forms and different from Kupfpfer cell or stellate cell in feature and had the increase of lysosome structure in cytoplasm. These cells were located on fiber in hepatic cord and repaired fibrosis. The other group cells were small size (2-5um) and located in destructive area and A6 positive cells,Liv2 positive cells,EpCAM positive cells were same. These cells were circular forms and had high N/C ratio and smaller than hepatocyte. These cells migrated into damaged cell area and had the phagocytic capacity. These cells were few F4/80 positive cells and smaller than Kupffper cell in size. We detected two kind of infused BMCs.The round BMCs repaired liver fibrosis and the small BMCs worked the phagocytized damaged hepatocyte and maintenance of liver.

MESENCHYMAL CELL LINEAGE ANALYSIS

T1036

INTRINSIC VARIABILITY IN THE IMMUNOMODULATORY POTENTIAL OF HUMAN NEONATAL WHARTON'S JELLY MESENCHYMAL STEM CELLS

Paladino, Fernanda Vieira, Mozetic, Isis, Sardinha, Luiz Roberto and Goldberg, Anna Carla, Hospital Israelita Albert Einstein, São Paulo, Brazil

Wharton's jelly mesenchymal stem cells (WJ-MSC) exhibit immunomodulatory effects on T cell responses. WJ-MSC are easy to process and proliferate rapidly in culture, but information on the variability of individual cell samples impacting upon in vitro expansion, immunomodulatory potential and aging processes is still needed. We evaluated the production profile of immunomodulatory molecules comparing WJ-MSC either undifferentiated or undergoing replicative senescence to analyze if proper responses by WJ-MSC are affected. WJ-MSC were cultured and samples were collected at an early stage (passage 5), at an intermediate stage (passage 15), and in replicative senescence (passage generally between 20 and 25). Gene expression by real-time PCR and secretion by flow cytometry (CBA) of IL-1α, IL-1β, IL-6, IL-8, IL-10, IL-11, and TGF-β1 were measured, in addition to IDO, HGF, and LIF gene expression (n=4), and to measure suppression of T cell proliferation (flow cytometry) samples from the same two peripheral blood mononuclear cells (PBMC), obtained from healthy platelet donors, were stimulated with phytohaemagglutinin (PHA) for 72 hours and tested against 3 different WJ-MSC. All genes were detected except IL-10. Expression of IL-1β, IL-6, and IL-8 were increased in passage 15 but decreased as cells reached senescence. As for secretion, only IL-6, IL-8, and IL-11 were detected. Surprisingly, senescence led to diminished secretion of both IL-6 and IL-8. IDO and LIF gene expression varied among samples with no specific pattern. In co-culture all three WJ-MSC were capable of inhibiting PHA mitogen-activated CD3+ T cell proliferation, albeit with different strengths and the extension of inhibition of the proliferative response differed markedly between the two PBMC. Moreover, in spite of similar CD4 and CD8 T cell counts in both PBMC, WJ-MSC did not inhibit CD4+ and CD8+ T cell subpopulations equally. Our results show that

neonatal, environment-protected WJ-MSC display different basal patterns of cytokine mRNA expression and protein secretion. In addition, T cell responses also presented different profiles according to the donor. Taken together, our data indicate that the therapeutic use of these cells may be impacted by the intrinsic variability present in donors (WJ-MSC) and recipients (lymphocytes).

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HEMATOPOIETIC CELLS

T1040

ENGINEERING HUMAN PLURIPOTENT STEM CELL-DERIVED NATURAL KILLER CELLS TO EXPRESS CLEAVAGE-RESISTANT CD16A FOR ENHANCED ANTI-TUMOR KILLING

Blum, Robert[1], Wu, Jianming[2], Walcheck, Bruce[2] and Kaufman, Dan S.[3], [1]University of California, San Diego, La Jolla, CA, U.S., [2]University of Minnesota, St. Paul, MN, U.S., [3]University of California, San Diego, La Jolla, CA, U.S.

CD16a is an IgG Fc receptor expressed by human natural killer (NK) cells. This receptor binds antibodies attached to target cells to initiate antibody-dependent cell-mediated cytotoxicity (ADCC), a key effector mechanism of NK cell-mediated killing of diverse tumor cells. CD16a undergoes a rapid down-regulation in expression by ADAM17 (A Disintegrin And Metalloprotease-17) mediated proteolytic cleavage upon cell activation. This loss of CD16a expression leads to reduced ADCC activity and decreased anti-tumor killing. Previous studies by our group identified a cleavage site in CD16a that are targeted by ADAM17 activity. Substitution of the serine at position 197 in the middle of the cleavage region for a proline (S197P) effectively blocks CD16a cleavage. Previous studies by our group also demonstrate efficient NK cell production from human pluripotent stem cells. We have developed a two-stage culture system to efficiently produce NK cells from human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs). These hESC and iPSC-derived NK cells have phenotype and function similar to NK cells isolated from peripheral blood. We have now used the *Sleeping Beauty* transposon system to stably express the cleavage-resistant CD16a(S197P) in iPSCs. NK cells derived from these iPSCs maintain expression of CD16a(S197P) without evidence of silencing. We demonstrate that the CD16a (S197P) is resistant to shedding upon NK cell activation. Additionally, we show that ADCC is enhanced in the engineered CD16a (S197P)-expressing hESC/iPSC-derived NK cells compared to NK cells from either peripheral blood or non-engineered iPSCs. Specifically, ADCC is tested by addition of an anti-CD20 antibody (rituximab) to NK cells targeting CD20-expressing Raji B cell leukemia cells. This cellular engineering of human pluripotent stem cell-derived lymphocytes provides an important strategic approach for cellular immunotherapies against refractory malignancies and chronic viral infections. Rather than requiring NK cells or other effector populations to be isolated from individual donors, we aim to produce NK cells from pluripotent stem cells to provide an unlimited source of lymphocytes as a standardized "off-the-shelf" immunotherapy product.

T1042

LOSS OF β7 INTEGRIN ALTERS ADHESION MOLECULE EXPRESSION AND PROMOTES HSC SELF-RENEWAL

Murakami, Jodi L[1,2], Kumar, Bijender[1,2], Garcia, Mayra[1,2] and **Chen, Ching-Cheng**[1,2], [1]Gehr Family Center for Leukemia Research at City of Hope, Duarte, CA, U.S., [2]Beckman Research Institute of City of Hope, Duarte, CA, U.S.

We recently established the importance of β7 integrin in HSC homing and subsequent engraftment after transplantation. However, it remains unclear if β7 integrin plays a role in HSC homeostasis. To investigate this, we conducted studies using a mouse model that lacks β7 integrin (β7KO) and we found that β7KO mice had significantly higher frequency and absolute number of HSCs in the BM at steady state compared to wildtype (WT) mice. Interestingly, we observed a faster hematopoietic recovery after 5-FU challenge in β7KO mice that resulted in enhanced survival, suggesting that β7KO HSCs may have enhanced stem cell functions. While we observed no significant difference in the cell cycle status of β7KO HSCs compared with WT HSCs using Ki67 and DAPI, single cell division assay revealed that β7KO HSCs have a longer mean time to first division in comparison to WT HSCs upon mitogenic stimulation, suggesting that β7KO HSCs may be in a more primitive state. Indeed, lower ROS and higher pimo staining confirmed that β7KO HSCs are in a more primitive state. RNAseq analysis and single cell qPCR revealed that adhesion molecule expression was altered and expression of primitive HSC markers was upregulated in β7KO HSCs. Moreover, immunofluorescent staining of the cell-fate determinant and polarity factor Numb showed that β7KO HSCs underwent significantly more symmetric self-renewal divisions and significantly fewer symmetric differentiation divisions than did WT HSCs. Together, these data strongly suggest that β7 integrin regulates HSC cell fate decisions by promoting self-renewal potential and suppressing differentiation commitment of HSCs.

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T1044

FUNCTIONAL AND MOLECULAR CHARACTERIZATION OF THE HUMAN ENDOTHELIAL-TO-HEMATOPOIETIC TRANSITION

Guibentif, Carolina[1], Ronn, Roger[1], Lang, Stefan[1], Böiers, Charlotta[1], Saxena, Shobhit[1], Soneji, Shamit[1], Enver, Tariq[2], Karlsson, Goran[1] and Woods, Niels-Bjarne[1], [1]Lund University, Lund, Sweden, [2]University College London, London, U.K.

Accumulating evidence suggests that hematopoietic development originates from an endothelial-to-hematopoietic transition (EHT) event during development. To molecularly dissect EHT, we coupled flow cytometry and single-cell qRT-PCR to analyze CD34$^+$ cells derived from human pluripotent stem cells. Gene expression analysis of 90 transcription factors, surface receptors and other relevant genes for endothelium as well as for hematopoietic stem/progenitor cell identity and regulation enabled the identification of distinct populations spanning a developmental program from endothelium to hematopoietic cells. The endothelial and hematopoietic groups displayed CD43$^-$ and CD43$^+$ surface expression, respectively. Interestingly, at the interface of these two lineages, a distinct subset of cells displayed dual endothelial/hematopoietic expression characteristics, with over 20 genes otherwise exclusively expressed in either the endothelial or hematopoietic groups, and showed intermediate CD43 expression. While overall retaining an endothelial signature, this putative EHT population displayed increased expression specifically of *SPI1*, *RUNX1*, *LIN28B*, *MEIS1*, *FLI1*, *ETV6*, and *VAV1* compared to the other endothelial groups. This EHT population's phenotype and transcriptional state were distinct and downstream of the previously described hemogenic endothelium (CD43$^-$CD34$^+$CD90$^+$CXCR4$^-$CD73$^-$) and upstream of the earliest hematopoietic progenitors (CD43$^+$CD34$^+$CD90$^+$). FACS sorting and sub-culturing the EHT population, we demonstrate a rapid and high conversion rate from an adherent endothelial morphology to a round CD43$^+$ cells expressing a hematopoietic specific WAS-GFP reporter. Further analysis revealed that this EHT cell population could be subdivided into 3 distinct transcriptional stages displaying a moderate but progressive downregulation of the endothelial genes, whilst also displaying upregulation of hematopoietic genes. This clearly indicates the stepwise establishment of a hematopoietic program within the EHT population. Thus, using single-cell analyses in conjunction with Index-Sorting, we show for the first time a molecular signature of cells undergoing EHT within a developmental hierarchy, demonstrating a distinct transcriptional profile and cellular function.

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T1046

CONFETTI REPORTER SYSTEMS TO EXAMINE THE CLONALITY OF HEMATOPOIETIC CLUSTERS LINING VESSELS

Hildebrand, Leanne Demers, California Institute for Regenerative Medicine, San Francisco, CA, U.S.

During embryogenesis, the precursors (pre) of hematopoietic stem cells (HSCs) emerge in one of two hematopoiesis waves called primitive (p) and definitive (d). The primitive hematopoietic wave occurs within the yolk sac to supply nucleated red blood cells to the embryo, and these yolk sac pHSCs are thought to be incapable of generating the HSCs found in adult bone marrow. The preHSCs that become HSCs found during adulthood are likely generated during the definitive wave. These dHSCs are largely produced within the dorsal aortic area of the aorta-gonad-mesonephros (AGM) region of the embryo, and form clusters of hematopoietic cells on the lumenal surface of the aorta. Our preliminary research has shown the yolk sac produces dHSCs, which circulate within the embryo body and seed other hematopoietic sites such as the fetal liver. In this study, we aim to investigate if yolk sac derived cells migrate and attach to the wall of the dorsal aorta to produce or contribute to the hematopoietic clusters observed there. To answer this question, we are using the Confetti reporter strain, which upon expression of the activating enzyme Cre, will randomly recombine to activate expression of either GFP, CFP, OFP or RFP, allowing us the ability to track the progeny of individual cells or "clones". By crossing the Confetti reporter line with two separate Cre/Lox systems with unique endothelial and hematopoietic expression patterns, we can observe the "clonality" of hematopoietic clusters that line the vasculature. If the clusters are produced by their underlying endothelium, they will have color patterns identical to their underlying endothelial cells. However, if the clusters are produced elsewhere and attach to the vessel wall during circulation, they will have different color patterns than the endothelial cells they are attached to. Our data suggest a diversity of hematopoietic clusters exists on the vascular surfaces of the yolk sac, dorsal aorta, and vitelline vessels.

Funding Source: CIRM

T1048

HUMANIZED ANIMAL MODEL FOR STUDYING THE EFFICACY OF VACCINE CANDIDATES AGAINST TULAREMIA

Kim, C-Yoon, Konkuk University, Seoul, Korea, South

Francisella tuarensis (F. tularensis) is a highly infectious pathogen and considered as a potential biological weap-

on due to no vaccine for human currently. Tul4 and FopA, which are outer membrane proteins of F. tularensis, play an important role and immunogenicity. In the present study, we evaluated the humoral immune response by cocktail of recombinant Tul4 and FopA (rTul4 and rFopA), which were codon-optimized and expressed in Escherichia coli. Moreover, NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice with human CD34+ cells (hu-mice) were applied to evaluation of human like immune response. Vaccination of hu-mice with cocktail of rTul4 and rFopA elicited significant human immunoglobulin response (total IgM, IgG and Tul4-specific IgM, IgG and FopA specific IgM) and total and spe. Furthermore, vaccinated hu-mice exhibited the prolonged survival against an attenuated live vaccine strain. These results suggest that cocktail of rTul4 and rFopA induces humoral immune response in hu-mice, in which demonstrates potential as a vaccine candidate for human use.

Funding Source: Research supports were provided by the Research Institute of Veterinary Science, and the BK21 Program for Veterinary Science, College of Veterinary Medicine, Seoul National University.

T1050

HEMATOPOIETIC STEM CELLS DEPEND ON AAT1-MEDIATED AMINO ACID HOMEOSTASIS

Li, Zhenrui[1], Qian, Pengxu[1], Zhao, Meng[1], Perry, John[1], He, Xi (CiCi)[1], TAO, Fang[2], Paulson, Ariel[1], Semerad, Craig L[3], Haug, Jeffrey S.[4] and Li, Linheng[1], [1]Stowers Institute for Medical Research, Kansas City, MO, U.S., [2]Stowers Institute for Medical Research, Kansas City, MO, U.S., [3]Stowers Institute for Medical Research, Kansas City, MO, U.S., [4]Stowers Institute, Kansas City, MO, U.S.

Specific amino acid metabolism has been shown to regulate the pluripotent state of mouse embryonic stem cells; however, whether specific amino acid transporters (AATs) are involved in stem cell maintenance is unknown. Here, using RNA sequencing to screen the gene expression profiles of 16 different hematopoietic cells including hematopoietic stem, progenitor cells, and mature lineage cells, among 45 AATs tested, we identified amino acid transporter 1 (AAT1) as a hematopoietic stem cell (HSC) specific AAT. Inhibition of AAT1 led to decreased HSC number and functionality, suggesting the amino acid (AA) substrates of AAT1 might be required for HSC maintenance. Indeed, individual deprivation of each essential AA (EAA) showed that withdrawal of Valine (Val), Methionine (Met), or Threonine (Thr), which are substrates of AAT1, caused a > 90% decline of HSCs. Furthermore, loss of AAT1 function impaired the capability of Val and Met to maintain HSCs. Mechanistically, AAT1 inhibition elicited the AA starvation response pathway, resulting in the phosphorylation of eIF2α (p-eIF2α) and inhibition of eIF2α-dependent general translation. p-eIF2α in turn induced translation of c-Myc, which caused HSC activation, differentiation, and apoptosis. Loss of c-Myc expression rescued the Val, Met, and Thr deprivation-mediated HSC reduction in ex vivo culture. Thus, HSC maintenance depends on AAT1, operating as a sensor and transporter of specific AAs required by HSCs.

T1052

IPSC BASED ADOPTIVE IMMUNOTHERAPY IN COMBINATION WITH GENOME EDITING STRATEGY

Minagawa, Atsutaka[1,2], Hotta, Akitsu[2], Uemura, Yasushi[3], Nakatsura, Tetsuya[3] and Kaneko, Shinn[1,2], [1]Kyoto University, Kyoto, Japan, [2]Department of Life Science Frontier, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [3]National Cancer Center, Kashiwa, Japan

We have reported that differentiating iPS cells established from antigen specific monoclonal CD8 T cells (T-iPSCs) enables us to produce large amount of young CD8 T cells that are comparable to original CD8 T cells in antigen specificity. This technology enables us to establish iPSC based adoptive cancer immunotherapy. However it turned out that loss of antigen specificity occurs during optimized differentiation method into functional CD8 T cells. As a solution for this , we have developed Recombination Activating gene2 (RAG2) knock-out strategy by using CRISPER Cas9 system.To show the value of this straategy We established Glypican-3 (liver cancer expressing antigen) specific TCR having T-iPSCs and knocked out RAG2 gene. By 2weeks of coculture with OP9 DLL1 mouse stroma cells we differentiated these cells into CD8 T cells. Analysis of TCRαβsequence of these T cells showed the positive effect of RAG2 KO for preserving Glypican-3 specificity. We also confirmed the specific killing function of differentiated RAG2 KO CD8 cells against Glypican-3 expressing tumor in vitro and in vivo.

We have succeeded in making safer and more practical iPS cells for clinical application by using genome editing strategy. This strategy would enables us to supply safe and effective cells for adoptive cancer immunotherapy in the future.

T1054

NOTCH SIGNALING MAINTAINS SURVIVAL AND ENABLES MATURATION OF FETAL HEMATOPOIETIC STEM CELLS

Pajcini, Kostandin V.[1], Gerhardt, Dawson[2], Xu, Lanwei[2], Jain, Rajan[2], Epstein, Jonathan[2], Speck, Nancy[2], Bigas, Anna[3] and Pear, Warren S.[2], [1]University of Illinois at Chicago, Chicago , IL, U.S., [2]University of Pennsylvania, Philadelphia, PA, U.S., [3]IMIM-Hospital Del Mar, Barcelona, Spain

Notch1 contains an evolutionarily conserved transcriptional activation domain (TAD) that is required for optimal Notch1 signaling in vitro and for Notch1 induced T-ALL. To determine its role in development, we generated transgenic knock-in mice lacking the Notch1 TAD (ΔTAD/ΔTAD). Unlike Notch1 null mice, ΔTAD/ΔTAD mice survive past E9.5, but die in late gestation due to multiple cardiac abnormalities. Although Notch1 is required to generate the earliest embryonic hematopoietic stem cell (HSC) at embryonic day 9.5 (E9.5) in the aorta-gonad-mesonephros, the lethality of Notch1 null mice between E9.5-10.5 has made it difficult to study the function of Notch1 at later times of fetal development. We now show that ΔTAD/ΔTAD fetal liver HSCs, as defined by the LSK and SLAM phenotypes, are markedly decreased compared to wild type fetal liver HSCs. HSCs purified from the fetal liver of ΔTAD/ΔTAD embryos are also impaired in their ability to reconstitute the hematopoietic compartment in competitive bone marrow transplants, suggesting a cell intrinsic defect of fetal HSCs with hypomorphic Notch signaling. The impairment in Notch HSC function by the ΔTAD/ΔTAD was confirmed in Vav-Cre x RBPJ$^{f/f}$ conditional mice. Mechanistically we determined that Notch targets Hes1 and the Notch ligand Jagged1 are essential components of Notch signaling in the fetal liver. Furthermore, we show that the intracellular Notch1-ΔTAD protein fails to efficiently form the core transcriptional complex due to its decreased binding of MAML. Together, these studies reveal an essential role of the Notch1-TAD in embryonic development and identify important functions for Notch1 signaling in fetal HSC function.

T1056

HUMAN AMNIOTIC FLUID STEM CELLS: A NOVEL FETAL HAEMATOPOIETIC STEM CELL SOURCE WITH POTENTIAL FOR THERAPY

Ramachandra, Durrgah L, Loukogeorgakis, Stavros P, Antoniadou, Eleni, Manson, Ania, Shangaris, Panicos, Tedeschi, Alfonso, Subramaniam, Sindhu, Carmo, Marlene, Blundell, Mike, Howe, Steven, L David, Anna and De Coppi, Paolo, University College London, London, U.K.

The demand for haematopoietic stem cells (HSC) in clinical applications is increasing. Amniotic fluid stem cells (AFSC) serve as a potential alternative cell source for therapy. The significant haematopoietic activity of murine AFSC led us to explore the potential of human CD117/c-Kit+ AFSC to reconstitute the haematopoietic system in vivo. Human AFSC (2nd and 3rd trimester) and cord blood HSC (CB-HSC; control) were selected for CD117 and CD34 respectively flow-cytometry. Sorted cells (10^4 in 200µl PBS) were injected intravenously into sub-lethally irradiated NOD-SCID/IL2rγ^{null} (NSG) mice (n=6/group). Haematopoietic engraftment of human cells (% of human CD45$^+$ within total CD45$^+$) and multi-lineage reconstitution (erythroid, myeloid and lymphoid) were assessed at 16 weeks in blood, bone marrow (BM) and spleen. BM mononuclear cells (MNC) from mice engrafted with human cells were used in secondary transplantation experiments ($1.5x10^7$ MNC in 200µl PBS; haematopoietic engraftment assessment 16 weeks post-transplantation; n=6/group). Human AFSC engrafted the haematopoietic system of sub-lethally irradiated NSG mice at levels similar to the ones achieved with CB-HSC (blood: AFSC 7.5±1.3% vs. CB-HSC 6.1±2.2%, p=0.6; BM: AFSC 46.3±7.9% vs. CB-HSC 38.3±8.2%, p=0.6; spleen: AFSC 39.6±9.3% vs. CB-HSC 34.7±10.5%, p=0.7). Importantly, the potential for multi-lineage haematopoietic reconstitution was comparable between groups at 16 weeks post primary transplantation. Moreover, at 16 weeks following secondary transplantation, AFSC-derived haematopoietic cells were detected in peripheral blood (8.9±1.6%) and other haematopoietic organs; engraftment levels were similar to these in the CB-HSC group (blood: 7.3±1.8%, p=0.8). Human CD117/c-Kit+ AFSC have functional, multi-lineage haematopoietic potential that is similar to the current "gold-standard" stem cell source for haematopoietic transplantation. The ease of isolation during early gestation, as well as their gene-engineering and expansion potential make human AFSC a novel autologous fetal cell source for pre- and post-natal therapy of inherited haematological disorders.

T1058

IDENTIFYING AND ENRICHING THE MATURE HUMAN STEM CELL-DERIVED MEGAKARYOCYTE POPULATION FOR PLATELET PRODUCTION

Sim, Xiuli[1,2], Jarocha, Danuta[1], Hanby, Hayley A.[2], Camire, Rodney M.[1,3], Marks, Michael S.[2,3], Poncz, Mortimer[1], French, Deborah L.[3] and Gadue, Paul J.[3], [1]The Children's Hospital of Philadelphia, Philadelphia, PA, U.S., [2]University of Pennsylvania, Philadelphia, PA, U.S., [3]Children's Hospital of Philadelphia, Philadelphia, PA, U.S.

Stem cell-derived platelets have the potential to replace donor platelets for transfusion purposes. The current technology enables platelet generation from various stem cell sources in vitro, but low yields and poor platelet function are obstacles that need to be overcome. Improving our understanding of megakaryocyte (MK) maturation and platelet formation using stem cell models can help to optimize in vitro platelet generation. Here, we present data identifying two distinct MK populations following the in vitro differentiation of human peripheral blood CD34+ hematopoietic stem cells and induced pluripotent stem cells (iPSCs). We designate these populations low and high granular MKs based on their distinct side scatter or granularity profiles by flow cytometry. We show that the low granular (LG) MKs appear first and become high granular (HG) MKs during culture. Following this transition, we found that a population of the HG MKs become damaged as shown by the loss of the adhesion receptor, designated GPIba (a.k.a. CD42b) and by the expression of apoptotic markers, annexin V and TUNEL. We designate these two subpopulations HG/CD42b+ and HG/CD42b-. Of interest is the finding that ~80% of the HG/CD42b+ MKs are functionally responsive to agonist stimulation while only ~30% of the LG/CD42b+ MKs are responsive and the HG/CD42b- MKs are unresponsive. From these data, we hypothesized that the HG/CD42b+ MKs may be the desired cells for platelet generation. Because over time, the HG/CD42b+ MKs become a small percentage of the larger population of damaged MKs, we wanted to mark these cells to follow for platelet generation. Human MKs endocytose coagulation factor V (FV) so we marked the cells with fluorescent-labeled FV. By confocal microscopy, we show colocalization of FV with von Willebrand factor in the alpha granules of the MKs. By using an established in vivo mouse model in which human platelets are analyzed in the blood of immunodeficient mice following MK infusion, we show that FV+ CD42b+ human platelets are detected. Studies are now being performed to examine whether the FV+ HG/CD42b+ MKs after a short pulse labeling may be useful in identifying that subpopulation of in vitro MKs that are primed to release long-lasting, functional platelets.

T1060

THE TRANSCRIPTION FACTOR HIF1ALPHACONTROLS EXPRESSION OF THE CYTOKINE IL-22 IN CD4 T CELLS

Zenewicz, Lauren A, Budda, Scott and Girton, Alanson, University of Oklahoma Health Sciences Center, Oklahoma City, OK, U.S.

IL-22 is expressed by activated lymphocytes and is important in modulation of tissue responses during inflammation. The cytokine induces proliferative and anti-apoptotic pathways in epithelial cells allowing enhanced cell survival. This can have positive effects, such as in the maintenance of epithelial barriers in the gastrointestinal tract, but also negative effects, such as contributing to colorectal tumorigenesis. As IL-22 can be dual-natured, we hypothesized that its biological activity should be tightly regulated in order to limit IL-22 expression to the sites of inflammation. One such environmental cue could be low oxygen, which often accompanies inflammation. We show that in CD4 T cells IL-22 expression is upregulated in hypoxia, and this upregulation is dependent on the transcription factor hypoxia-inducible factor 1α (HIF-1α). This finding has implications on the regulation of Il22 gene expression and the cytokine's presence in different inflammatory environments.

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CARDIAC CELLS

T1064

HYPOXIA AND AKT INDUCED STEM CELL FACTOR IS A BIASED AGONIST OF THE INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR.

Bareja, Akshay[1], Hodgkinson, Conrad[1], Payne, Alan[1], Pratt, Richard [1] and Dzau, Victor J.[2], [1]Duke University, Durham, NC, U.S., [2]Institute of Medicine, Washington, DC, U.S.

Ischemic heart disease is the most common type of heart disease and is the leading cause of mortality in the world. An immediate consequence of acute ischemic injury is severe cell death. A promising therapeutic approach is the delivery of stem cells, such as mesenchymal stem cells, that release a range of cytoprotective and pro-regenerative factors. One of these so-called paracrine factors that our lab has identified is Hypoxia and Akt induced Stem cell Factor (HASF). Our lab has recently shown that HASF promotes cardiomyocyte proliferation and pre-

vents apoptosis. Injection of the purified protein directly into the heart following myocardial infarction resulted in reduced injury and fibrosis by preventing cardiomyocyte cell death. Although the therapeutic effects of HASF have been clearly demonstrated, its precise mechanism of action is largely unknown. To this end we sought to identify the target cell-surface receptor to which HASF binds. A yeast two-hybrid screen identified a partial fragment of the Insulin-like Growth Factor 1 Receptor (IGF1R) as a binding partner of HASF. Subsequent co-immunoprecipitation experiments showed that HASF bound to the full-length IGF1R. Binding assays revealed a high affinity of HASF for the IGF1R. In vivo overexpression of HASF was shown to activate the IGF1R. Interestingly, treatment of neonatal ventricular cardiomyocytes with HASF resulted in the activation of the IGF1R and ERK but not of Akt, unlike IGF1 which induces the activation of all three. This activation was abrogated in the presence of a pharmacological inhibitor to the IGF1R and after IGF1R-specific siRNA-mediated knockdown. However, siRNA-mediated knockdown of either the IGF2R or the insulin receptor had no effect on HASF-induced cell signaling. We are currently conducting studies to further elucidate the molecular underpinnings of the biased activation of the IGF1R by HASF. Our larger goal is to use HASF as a tool to further our understanding of biased agonism at the IGF1R. We hope that these experiments will provide instructions for how to modulate the IGF1R so as to specifically activate therapeutically-relevant signaling pathways while avoiding those that induce deleterious side-effects.

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T1066

SEQUENTIAL STIMULATION AND INHIBITION OF LYSOPHOSPHATIDIC ACID RECEPTOR 4 ENHANCES CARDIAC DIFFERENTIATION OF PLURIPOTENT STEM CELLS AND REPAIRS THE INFARCTED HEART

Cho, Hyun-Jai[1], Lee, Jin-Woo[1], Lee, Choon-Soo[1], Yang, Han-Mo[1], Kwon, Yoo-Wook[1] and Kim, Hyo-Soo[2], [1]Seoul National University Hospital, Seoul, Korea, South, [2]Seoul National University Hospital, Seoul, Korea, South

Discovering a lineage-specific marker based on a comprehensive understanding of development is a key process in which allows an efficient induction of differentiation. We here report a new cardiac progenitor stage-specific marker, lysophosphatidic acid receptor 4 (LPAR4), and show its functional significance of cardiac differentiation and therapeutic implication. Methods and results: To screen cell-surface expressing molecules on cardiac progenitor cells compared to undifferentiated cells, we isolated Flk1+/PDGFRα+ cells at differentiation day 4 and performed microarray analysis as compared with controls. Among candidates, we identified a new G protein-coupled receptor, LPAR4. Consistent with LPAR4 expression in mouse iPSCs where it peaks at day 3 after differentiation and shuts down immediately, a heart-specific expression of LPAR4 in mouse embryo disappears completely in adults, suggesting the role of LPAR4 in stimulating differentiation at an early stage and must be shut off for further progression of differentiation. In mouse iPSCs, LPA (LPAR4 agonist) followed by antagonist (AM966 and BrP-LPA) treatment significantly increased cardiac differentiation efficiency. Furthermore, there was a substantial increase in LPAR4 (+) cells in the adult mouse after myocardial infarction (MI). In vivo sequential stimulation and inhibition of LPAR4 resulted in the reduction of infarct size and improvement of heart dysfunction after MI. Conclusions: We demonstrate that LPAR4 is a new cardiac progenitor stage-specific marker, as expressed on the cell surface. The manipulation of LPAR4 signaling enhances in vitro differentiation of iPSCs and ESCs into cardiac cells and shows in vivo therapeutic potential in adult mice after MI. Our findings provide a new insight into embryonic cardiac development and regeneration after injury.

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T1068

SERUM-FREE HUMAN IPSC-DERIVED CARDIOMYOCYTES FOR CONTACTLESS IN VITRO TESTING

Gibbons, George[1], Zuppinger, Christian[2], Chang, Yu-Fen[3], Robertson, Abigail[4], Dutta Passecker, Priyanka[1], Wessel-Carpenter, Naomi[1] and Daniels, Matthew[3], [1]Axol Bioscience Ltd., Cambridge, U.K., [2]Universitätsspital Bern, Bern, Switzerland, [3]University of Oxford, Oxford, U.K., [4]University of Manchester, Manchester, U.K.

Using defined factors Oct3/4, KLF4, Sox2 and c-Myc, adult cells from healthy and patient donors can be reprogrammed to generate induced pluripotent stem cells (iPSCs). Subsequently, these can be differentiated into a variety of cell types including cardiomyocytes. Human iPSC-derived cardiomyocytes (iPSC-CMs) can be cultured in vitro under serum-free conditions and as such, offer a platform investigate the effect of growth factors, cytokines and drugs on the development and functionality of human cardiomyocytes in vitro. Following differentiation, spontaneously beating iPS-CM's were evaluated in 2D and 3D culture. Expression profiling by immunohistochemistry confirms the expression of cardiomyocyte selective markers including α-actinin, myosin heavy chain, atrial and ventricular myosin light chains, troponin-T and -I, β-cat-

enin, vimentin, L-type calcium channels, connexin-40 and -43, telethonin and ankyrin repeat domain-1 (ANKRD1). Immunohistochemistry findings were validated by Western Blot for α-actinin and cardiac troponin-T expression. Additional analyses conducted, include bi-nucleate cell counts, cell form factor measurements and a comparison of plating efficiencies across a variety of substrates. The electrical activity of the iPSC-CMs was confirmed using a multi-electrode array (MEA), and the calcium dye Fluo4. One application of these cells is drug toxicity testing. To show proof of principle that this can be undertaken in a contactless manner using only genetically encoded tools, which offers several advantages compared to low throughput contact based methods with chemical dyes, we developed a simultaneous optical control/calcium imaging approach to replace the need for electrode stimulation and dyes. We are able to control the beat frequency of iPSC-CMs across the physiological range (0.3Hz – 2Hz) and can observe the anticipated effects of compounds such as Dofetilide, a known hERG inhibitor. Here, we have identified a range of characteristics in these human iPSC-CMs that confirms their ability to function as a highly-pure population of single beating human cardiomyocytes in vitro and presented evidence of a technically simple and scalable platform for cardiotoxicity screening assays.

T1070

DRUG DISCOVERY AGAINST HEART FAILURE USING HUMAN IPS-DERIVED CARDIOMYOCYTES

Naito, Atsuhiko, **Ito, Masamichi**, Nakata, Ryo, Yamaguchi, Toshihiro, Morita, Hiroyuki and Komuro, Issei, The University of Tokyo, Tokyo, Japan

Heart failure is a clinical condition when the heart become unable to eject/fill enough blood to/from the body. Heart failure is observed as a final common consequence of various cardiac diseases, such as myocardial infarction, hypertension, and cardiomyopathy. Current pharmacotherapy for heart failure includes diuretics, b-adrenergic receptor antagonist, and the inhibitors of renin-angiotensin-aldosterone axis, however, prognosis of the chronic heart failure is still poor and discovery of the new drug against heart failure is awaited. Induced pluripotent stem (iPS) cell technology is expected to provide a breakthrough to various research fields including drug discovery. Phenotypic drug discovery (PDD) using human iPS cell-derived cells might be advantageous over traditional PDD using immortalized cell lines in that we can observe the native molecular and physiological response against various drug candidates in the cell type of interest. We may also recapitulate the 'disease-phenotype' using iPS cells established from the patients of genetic disorders and perform phenotypic screening to discover a new drug against those diseases. iPS-derived cardiomyocytes from the patients of cardiomyopathy have been reported to exhibit several disease-specific phenotypes. We are currently challenging to investigate a new heart failure drug target using iPS cells established from the patients of cardiomyopathy. We have established the iPS cell lines from more than 30 cardiomyopathy patients with various genetic backgrounds. We then selected several iPS cell lines that exhibit the reported disease-specific phenotype with high signal to noise ratio and currently performing an initial screening using commercially available chemical library to test the feasibility of our strategy.

Funding Source: This research is supported by "The Program for Intractable Diseases Research utilizing Disease-specific iPS cells" and "Practical Research Project for Rare / Intractable Diseases" from Japan Agency for Medical Research and De

T1072

NATURAL COMPOUNDS CAN INDUCE CARDIOMYOCYTES DIFFERENTIATION.

Lee, Jin A, Sungkyunkwan University, Suwon-si, Korea, South

Pluripotent stem cells have enormous potential to be used in regenerative medicine for many otherwise incurable diseases. Cardiovascular diseases are one of the major causes of morbidity and mortality worldwide. Owing to the fact that terminally differentiated cardiomyocytes have limited regeneration potential, in case of several cardiovascular pathologies, like congenital defects, myocardial infraction, cardiomyopathy etc, use of clinical grade healthy cardiac cells becomes the only alternative to achieve complete cure. Use of differentiated cardiomyocyte for clinical applications thus depends on through knowledge about signaling pathways that regulate the efficiency of cardiomyocyte commitment and is needed to modulate the overall process of generating healthy cardiac cells. To this end, we aim to screen novel chemical agents which are capable to enhance the overall efficiency of cardiomyocte differentiation when compared to known inducers. In this study we used mouse embryonal carcinoma cells and an efficient cardiomyocyte specific readout driven by myosin heavy chain promoter (a-MHC) to screen commercially available chemical library of natural compounds to identify cadiomyocyte specific inducers. Using this system, we screened 800 small molecules and found Lupinie and Ursinoic acid, two naturally occurring alkaloids capable of inducing cardiomyocyte in excess to that of known inducer Oxytocin by18.4% and 23.4% respectively. We characterized the enhancement of cardiac induction by probing increase in expression of cardiac specific markers, mechano-chemical property like spontaneous beating and physiochemical property like calcium transients. We further noticed similar induction of cardiac specific program in mouse ES cells. This study offers a way to develop novel differentiation agents which

might play important role in developing defined conditions and parameters for high efficiency lineage commitments and also provide ample opportunity to understand the key molecular mechanism which control the process.

Funding Source: Brain Korea 21 plus

T1074

A DEFINED MEDIUM AND METHOD FOR THE GENERATION OF CARDIOMYOCYTES FROM HUMAN PLURIPOTENT STEM CELLS

Macri, Vincenzo S[1], Lin, Eric[2], Shafaat Talab, Sanam[2], Sampaio, , Arthur[3], Tibbits, Glen[2], Thomas, Terry E.[3], Eaves, Allen C.[3,4], Szilvassy, Stephen J[3] and Louis, Sharon A.[3], [1]STEMCELLTechnologies, Vancouver, BC, Canada, [2]Simon Fraser University, Burnaby, BC, Canada, [3]STEMCELL Technologies Inc., Vancouver, BC, Canada, [4]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) are used to study cardiomyocyte biology and disease. Several protocols to produce cardiomyocytes from human induced-pluripotent stem (hiPS) cells and human embryonic stem (hES) cells have been reported. However, the methods and efficiency of producing hPSC-CMs are variable. To overcome this limitation, a defined medium and simple method were developed to efficiently produce functional cardiomyocytes from both hiPS and hES cells. The medium and method were tested with three hiPS and two hES cell lines maintained in TeSR(TM)-E8(TM) or mTeSR(TM)1 media. The hiPS and hES cell lines were seeded at ~3x105 cells/well with Y-27632 ROCK inhibitor in TeSR(TM)-E8(TM) or mTeSR(TM)1 on Matrigel®-coated 12-well plates and maintained with daily media changes until a confluent monolayer was achieved (~3-4 days). Next, a sequential three-step, fourteen-day differentiation method was used with the new medium to generate hPSC-CMs. Beating cardiomyocytes were observed as early as day 8 and beating cardiomyocyte sheets were produced by day 14. More than 85% of the cells from all hiPS and hES cell lines tested were positive for cardiac troponin T (cTNT+) on day 18 when assayed by flow cytometry. On average, each well produced >1x106 cTNT+ cells. Optical mapping electrophysiology of 40 day-old hPSC-CMs indicated generation of nodal, atrial, and ventricular cardiomyocytes. In summary, we have developed a defined medium and user-friendly method to generate a high percentage of hPSC-CMs from several hiPS and hES cell lines.

T1076

ANALYSIS OF DEVELOPMENT OF LEFT AND RIGHT VENTRICULAR CARDIOMYOCYTES USING HIPSCS

Okubo, Chikako[1], Miki, Kenji[2], Funakoshi, Shunsuke[3], Takaki, Tadashi[4], Hatani, Takeshi[4], Nishikawa, Misato[4], Takei, Ikue[4], Inagaki, Azusa[4], Narita, Megumi[4], Yamanaka, Shinya[5] and Yoshida, Yoshinori[6], [1]Center for iPS Research and Application, Kyoto, Japan, [2]Center for iPS Research and Application, Kyoto University, Sakyo-ku, Kyoto, Japan, [3]Kyoto University, Kyoto, Japan, [4]Center for iPS Cell Research and Application, Kyoto, Japan, [5]Center for IPS Cell Research & Application, Kyoto, Japan, [6]Center for IPS Cell Research and Application, Kyoto University, Kyoto, Japan

During heart development, cardiovascular progenitors in first and second heart fields (FHF and SHF) contribute to left and right ventricles (LV and RV) respectively. However it is unclear whether induced cardiomyocytes from iPSCs recapitulate this process. For providing insights into development of cardiomyocytes in LV and RV, here we established HAND1-EGFP/HAND2-mCherry double reporter line. HAND1 and HAND2 are expressed in developmental heart, especially in LV and RV, respectively. We observed that HAND1 is expressed from day3 and HAND2 is expressed from day8 during cardiac differentiation from pluripotent stem cells. In addition at day8, almost all the HAND1 positive cells expressed surface makers of early cardiovascular progenitor. On the other hand, C-KIT was expressed in HAND1 negative cells. At day30, Troponin T, a cardiac marker, positive cells were divided into four fractions based on HAND1 and HAND2 expressions. These results suggest the possibility of applying this system to analyzing plasticity of HAND1 and HAND2 positive cells from cardiovascular progenitors in FHF and SHF to cardiomyocytes in LV and RV. It could provide insights into cardiac development and the mechanism of LV and RV specific diseases.

T1078

MYOSIN HEAVY CHAIN IDENTIFIES HUMAN MYOCYTE DIFFERENTIATION BEYOND TROPONIN T EXPRESSION: SINGLE-CELL PROFILING OF RNA, PROTEIN AND FUNCTION IN HIPSC-DERIVED CARDIAC MYOCYTES

Sharma, Janhavi[1], De la Cruz, Omar[2], Zhang, Xiaodong[1], Chiamvimonvat, Nipavan[3] and Lopez, Javier E.[1], [1]UC Davis, Davis, CA, U.S., [2]Case Western Reserve University, Cleveland, CA, U.S., [3]Department of Veterans Affairs, Mather, CA, U.S.

Generating adult cardiac myocytes from pluripotent stem cells (hiPSC-CM) is essential for myocardial repair and regenerative therapies, and provides a unique tool for understanding the biology of cardiac diseases in their proper developmental context. Targeting the single-cell maturation profile, we developed a non-genetic and non-pharmacological approach to promote hiPSC-CM differentiation. By careful optimization, we obtained 3 distinctive cultures (#1, #2 and #3) with varying maturation profiles. We studied the single-cell transcriptome (RNA Seq.), protein expression and electrophysiological (EP) signature of hiPSC-CMs from these 3 cultures at 30 days of differentiation. RNA expression for troponin T (cTnT) and total myosin heavy chain (T-MyHC), showed a significant increase across the cultures (#1= 25%, #2= 70% and #3=85%, n=64, $p< 0.01$). cTnT transcript numbers for culture #3 plateaued while T-MyHC transcript levels continued increasing. The increase of transcripts was paralleled by the increase of both proteins (#1= 24%, #2=65%, #3=88%, ~5K cells per group, $p <0.01$). Further patch clamp recording showed that hiPSC-CMs in #3 had more hyperpolarized maximum diastolic membrane potentials, larger AP amplitude and bigger maximal upstroke velocity as compared to #2, indicating a more mature state. In conclusion, we promoted hiPSC-CM maturity by 30 days. Single-cell profiling demonstrated that our approach significantly increased myogenesis and maturation of hiPSC-CMs. The cTnT transcriptional plateau with persistent increment in T-MyHC transcript may indicate more mature state of the cells, which could be used as a biomarker for further hiPSC-CM differentiation and maturation. Our findings provided a mechanistic understanding of the hiPSC-CM maturation based on the integration of single-cell transcriptome analysis, single-cell electrophysiological recordings as well as the correlated protein level analysis.

T1080

CDX2 CELLS FROM MOUSE PLACENTA EXHIBIT CARDIOMYOGENIC POTENTIAL

Vadakke-madathil, Sangeetha[1], Ranjan, Amaresh Kumar[1], Yoon, Jesse[1], Raedschelders, Koen[2], Parker, Sarah[2], Van Eyk, Jennifer[2] and Chaudhry, Hina[1], [1]Icahn School of Medicine at Mount Sinai, New York, NY, U.S., [2]2Cedars Sinai Medical Center, Los Angeles, CA, U.S.

The placenta serves as a reserve of stem/progenitor cells, which are likely superior to other adult stem cells in terms of stemness and plasticity. We have previously shown that injury to the maternal heart in pregnancy elicits migration of fetal-derived cells and their differentiation into cardiomyocytes and vascular cells. Interestingly, 40% of these cells expressed Cdx2, a trophoblast stem cell marker known solely to form placenta. We hypothesize that placental Cdx2 could be a novel cell target that may have cardiomyogenic potential. In the current study, we confirmed the expression of Cdx2 in the murine end-gestation placenta and isolated these cells using a transgenic mouse model that labels fetal-derived Cdx2 cells with eGFP. When cultured on neonatal cardiomyocyte feeders, these cells differentiated into spontaneously beating cardiomyocytes and expressed cardiac structural proteins cardiac troponin T and alpha sarcomeric actinin. In addition, cdx2 cells clonally expand and give rise to endothelial (CD31) and smooth muscle (αSMA) cells implying a multipotent nature. We further demonstrated that Cdx2 cells exhibit a unique proteome that confers roles in cell movement, migration, growth and survival compared to the embryonic stem cells. Furthermore, Cdx2 cells displayed a gene signature suggesting an ability to evade host immune surveillance. In summary, we have uncovered a multipotent role for Cdx2 cells from placenta in that they appear to have cardiomyogenic and vasculogenic potential. Their immune signature suggests that Cdx2 cells may be a favorable source for allogeneic cell therapy for cardiac regeneration, endowed with a unique proteome that facilitates homing and survival.

Funding Source: NYSTEM contract C029565

MUSCLE CELLS

T1084

FOXO SIGNALING IS REQUIRED FOR PRESERVING THE MUSCLE STEM CELL QUIESCENCE STATE

Garcia-Prat, Laura[1,2], Ortet, Laura[1], Perdiguero, Eusebio[1], Sandri, Marco[3,4], Sartorelli, Vittorio[5] and Muñoz-Cánoves, Pura[1,2], [1]Pompeu Fabra University, Barcelona, Spain, [2]CNIC, Madrid, Spain, [3]Venetian Institute of Molecular Medicine (VIMM), Padova, Italy, [4]Consiglio Nazionale delle Ricerche (CNR) Institute of Neuroscience, Padova, Italy, [5]Laboratory of Muscle Stem Cells and Gene Regulation, National Institutes of Health, Bethesda, MD, U.S.

Skeletal muscle has a remarkable capacity to regenerate by virtue of its resident stem cells (satellite cells). These Pax7-expressing stem cells are normally quiescent in the adult. Upon injury, quiescent satellite cells activate and proliferate, to subsequently either withdraw from the cell cycle (to differentiate and fuse to form new myofibers) or self-renew restoring the quiescent satellite cell pool. Maintaining the quiescent state is vital for satellite cell stemness, as a quiescence-to-senescence switch has been shown as a cause of satellite cell aging and loss of regenerative functions (Sousa-Victor et al, Nature 2014; García-Prat et al. Nature 2016). How muscle stem cells maintain quiescence and therefore preserve stemness is still largely unknown. Through a combination of global gene expression/bioinformatics and molecular analyses, we found that the FoxO family members of transcription factors (and in particular, FoxO1, FoxO3a and FoxO4) are enriched in quiescent satellite cells and play a key and cooperative role in the regulation of stem cells in skeletal muscle. Simultaneous deletion of the three FoxO genes specifically in mouse satellite cells (hence abrogating FoxO signaling) demonstrated that FoxO activity is essential for both establishment of the adult muscle stem cell pool during mouse postnatal life and for maintenance of their quiescence state throughout adult life. Through RNAseq comparative analysis between quiescent satellite cells proficient or deficient in FoxO signaling, we discovered that FoxO-dependent preservation of the quiescent state in these cells depends on the repression of the muscle differentiation gene program. In the absence of FoxO signaling, the expression of the master regulator of myoblast differentiation, Myogenin, is induced in quiescent satellite cells, which undergo spontaneous activation and subsequent differentiation, and eventual fusion with pre-existing myofibers. Thus, FoxO activity prevents muscle stem cell depletion and loss of function by maintaining the undifferentiated state of quiescence through Myogenin repression. This pathway has implications in the loss of muscle stem cell number and function in aging.

T1086

SKELETAL MUSCLE INJURY REPAIR USING IN SITU CASTING OF FIBRIN GEL SEEDED WITH MUSCLE DERIVED STEM CELLS (MDSCS)

Matthias, Nadine, Hunt, Samuel D. and Darabi, Radbod, University of Texas Health Science Center at Houston, Houston, TX, U.S.

Muscle mass-loss injury, caused by trauma or combat-induced injuries, is a major health concern as it leads to severe morbidity due to incomplete tissue repair and massive muscle fibrosis. The damaged muscle is characterized by partial or full thickness loss of muscle and its bio-scaffold, which results in extensive fibrosis and scar formation. Therefore, one potential therapeutic option is to use bio-scaffolds in combination with muscle stem cells to reconstitute new muscle tissue structure. For this purpose, muscle derived stem cells (MDSCs) are a very fit candidate as they can be isolated and expanded abundantly and are endowed with great multi-lineage differentiation potential into different tissues such as skeletal muscle and vascular lineages. Therefore, in this study, we have evaluated the regeneration potential of MDSCs combined in a fibrin-based hydrogel for muscle mass-loss injury repair in a murine model. The muscle mass injury was created by generation of a partial thickness defect using a partial wedge resection in the tibialis anterior (TA) muscle of NSG mice. This defect, if left untreated, led to severe muscle fibrosis and scar formation as our results indicated. In next step, LacZ labeled murine MDSCs were combined in a fibrin-based hydrogel and used for in situ casting of the muscle defects. The defect was casted in situ using the cell-fibrin gel and after gel formation, the wound was closed. The TAs were evaluated after 6-8 weeks after damage using histological and immunofluorescent analysis to evaluate the engraftment and tissue repair. Interestingly, our results demonstrated that in situ gel-casted MDSCs were able to survive and engraft efficiently into the muscle defect. In addition, transplanted cells contributed to the local satellite cell pool seeding as well as new muscle blood vessel reconstitution. Furthermore, damaged bio-scaffold was reconstituted in engrafted area supporting donor derived LacZ positive myofiber formation. This is the first successful report of the therapeutic efficiency of in situ stem cell- fibrin gel casting for muscle-loss injury repair which provides a great therapeutic option for future applications.

T1088

PURIFICATION OF ISOLATED SATELLITE CELLS USING MICROFLUIDIC SORTING

Syverud, Brian Chen, Lin, Eric, Nagrath, Sunitha and Larkin, Lisa, University of Michigan, Ann Arbor, MI, U.S.

As the resident skeletal muscle stem cell, the satellite cell plays a key role in repairing muscle damage. Due to this regenerative ability, satellite cells have tremendous therapeutic potential. In addition to cell therapy approaches, satellite cells are applied in skeletal muscle tissue engineering for repairing traumatic injuries. In both cases, however, obtaining a sufficiently large and highly pure population of satellite cells presents a significant challenge. We hypothesized that the size difference between satellite cells and fibroblasts, the two primary cell types obtained from a muscle isolation, would allow for microfluidic separation of these two populations and purification of the satellite cells. Using label-free, inertial separation in a microfluidic device, satellite cells and fibroblasts were sorted, and the purified satellite cell population was then used to engineer skeletal muscle. Twenty-four hours after isolation and sorting, aliquots of cells were fixed for analysis. These cells were stained for Pax7 to identify satellite cells and DAPI to identify all nuclei. Remaining cells were cultured for 14 days to form a 3D construct. Light microscopy images of the muscle monolayer were recorded on Day 11 to evaluate myotube density. After 3D construct formation, muscle function was assessed by measurement of isometric tetanic force production. Prior to microfluidic sorting, the cell isolation process yielded a satellite cell purity of 45 ± 4.7%. This purity was enriched to 78% ± 5.0% by sorting. Sorted cells proliferated normally and exhibited increased myogenic differentiation. Myotube density in the sorted cell population was 29.8 ± 1.36 myotubes/mm^2, significantly greater than the 5.91 ± 1.80 myotubes/mm^2 in unsorted controls. In terms of force production, 3D constructs fabricated from sorted cells produced significantly greater tetanic forces (117.2 ± 5.65 μN) than unsorted controls (57.1 ± 9.43 μN). These results show the promise of microfluidic sorting in purifying isolated satellite cells. Sorted satellite cells exhibited increased myogenic differentiation and improved contractile function compared to unsorted cells. This unique technology could assist researchers in translating the regenerative potential of satellite cells to cell therapy and tissue engineering therapies.

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T1090

ESTABLISHMENT OF MYOGENIC STEM CELLS WITH FOUR FACTORS

Lee, Eun-Joo[1], Kim, Ah-Young[2], Lee, Eun-Mi[3], Elfadl, Ahmed[3], Kim, Hyeong-Mi[3] and **Jeong, Kyu-Shik**[3], [1]Kyungpook National University, Daegu city, Korea, South, [2]Kyungpook Natl University, Daegu, Korea, South, [3]Kyungpook National University, Daegu, Korea, South

A lot of efforts have been made to supply myogenic stem cells. It is very invasive to getting muscle sample from patient to obtain myoblast or satellite cells. Finding out new method to supply myogenic stem cells is necessary. We screened out 12 transcriptional factors which are related to myogenic differentiation and self-renewal capacity. Among them, we find out four transcriptional factors which are necessary to obtain myogenic and proliferation ability. We transduced the four transcriptional factors to mouse embryonic fibroblasts with lentivirus. And we did single cell sorting using cell surface marker CD90.2 to obtain homogeneous cells. After establishing cells with normal karyotype, we checked myogenic differentiation ability and proliferation capacity of the cells. With the four transcription factors, mouse embryonic fibroblasts shows high expression level of Pax7, Myf5, MyoD, Myogenin and MHC. Also the cells form myotubes when put into muscle differentiation media. The transduced cells have higher proliferation capacity than both MEF and MyoD transduced MEF. All the four factors are important to induced myogenic stem cells. Omitting one of the four transcription factors hinders myogenic differentiation or proliferation of transduced cells. Thus using these four transcriptional factors, we could establish and supply myogenic stem cells.

Funding Source: This research was supported by Agricultural Biotechnology Development Program, Ministry of Agriculture, Food, and Rural Affairs (312062-5).

PANCREATIC, LIVER, LUNG, OR INTESTINAL/GUT CELLS

T1092

STEM CELL-DERIVED HEPATOCYTE-LIKE CELLS: AN ALTERNATIVE FOR HIGH THROUGHPUT TOXICITY SCREENING

Aho, Joy L., Galitz, David, Swaminathan, Preethi and Rinaldi, Fabrizio, R&D Systems, Minneapolis, MN, U.S.

Secondary liver toxicity is a leading cause of commercial pharmaceutical drug recalls and compound failures during drug development. Primary hepatocytes, the most common in vitro model for drug-induced liver toxicity

testing, are not ideal for high throughput screening due to their limited availability, difficulty to culture, and functional instability in vitro. Hepatocyte-like cells, derived from human pluripotent stem cells, are emerging as a stable and renewable model for drug-induced liver toxicity testing. In this study, we hypothesize that hepatocyte-like cells derived from induced pluripotent stem cells using the StemXVivo® Hepatocyte Differentiation Kit can be used for high throughput toxicity screens. We have previously demonstrated that the StemXVivo® Hepatocyte Differentiation Kit efficiently and consistently generates functional hepatocyte-like cells. To provide further functional validity of these cells we first show that Cyp3A4, an essential hepatic enzyme often analyzed in drug toxicity studies, can be induced in kit-derived hepatocytes. We then used high content imaging to quantitate cell viability against a panel of known hepatotoxic molecules. Our data indicate that the StemXVivo® Hepatocyte Differentiation Kit provides a reliable and renewable source of hepatocyte-like cells that can be utilized for high throughput toxicology and drug discovery.

T1094

HIGH FAT DIET ENHANCES STEMNESS AND TUMORIGENICITY OF INTESTINAL PROGENITORS

Beyaz, Semir[1], Mana, Miyeko[2], Roper, Jatin[3], Orkin, Stuart H.[4], Sabatini, David[5] and Yilmaz, Omer[3], [1]Harvard Medical School, Boston, MA, U.S., [2]Massachusetts Institute of Technology (MIT), Cambridge, MA, U.S., [3]Massachusetts Institute of Technology (MIT), Cambridge, MA, U.S., [4]Boston Children's Hospital, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, U.S., [5]Whitehead Institute, Cambridge, MA, U.S.

Little is known about how pro-obesity diets regulate tissue stem and progenitor cell function. Here we show that high-fat diet (HFD)-induced obesity augments the numbers and function of Lgr5+ intestinal stem cells of the mammalian intestine. Mechanistically, a HFD induces a robust peroxisome proliferator-activated receptor delta (PPAR-d) signature in intestinal stem cells and progenitor cells (non-intestinal stem cells), and pharmacological activation of PPAR-d recapitulates the effects of a HFD on these cells. Like a HFD, ex vivo treatment of intestinal organoid cultures with fatty acid constituents of the HFD enhances the self-renewal potential of these organoid bodies in a PPAR-d dependent manner. Notably, HFD- and agonist-activated PPAR-d signalling endow organoid-initiating capacity to progenitors, and enforced PPAR-d signalling permits these progenitors to form in vivo tumours after loss of the tumour suppressor Apc. These findings highlight how diet-modulated PPAR-d activation alters not only the function of intestinal stem and progenitor cells, but also their capacity to initiate tumours.

Funding Source: HHMINIH

T1096

THE ROLE OF LONG NONCODING RNAS IN ENDODERMAL ORGAN SPECIFICATION

Gaertner, Bjoern Christian[1], Xie, Ruiyu[1], Liu, Patrick[1], Yeo, Gene[1] and Sander, Maike[2], [1]University of California, San Diego, La Jolla, CA, U.S., [2]University of California, San Diego, La Jolla, CA, U.S.

Long non-coding RNAs (lncRNAs) constitute a heterogeneous group of developmentally regulated and highly cell type-specific transcripts. More recently, lncRNAs have emerged as important regulators of lineage commitment and cell fate maintenance. However, it is currently unclear how lncRNAs drive cell lineage diversification during endoderm organ development. At this time, how lineage-specific programs are initiated as liver and pancreas arise from common progenitors during embryonic development is poorly understood. Our exceptionally efficient human embryonic stem cell (hESC) differentiation system allows us to generate endodermal organ cell types through multiple lineage intermediates and provides a unique system to characterize the role of lncRNAs in cell type specification and diversification. This system enables us to follow the progressive differentiation of both the pancreatic and hepatic lineages from a common progenitor cell population. Taking advantage of our hESC differentiation system, we have generated genome-wide maps of chromatin state, transcription factor binding, and transcription in pancreatic endoderm, hepatic endoderm, and their common bipotential progenitor cells. Integrative analysis of this comprehensive atlas suggests that many pancreas-specific lncRNAs are directly regulated by the pancreas-determining transcription factor PDX1, as the promoters of pancreas-restricted lncRNAs are occupied by PDX1, and PDX1 knockdown diminishes their expression. Based on this analysis, we identified nine stage-specifically expressed lncRNAs that are predicted to regulate human pancreas differentiation. Ongoing experiments aim to determine the mechanisms by which these lncRNAs control cell fate decisions during endoderm development. These studies will provide novel insight into how lncRNAs are regulated during developmental transitions and how lncRNAs influence cell type-specific programs at lineage bifurcations for related cell fates.

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T1098

3D PRINTED HUMAN LIVER TISSUE MAINTAINS DRUG AND OTHER METABOLIC FUNCTIONS USEFUL FOR DRUG DISCOVERY RESEARCH AND DEVELOPMENT FOR LONG PERIODS

Kizawa, Hideki, Nagao, Eri and Zhang, Guangyuan, Cyfuse Biomedical, Tokyo, Japan

Pharmaceutical companies need in vivo-like human liver model precisely predictable clinical results and enhancing efficiency of drug discovery research and development because several liver functions vary according to the species. Various types of human liver models have always been used, but these are limited in functional sustainability and/or coverage. Recently, many researchers have showed that coculturing hepatocytes with fibroblasts or liver non-parenchymal cells significantly improve hepatic function and its sustainability. And also, it has been generalized that 3D culture has a great advantage in eliciting in vivo-like functionality compared to 2D culture. Therefore, at this time, it is thought that 3D coculture of hepatocytes with fibroblasts is one of the ideal choice for making functional and in vivo-like liver 3D models. Recently, reports on the development of bio 3D printer are increasing year by year. Existing bio 3D printing technologies have been largely pursued for the novelty of the printing technology itself. To our knowledge, there is no information about in vivo-like functional liver model constructed using bio 3D printer. Here we report novel 3D printed human liver tissue showing drug and other metabolic functions useful for drug discovery research and development for long periods. We used our original bio 3D printer, "Regenova" for constructing human 3D liver tissue. Printed liver tissue maintained high-level CYP3A4 gene expression and the enzyme activity more than 2 months. Microarray analyses showed the same or higher expression of typical genes related to life-style disease as well as drug metabolism in printed liver tissue at day 22 than those in primary hepatocyte at day 0. Drug-induced hepatotoxicity found in clinical was recapitulated at the in vitro assay using printed liver tissue. Printed liver tissue showed typical structural characteristics like in vivo human liver. Together, our printed liver tissue is promising as novel 3D liver models useful for drug discovery researches at the various stages in preclinical studies. Our results provide novel link of bio 3D printing technology with a wide range of application prospects in basic, pharmaceutical and medical studies.

T1100

HUMAN IPS CELL-DERIVED HEPATOCYTE-LIKE CELLS ACHIEVE ZONE-SPECIFIC HEPATIC PROPERTIES BY MODULATION OF WNT SIGNALING

Mitani, Seiji[1], Takayama, Kauzo[2], Tachibana, Masashi[2], Sakurai, Fuminori[2] and Mizuguchi, Hiroyuki[2], [1]Graduate School of Pharmaceutical Sciences, Osaka University, Suita, Osaka, Japan, [2]Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan

It is well known that hepatocytes show a remarkable heterogeneity in metabolic functions, and liver lobule can be divided into three zones (zone 1, 2, and 3) depending on difference of their metabolic functions. Although the method of hepatocyte differentiation from human induced pluripotent stem (iPS) cells has been largely improved over the past decade, there remains no technique for generating the human iPS cell-derived hepatocyte-like cells (iPS-HLCs) with zone-specific hepatic properties. Because it is known that hepatocytes interact with other hepatocytes and non-parenchymal cells in vivo, in this study the human iPS-HLCs were cultured with hepatocyte- or non-parenchymal cell-conditioned medium (CM) to achieve zone-specific properties. We found that the gene expression levels of zone 1 markers (Arginase 1 and transthyretin) or zone 3 markers (cytochrome P450 1A1 and constitutive androstane receptor) in the human iPS-HLCs were increased by culturing with cholangiocyte-CM or hepatocyte-CM, respectively. Urea or lipid synthesis capacities, which are known to be high in zone 1 and 3 hepatocyte, respectively, in the human iPS-HLCs were also enhanced by culturing with cholangiocyte-CM or hepatocyte-CM, respectively. Moreover, expression levels of β-catenin, which is known to be highly expressed in zone 3 hepatocytes, in the human iPS-HLCs were upregulated by culturing with hepatocyte-CM. These results suggest that the human iPS-HLCs acquired zone 1-specific or zone 3-specific properties by culturing with cholangiocyte-CM or hepatocyte-CM, respectively. To elucidate the mechanism underlying this phenomenon, WNT ligand and inhibitor expression levels in cholangiocytes and hepatocytes were analyzed. The WNT inhibitor factor 1 (WIF-1) expression level in cholangiocytes was significantly higher than that in hepatocytes, although the WNT ligands, WNT7B and WNT8B, were highly expressed in both hepatocytes and cholangiocytes. Furthermore, zone 3 human iPS-HLC conversion was promoted by hepatocyte-CM through WNT7B and WNT8B, while zone 1 human iPS-HLC conversion was promoted by cholangiocyte-CM through WIF-1. In conclusion, WNT signal modulators secreted from hepatocytes or cholangiocytes conferred zone-specific hepatic properties onto the human iPS-HLCs.

T1102

HIGHLY EFFICIENT AND REPRODUCIBLE DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO PDX1+/NKX6.1+ PANCREATIC PROGENITORS

Riedel, Michael J.[1], Lam, Stephanie[1], Luu, Yvonne[1], Thomas, Terry E.[1], Eaves, Allen C.[1,2] and Louis, Sharon A.[1], [1]STEMCELL Technologies, Inc., Vancouver, BC, Canada, [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

During human development, all pancreatic cells arise from a common progenitor that co-expresses PDX-1 and NKX6.1. Many protocols have been developed, with varying efficiencies, to direct the differentiation of human pluripotent stem cells (hPSCs) to PDX1+/NKX6.1+ pancreatic progenitor cells (PPCs). These PPCs can then be further matured in vitro or in vivo towards more specific cell lineages, including endocrine cells. hPSC-derived PPCs are useful for studying pancreas development and disease and are currently being evaluated for clinical use as a treatment for type 1 diabetes. To standardize generation of hPSC-derived PPCs, we developed the STEMdiff™ Pancreatic Progenitor Kit, consisting of a serum-free and defined medium and supplements that supports reproducible and efficient generation of PDX1+/NKX6.1+ PPCs from multiple hPSC lines. hPSCs maintained under defined, feeder-free conditions in mTeSR™1 on Corning® Matrigel® were seeded as single cells at 2.1×10^5 cells/cm^2 into 12-well plates. Cells were first differentiated to definitive endoderm (DE) for 2 days (Stage 1), which consistently generated >75% CXCR4+/SOX17+ DE cells for further differentiation. DE-enriched cells were then differentiated through Stages 2 to 4 over 11 days with daily full medium exchanges. At end Stage 4, expression of key pancreatic progenitor transcription factors was assessed by flow cytometry (FC) and qPCR. By FC, co-expression of PDX1 and NKX6.1 was: H1, 69.3 ± 3.8% (n=10); H9, 77.7 ± 3.8% (n=9); WLS-4D1, 66.0 ± 6.5% (n=7) and WLS-1C, 61.5 ± 6.0% (n=5; mean ± SEM). Essentially all NKX6.1-positive cells were PDX1-positive. On average, each well yielded $3.5 \times 10^6 \pm 0.2 \times 10^6$PPCs (n=28; mean ± SEM), indicating an average generation of 4.4 PPCs per hPSC. PPCs generated with this novel medium show appropriate upregulation of PDX1, NKX6.1, and SOX9, with minimal expression of NGN3, and are capable of maturing to monohormonal endocrine cells and ductal tissue in vivo. We have developed a complete, serum-free and defined medium and supplements that promote efficient differentiation of hPSCs to PDX1+/NKX6.1+ pancreatic progenitor cells. The protocol described here is robust across multiple hPSC lines, and can be reliably used in studies aimed at understanding pancreatic development and disease or for further maturation towards β-cells.

T1104

BMP SIGNALING AND CELLULAR DYNAMICS DURING REGENERATION OF AIRWAY EPITHELIUM FROM BASAL PROGENITORS

Tadokoro, Tomomi[1], Gao, Xia[2], Hong, Charles C.[3], Hogan, Brigid L.[2] and Hotten, Danielle[2], [1]Yokohama City University Graduate School of Medicine, Yokohama, Japan, [2]Duke University Medical Center, Durham, NC, U.S., [3]Vanderbilt University School of Medicine, Nashville, TN, U.S.

The pseudosratified epithelium lining the conducting airway of the human lung, and the trachea and main bronchi of the mouse lung, are mainly composed of ciliated and secretory luminal cells. A population of basal stem/progenitor cells located close to the basal lamina has a critical function on maintenance of the mucociliary epithelium at steady state and repair of luminal cells after injury. Evidence suggests that the self-renewal and differentiation of the basal cells are influenced by signals from luminal cells and from the underlying mesenchyme. To identify these regenerative signals, we developed the 3D clonal organoid culture system. This assay revealed that inhibitors of canonical BMP signaling pathway stimulate cell proliferation but do not affect differentiation. By contrast, exogenous BMP4 ligand dramatically inhibits cell number. Given these findings we followed changes in the levels of phosphoSMAD1/5/8 protein and transcripts for BMP signaling pathway components in vivo in the mouse trachea during regeneration of the mucociliary epithelium from basal cells. The findings are consistent with a model in which BMP from the mesenchyme places a break on the proliferation of the epithelium at steady state. This break is released transiently during repair by the upregulation of BMP antagonists. As repair proceeds there is an accumulation of suprabasal epithelial cells, so that cell density along the basal lamina increases almost twofold over steady state. However, during the next phase of repair the density of epithelial cells is restored to normal by the active extrusion of apoptotic cells. If the BMP antagonist LDN-193189 is given systemically during repair, the maximum cell number during the early phase is increased over controls. However, following the cell shedding phase normal cell density is restored. Taken together, these results reveal a critical role for both BMP signaling and cell shedding in homeostasis of the respiratory epithelium.

T1106

GENOME EDITING IN EMBRYONIC STEM CELLS FOR UNDERSTANDING HUMAN PANCREATIC DEVELOPMENT AND NEONATAL DIABETES

Zhu, Zengrong, Li, Qing, Lee, Kihyun, Gonzalez, Federico and Huangfu, Danwei, Memorial Sloan Kettering Cancer Center, New York, NY, U.S.

The generation of disease-relevant cell types from human embryonic stem cells (hPSCs) is highly valuable for studying disease pathophysiology at the cellular level. However, the potential of hPSCs for understanding more complex biological processes such as a multistep developmental process remains uncertain. It challenges not only in the need for faithfully recreation of the complexity of embryonic development in a petri dish but also the demand for efficient genetic manipulation tools. Here we systematically examined the roles of 8 pancreatic transcription factors (PDX1, RFX6, PTF1A, GLIS3, MNX1, NGN3, HES1 and ARX) through TALEN and CRISPR/Cas-mediated gene editing and hPSC directed differentiation into functional β cells. Our analysis not only defines the specific developmental step(s) affected by these mutations, but also revealed a number of novel insights into disease mechanisms, including a role of RFX6 in regulating the number of pancreatic progenitors, a dosage-sensitive requirement for PDX1 in pancreatic endocrine development, and a divergent role of NGN3 in humans and mice. Taking full advantage of the power of genome editing, we further performed temporal rescue studies to investigate the competence window for NGN3-dependent pancreatic β cell formation as well as gene correction experiments to verify CRISPR targeting specificity. Thus our study not only supports the application of hPSC-based genetic model for studying developmental defects that complements classic model organisms, but also demonstrates the speed and sophistication necessary to meet the growing demand for validating disease causality.

ENDOTHELIAL CELLS/ HEMANGIOBLASTS

T1110

ETV2 AND SCL CO-OPERATE TO SPECIFY HEMOGENIC ENDOTHELIUM AND OVERRIDE CARDIAC FATE CHOICE DURING MESODERM DIVERSIFICATION

Duan, Dan, Org, Tonis and Mikkola, Hanna K A, University of California, Los Angeles, CA, U.S.

Understanding the mechanisms determining hematopoietic and cardiac lineages from mesoderm is needed for developing cell-based therapies for blood and heart diseases. We discovered that the bHLH transcription factor Scl has a dual function in mesoderm to direct hematopoietic specification and repress ectopic cardiomyogenesis in endothelium in hemogenic tissues and the heart. Apart from Scl, the only transcription factor whose loss induces ectopic cardiac program in hematovascular mesoderm is Etv2, the master regulator of vascular development that activates Scl. However, even the high Etv2 expression in Scl deficient endothelial cells is unable to rescue hematopoietic vs cardiac fate choice in the absence of Scl. To assess whether Etv2 is essential for hematopoiesis and cardiac repression, or merely regulates these processes by inducing Scl, we overexpressed Scl or Etv2 in Etv2^{KO} ES cells during embryoid body (EB) differentiation. Similar to Etv2 induction, Scl induction was sufficient to rescue endothelial and hematopoietic progenitor cell generation in Etv2^{KO} EBs. Moreover, in contrast to Scl^{KO} endothelial cells that gave rise to cardiomyocytes, both Etv^{KO}iEtv2 and Etv^{KO}iScl endothelial cells were protected from misspecification to cardiac fate. Interestingly, RNA-seq analysis of day4.75 endothelial cells showed incomplete molecular rescue in Etv^{KO}iScl cells. Several key hematovascular genes such as Sox17 and Ets2 were not activated, and several major cardiac genes such as Hand1 and Sox9 failed to be repressed as in WT and Etv2^{KO}iEtv2 endothelial cells. However, RNA-seq of day7 endothelial cells showed that most dysregulated genes in day 4.75 EB Etv2^{KO}iScl cells were rescued to similar levels as WT and Etv2^{KO}iEtv2 cells by day 7. Moreover, correlating gene expression and chromatin accessibility in day D4.75 endothelial cells with Etv2 binding in mesoderm showed that significant number of genes whose activation was delayed in Etv2^{KO}iScl endothelium were bound by Etv2, and showed diminished DNA accessibility in the absence of Etv2. These data suggest that although Scl induction can functionally rescue hematovascular fate and repress cardiac fate independent of Etv2, Etv2 paves the way for Scl mediated hematopoietic vs. cardiac fate choice by opening chromatin for efficient and timely gene activation.

Funding Source: American Heart Association

T1112

IDENTIFICATION OF ANGIOGENIC PEPTIDES FROM THE FIRST FAS I DOMAIN OF PERIOSTIN AND THERAPEUTIC APPLICATION IN WOUND HEALING

Bareun Kim[1], Yang Woo Kwon[2] and Jae Ho Kim[3], [1]School of Medicine, Pusan National University, Yangsan, Gyeongsangnam-do, Korea, The Republic of, [2]Pusan National University, Yang san-si, Korea, South, [3]Busan National University, Yangsan-si, Gyeongsangnam-do, Korea, South

Angiogenic peptide drugs have a potential of clinical application in treating severe ischemic diseases. Periostin, an extracellular matrix protein expressed in injured tissues, has been reported to promote angiogenesis and tissue repair. In previous study, we showed that the first FAS-1 domain in periostin is responsible for periostin-induced

migration of endothelial colony forming cells (ECFCs) and demonstrated that periostin could be a therapeutic agent for the treatment of peripheral artery occlusive disease by promoting homing of ECFCs. In this study, we further characterized the first FAS-1 domain of periostin to identify a minimum region responsible for promoting ECFCs migration and angiogenic activity. A peptide from 142th amino acid to 151th amino acid in the first FAS-1 domain of periostin was critical for increasing chemotatic migration, adhesion, proliferation and tubulogenesis of human ECFCs in vitro. Chemotatic migration of ECFCs induced by the periostin peptide was abrogated by blocking antibody against β5 integrin. In addition, the periostin peptide activated AKT/ERK signaling pathway. Moreover, in the skin wound, treatment of the periostin peptide accelerated wound closure compared with HBSS-control group. These results suggest that the periostin peptide has a potential as a drug candidate for enhancing angiogenesis and wound closure.

T1114

HUMAN NAÏVE DIABETIC-IPSC DERIVED VASCULAR PROGENITORS FOR TREATMENT OF DIABETIC RETINOPATHY

Park, Tea Soon, Bhutto, Imran Ahmed, Zimmerlin, Ludovic, Minn, IL, Pomper, Martin, Lutty, Gerard A. and Zambidis, Elias, Johns Hopkins School of Medicine, Baltimore, MD, U.S.

Successful cellular therapy of diabetic vascular complications (DVC) will require novel sources of angiogenic progenitors that can sustain long-term functional recovery in a clinical setting. Translation of human induced pluripotent stem cell (hiPSC)-derived vascular progenitors (VP) to the clinic requires safe and efficient protocols for reprogramming and derivation. Since adult-driven sources such as diabetic endothelial progenitor cells (D-EPC) have proven to be scarce or impaired, iPSC technology may offer unlimited amounts of VP to re-vascularize ischemic diabetic tissue. To test this potential, we generated diabetic iPSC (D-iPSC) from type-I diabetic patients' skin fibroblasts using non-integrative episomal 7-factor (SOX2, OCT4, KLF4, MYC, NANOG, Lin28, and SV40-t antigen) system. Short exposure to a GSK3β inhibitor and ascorbic acid dramatically facilitated the reprogramming kinetics. Additionally, we recently developed novel chemical methods (GSK3β, ERK and tankyrase inhibition (LIF-3i)) that stably revert human pluripotent stem cells (hPSC) to a mouse ESC-like naïve state. We demonstrated that LIF-3i-reverted hPSC acquired transcriptomic, epigenetic, and signaling signatures of mESC, and significantly improved multi-lineage differentiation efficiencies. This LIF-3i naïve reversion method was successfully applied to generating naïve D-iPSC to obtain higher quality of therapeutic VP. To investigate the regenerative capacity of naïve D-iPSC-VP in a DVC-relevant model, we employed a streptozotocin (STZ) injected diabetic athymic nude rat model that exhibited hyperglycemia over 24 weeks and decreased retinal function as measured by electro-retinography. Our preliminary results showed that injection of healthy hiPSC-VP into this diabetic rat model validated human cell capacity to migrate and engraft to injured sites. We are currently comparing recovery of retinal function and acellular capillaries post-injection of D-iPSC-VP, naïve-D-iPSC-VP, and D-EPC. Intra-vitreous injection of hiPSC-VP expressing a luciferase transgene allowed detection of human cell migration using non-invasive live animal bioluminescent imaging. This STZ-induced diabetic rat model provides a translational opportunity to evaluate the use of patient specific iPSC-VP for treatment of DVC.

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EPITHELIAL CELLS (NOT SKIN)

T1116

THE EFFECTS OF SLC26A4 MUTATION ON EXPRESSIONS OF PROGENITOR CELL MARKERS IN THE MOUSE INNER EAR

Chan, Yen-Hui[1,2], Chen, You-Tzung[3], Yu, I-Shing[3], Lin, Shu-Wha[3], Hsu, Chung-Jen[1,2], Wu, Chen-Chi[1] and Lu, Ying-Chang[1], [1]National Taiwan University Hospital, Taipei, Taiwan, [2]Taichung Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taichung, Taiwan, [3]National Taiwan University College of Medicine, Taipei, Taiwan

Damage of hair cells or neurons of the inner ear is the major cause of irreversible sensorineural hearing impairment (SNHI) in humans, as both types of cells are highly differentiated and cannot regenerate after injury. Among the plethora of genes which have been linked to hereditary hearing impairment, recessive mutations in SLC26A4 are highly prevalent across different populations worldwide. SLC26A4 mutations are responsible for non-syndromic enlarged vestibular aqueduct and Pendred syndrome, leading to SNHI and episodic vertigo in humans. To date, several mouse models with Slc26a4 mutations have been established. Corresponding to the human counterpart, mice with defected Slc26a4 revealed SNHI, vestibular dysfunction, and inner ear malformations. Although some studies have investigated the pathogenetic mechanisms in these Slc26a4-defected mice, the implications of regenerative medicine in SLC26A4-associated SNHI remain largely unexplored. In the present study, we harvested the inner ear tissues from the Slc26a4-defected and wild-type mice, and examined the expression of progenitor cell markers. The cochlear and vestibular sensory epi-

thelia were dissected from the inner ears of mutant and wild-type mice at Day 1 after birth. The cochlear sensory epithelia included the organ of Corti with connected membranes, while the vestibular sensory epithelia included the saccule, utricle, lateral crista, anterior crista, and semicircular ducts. RNA was extracted from the harvested tissues to analyze the gene expression patterns. The tissues were also fixed by paraformaldehyde to perform immunohistochemistry staining of progenitor cell markers including OCT3/4, SOX2, and NANOG. Our results showed that the total number of progenitor cells was at the same level between Slc26a4-defected and wild-type mice. However, the expression pattern was different in the organ of Corti within the cochlea. These results provide insights into the maturing process of the inner ear in Slc26a4-defected mice and help in developing strategies for rescuing SLC26A4-associated SNHI .

T1118

DEFINING CANCER-PRONE STEM CELL NICHE IN THE HUMAN TUBAL EPITHELIUM

Flesken-Nikitin, Andrea[1], Odai-Afotey, Ashley[1], Schmoeckel, Elisa[2], Do, Thu-Vy[3], Madan, Rashna[3], Naima, Zaid[3], Grenier, Jen[1], Gupta, Divya[4], Holcomb, Kevin[4], Ellenson, Lora[4], Mayr, Doris[2], Godwin, Andrew[3] and Nikitin, Alexander Yu.[1], [1]Cornell University, Ithaca, NY, U.S., [2]Ludwig Maximilians University, Munich, Germany, [3]University of Kansas Medical Center, Kansas, KS, U.S., [4]Weill Cornell Medical College, New York, NY, U.S.

Stem cell (SC) niches of the female reproductive tract remain insufficiently elucidated. This precludes comprehensive understanding of the pathogenesis of the ovarian/extra-uterine high-grade serous carcinoma (HGSC), the most lethal gynecological malignancy. Previously we have identified a cancer-prone SC niche of the mouse ovarian surface epithelium (OSE). The OSE-SCs contribute to the OSE homeostasis in long-term lineage tracing experiments and give rise to carcinomas closely similar to human HGSC. Due to its different anatomy, the human equivalent of mouse cancer-prone SC niche remains unknown. It has been reported that likely precursor of HGSC, serous tubal intraepithelial carcinoma, is commonly found in the distal (fimbriated end) but not proximal region of the fallopian (aka uterine) tube. In search of putative SC niches for the human tubal epithelium (TE) we determined the expression of SC markers, such as KRT5/6, LEF1, and ALDH1, in the distal and proximal regions of the fallopian tube. We have also examined gene expression profiles of cells isolated from both regions, and have sorted those cells according to their ALDH enzymatic activity with ALDEFLUOR (AF). Both regions contained areas expressing SC markers and yielded AF+ cells. Consistently, isolated AF+ cells preferentially expressed SC markers. Primary cultures of both distal and proximal TE cells were differentiation competent, as evidenced by growth of both ciliated (AC-α-TUB+, FOXJ1+) and secretory (PAX8+) cells. However, in spite of these similarities, primary distal cells showed lower cellular senescence and increased proliferative potential, as compared to proximal cells. Furthermore, distal but not proximal cells were immortalized by concurrent TP53 knock out and MYC overexpression. In sum, these results support the notion of preferential origin of HGSC from the distal region of the fallopian tube. Furthermore, they suggest presence of two distinct pools of TE-SCs marked by their disparate susceptibility to the malignant transformation.

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T1120

CROSSTALK BETWEEN AUTOPHAGY AND OCULAR PAX6 CONFERS LIMBAL STEM CELLS ANTIOXIDATIVE DEFENSE AGAINST ULTRAVIOLET-A

Laggner, Maria, Pollreisz, Andreas, Schmidinger, Gerald, Schmidt-Erfurth, Ursula and Chen, Ying-Ting, Medical University of Vienna, Vienna, Austria

Autophagy, a major lysosomal degradation system, has been implicated in stem cell's functions, such as tissue homeostasis and post-injury regeneration. How the ocular surface, a tissue frequently exposed to ambient UV light, copes with photo-oxidative stress is largely unknown. Recently, we reported a crucial role of autophagy for the cellular defense of limbal stem cells (LSCs) against UVA-elicited oxidative stress. The molecular mechanism of this cytoprotective effect, however, remains elusive. PAX6 is a pleiotropic transcriptional regulator balancing the self-renewal, lineage commitment and differentiation of LSCs in the ocular surface. In face of oxidative stress, the transcriptional activity of PAX6 is regulated by dynamic shuttling between nuclear and cytoplasmic compartments. Herein, we sought to dissect the interplay between autophagy and Pax6 in LSC's stress response. To this end, we used three autophagy-deficient LSC models: (1) Krt14-Cre;Atg7$^{f/f}$ mouse cornea, (2) siRNA-assisted ATG7-knockdown human LSCs and (3) human LSCs with autophagy pharmacologically abrogated. In autophagy-competent mouse and human LSCs, 20 J·cm^{-2} of UVA irradiation activated autophagy, evidenced by a significant increase of autophagosome^{+} cells, as compared to the autophagy-deficient counterparts (35+ 4% vs. 8+5%, p<.05). By comparison, CM-H_2DCFDA-based flow cytometry analysis revealed a 1.13-fold and 3.47-fold increase of oxidative stress in irradiated Atg7$^{f/f}$ and Krt14-Cre;Atg7$^{f/f}$ LSCs, respectively (p<.05). Following irradiation, immunolocalization of Pax6 revealed a nucleocytoplasmic translocation in Atg7$^{f/f}$ LSCs. Meanwhile, cytokeratin K12 formed a perinuclear filamentous network replacing the

primitive K14. In contrast, Pax6 in Krt14-Cre;Atg7$^{f/f}$ LSCs maintained a nuclear expression, unaltered by irradiation. Interestingly, the de novo synthesized K12 revealed an aberrant, nucleus-restricted accumulation with unchanged cytoplasmic K14, indicative of a premature senescent phenotype. Collectively, our data suggest that autophagy synergizes with Pax6 in guiding the UV-distressed LSCs towards corneal phenotype differentiation.

T1122

IN PURSUIT OF AUTHENTICITY: IPS CELL-DERIVED RPE FOR CLINICAL APPLICATIONS

Miller, Sheldon S.[1], Miyagishima, Kiyoharu Joshua[1], Wan, Qin[2], Corneo, Barbara[3], Sharma, Ruchi[4], Lotfi, Mostafa[1], Boles, Nathan[5], Hua, Fang[6], Maminishkis, Arvydas[7], Zhang, Congxiao[1], Blenkinsop, Timothy A[8], Khristov, Vladimir[6], Jha, Balendu[4], Memon, Omar[4], D'Souza, Sunita[9], Temple, Sally[5] and Bharti, Kapil[10], [1]National Institutes of Health, Bethesda, MD, U.S., [2]NEI/NIH, Bethesda, MD, U.S., [3]Columbia University Medical Center, New York, NY, U.S., [4]NEI/NIH, Bethesda, MD, U.S., [5]Neural Stem Cell Institute, Rensselaer, NY, U.S., [6]National Eye Institute, National Institutes of Health, Bethesda, MD, U.S., [7]NIH/NEI, Bethesda, MD, U.S., [8]Icahn School of Medicine at Mount Sinai, New York, NY, U.S., [9]Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, U.S., [10]NEI, NIH, Bethesda, MD, U.S.

The RPE is a monolayer of pigmented, hexagonal cells connected by tight junctions that comprise part of the outer blood - retina barrier and that support photoreceptor function by the coordinated action of multiple regulatory mechanisms that act in and around the subretinal space (SRS). Induced pluripotent stem cells (iPSCs) can be efficiently differentiated into retinal pigment epithelial cells (RPE), offering the possibility of autologous cell replacement therapy for retinal degeneration stemming from RPE loss. The generation and maintenance of epithelial apical/basolateral polarity is fundamental for iPSC- derived RPE (iPSC- RPE) to recapitulate native RPE structure and function. Presently, no criteria have been established to determine clonal or donor based heterogeneity in the polarization and maturation state of iPSC-RPE. Here we provide an unbiased structural, molecular, and physiological evaluation of 15 iPSC- RPE that have been derived from distinct tissues from several different donors. We assessed the intact RPE monolayer in terms of an ATP- dependent signaling pathway that drives critical aspects of RPE function, including calcium and electrophysiological responses, as well as steady - state fluid transport. These responses have key in vivo counterparts that together help determine the homeostasis of the distal retina. In particular, the measurement of fluid flow from the apical to basal side of the RPE monolayer is a sensitive and informative assay to authenticate iPSC-RPE polarization and function. We characterized the donor and clonal variation and found that iPSC- RPE function was more significantly affected by the genetic differences between different donors than the epigenetic differences associated with different starting tissues. This study provides a reference dataset to authenticate genetically diverse iPSC- RPE derived for clinical applications.

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T1124

SCORING SYSTEM FOR ANALYZING VESSEL DEVELOPMENT IN EMBRYOID BODIES

Schmidt, Annette, Pharmacology and Toxicology, Munich, Germany and Schinköthe, Timo, Institute for Innovation and Medicine, Kirchheim, Germany

Embryoid bodies (EB) derived from pluripotent embryonic stem cells (ES) are powerful tools for different purposes. One interesting aspect is vessel development and the use for simulating angiostatic effects of drugs. Unfortunately, it is difficult to compare the impacts of different drugs. Current methods allow the description of observations, but quantifying methods are missing.

To overcome that gap, we have developed a score system to transfer microscopic observations into quantifiable values. The score system summarizes different vessel characteristics within few categories and allows the comparison of drug effects in view of time and dosage. We analysed score behaviour with respect to normal vessel development as well as under presence of angiotstatic drugs. The vessel score showed a time-dependent increase under normal conditions while under presence of an angiogenesis-inhibitor the score increase was slowed, and under angiogenesis enhancing conditions the score increase was accelerated.

The presented vessel development score seems to be a helpful tool to transfer microscopic observations in EB vessel development into a quantifiable and comparable value.

T1126

RECONSTRUCTING LINEAGE HIERARCHIES OF MICE UTERINE EPITHELIA DURING DEVELOPMENT USING SINGLECELL RNA-SEQ

Wu, Bingbing[1,2], An, Chengrui[3,4], Li, Yu[1,4], Gong, Lin[3,4], Ouyang, Hongwei[3,4] and Zou, Xiaohui[1,3], [1]Department of Gynecologythe First Affiliated Hospital, Hangzhou, Zhejiang Province, China, [2]Department of Gynecology the First Affiliated Hospital, Hangzhou, Zhejiang Province, China, [3]Key Laboratory of Tissue Engineering and Regenerative Medicine of Zhejiang Province, Hangzhou, Zhejiang Province, China, [4]Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cell and Regenerative Medicine, Hangzhou, Zhejiang Province, China

Uterine glands are indispensable to embryo implantation and growth. Uterine differentiation and adenogenesis begins postnatally involving budding, tubulogenesis, coiling and branching of the luminal epithelia. Mechanisms orchestrating uterine differentiation and adenogenesis are complex. Though a number of genes were reported to be involved, systematic molecular program controlling uterine differentiation and cellular hierarchies during adenogenesis are still incompletely known. Here we show that single cell transcriptome analysis surmounts these problems. We used single-cell RNA sequencing (RNA-seq) on 960 single mouse uterine epithelial cells from five developmental stages. Cells from different developmental stages not only possessed specific transcriptional markers, gene ontology analysis also showed distinct developmental signature, signal pathway, epigenetic activity,metabolism,which was further confirmed by small molecule inhibitors.These cells could be further classified into subpopulations within each developmental stage. There was a significant change in terms of subpopulations from one group showing fast proliferation property into another group showing characteristic of maturation between cells from postnatal day 7 and 14. Single cell Q-PCR was performed to further characterize cells from postnatal day 7, and identify candidate stem/progenitor markers responsible for adenogenesis afterwards. Our study unrevals the molecular mechanisms regulating early uterine differentiation and adenogenesis at single cell level for the first time, it would shed new light on disorder of uterine glandular development and help to better understand infertility issues related with uterine gland.

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T1128

CHARACTERIZATION OF THE MOUSE INCISOR STEM CELL NICHES USING GENE CO-EXPRESSION NETWORK ANALYSIS

Seidel, Kerstin[1], Tang, Cynthia[1], Houshmand, Bahar[1], Maas, Richard[2], Oldham, Michael[1] and Klein, Ophir[1], [1]University of California, San Francisco, San Francisco, CA, U.S., [2]Brigham and Women's Hospital, Harvard Medical School, Boston, MA, U.S.

The continuously renewing mouse incisor provides a unique system for studying the biology of adult stem cells. Its unidirectional growth, with stem cell progeny at progressively increasing stages of maturity arrayed in a linear fashion, enables the dissection of stage-specific mechanisms. To date, in-depth analysis of this system has been hindered by our limited understanding of the cellular diversity present in the incisor and by the absence of markers that allow a clear discrimination between distinct cell types. Therefore, we set out to identify novel, cell type-specific markers by analyzing the gene co-expression network organization in the proximal incisor, where mesenchymal and epithelial stem cell pools are located. In this study, expression profiles were generated from individual tissue samples micro-dissected from the proximal incisor region of 94 adult wild-type mice and used for gene co-expression network analysis to identify modules of co-varying genes. Previous work has shown that co-expression modules identified in heterogeneous tissues frequently relate to distinct biological processes and are often driven by discrete cell types. Therefore, we have been validating cell type specificity and co-expression of the highest ranked factors contributing to each identified module by visualization and comparison of gene expression patterns. Initial data analysis has led to the identification of modules representing distinct differentiated cell types and modules of co-expressed factors that appear to be specific to distinct but subsequent stages of maturity of stem cell progeny. Surprisingly, we uncovered multiple co-expression modules specific to the transient-amplifying cell population; this likely reflects the multitude of biological processes that are active in this population, and our data may enable us to better understand these processes and how they are intertwined. The wealth of new information obtained by this study will greatly enhance our understanding of the cellular composition of the incisor system and facilitate our studies of adult stem cells by rendering effects of experimental perturbations more interpretable.

Funding Source: 5RL9EB008539 (NIH/NIBIB), K99DE024214 (NIH/NIDCR), R01-DE021420 (NIH/NIDCR)

EPIDERMAL CELLS

T2002

3D SPINNING DISK IMAGING OF HUMAN HAIR FOLLICLE EPIDERMAL PRECURSOR CELLS

Dimitrov, Ariane, Genty, Gaïanne and **Paris, Maryline**, L'Oreal R&I, Aulnay-sous-Bois, France

Human hair follicles (hHF) possess several populations of epidermal precursor cells in the outer root sheath (ORS). Cells with the highest regenerative potential, expressing high levels of CD200 and K15, are located in the upper ORS. Epidermal precursor cells of the lower ORS express a high level of CD34. These populations are affected differentially in androgenic alopecia where the stem cell population is not altered whereas epidermal precursor cells number decreases. The precursors are usually quantified by flow cytometry and localized by immunofluorescence on thin transversal sections. Due to the small amount of precursors these two methods may lead to loss of information. In addition, they are technically laborious, time consuming and require a large number of samples. We therefore developed a new method where the immunostaining is performed without sectioning. hHF are dissected and detached from the dermal sheath to enable antibody penetration. Images of the whole hHF are acquired on a spinning disk confocal microscope and reconstructed by stitching algorithms. Optical sections are acquired at higher magnification throughout the sample in several regions of interest. Ultimately, this technique will allow us to study different populations of precursors, to characterize their distribution in the hHF and to analyze the intracellular localization of their proteins, therefore complementing flow cytometry and transversal sections approaches.

T2004

BNIP3 ACTIVATION VIA STIMULATION OF ERK AND JNK ACTIVITIES IS REQUIRED FOR THE PROTECTION OF KERATINOCYTES FROM UVB-INDUCED APOPTOSIS

Moriyama, Mariko[1], Uda, Junki[1], Hayakawa, Takao[1] and Moriyama, Hiroyuki[2], [1]Kindai University, Higashi-Osaka, Japan, [2]Kindaii Univ, Higasi-Osaka, Japan

The human skin plays an important role in a barrier function. Ultraviolet rays (UV) from sunlight exposure may cause cell apoptosis in skin epidermis, resulting in the disruption of the barrier. Our recent work has demonstrated that BNIP3 expression and 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 significantly suppressed the BNIP3-stimulated differentiation of keratinocytes, suggesting that autophagy is involved in the process. Moreover, we also found that overexpression of BNIP3 induced autophagy and mitophagy in HPEK. These data clearly suggest that BNIP3 plays a crucial role in keratinocytes differentiation by inducing autophagy.

In this study, we found that BNIP3 is also indispensable for maintenance of skin epidermis. UVB irradiation stimulated BNIP3 expression and cleavage of caspase3 in HPEK. 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. Our data also demonstrated that accumulation of reactive oxygen species (ROS) by UVB irradiation is sufficient to trigger an activation of JNK and ERK mitogen-activated protein kinase (MAPK) in HPEK. ROS also upregulated BNIP3 expression, which was mediated by JNK and ERK MAPK. Treatment of anti-oxidant reagent or specific inhibitor of MAPK, U0126 and JNK inhibitor significantly attenuated the expression of BNIP3 by UVB, followed by the induction of cell death by apoptosis. Furthermore, UVB-induced apoptosis was significantly stimulated by chloroquine or bafilomycin A1, an inhibitor of autophagy. These data clearly indicated that BNIP3-induced autophagy plays crucial roles in protection of skin epidermis against UVB irradiation through JNK and ERK MAPK, whose activations are mediated by UVB-generated ROS. Overall, our data shed light on functions of BNIP3, an inducer of autophagy, in both differentiation and maintenance of epidermal keratinocytes.

T2006

SECERETORY PHOSPHOLIPASE A2-IIA: EMERGING ROLES IN HAIR FOLLICLE STEM CELLS REGULATION AND CELLULAR DIFFERENTIATION

Waghmare, Sanjeev K, ACTREC, TATA MEMORIAL CENTRE, MUMBAI, Maharashtra, India

Epidermis homeostasis is maintained throughout the life by stem cells that self-renew in their niche and give rise to different progenitors. Secretory phospholipase A_2 Group-IIA (sPLA_2-IIA) catalyzes the hydrolysis of the sn-2 position of glycerophospholipids to yield fatty acids and lysophospholipids. sPLA_2-IIA is deregulated in various cancers; however, its role in epidermal homeostasis and stem cell regulation is still obscure. Here we show a transgenic mice overexpressing sPLA_2-IIA (K14-sPLA_2-IIA) showed increased proliferation and higher differentiation that led to a gradual loss of hair follicle stem cells with age. BrdU pulse-chase label retaining cell (LRC) study showed decrease in slow-cycling population indicating loss of stem cells quiescence. The colony forming efficiency of transgenic keratinocytes was significantly reduced. Further,

K14-$sPLA_2$-IIA keratinocytes showed enhanced activation of EGFR and JNK1/2 that led to c-Jun activation, which co-related with enhanced differentiation. Our results for the first time unravelled that overexpression of $sPLA_2$-IIA lead to depletion of hair follicle stem cells and abnormal epidermal components associated with increased differentiation. Thus roles of $sPLA_2$-IIA in various cancers and stem cell regulation suggest its prospective implications in cancer pathogenesis.

Funding Source: Indian Council of Medical Research

EYE OR RETINAL CELLS

T2010

TOWARDS A NEW TOOL FOR BASIC RESEARCH AND PRECLINICAL STUDIES IN THE RETINA FIELD: HUMANIZED PHOTORECEPTORS MOUSE MODELS

Golfieri, Cristina[1], Santos-Ferreira, Tiago[2], Borsch, Oliver[2], Ader, Marius[2] and Karl, Mike O.[1,2], [1]German Center for Neurodegenerative Diseases (DZNE), Dresden, Germany, [2]Technische Universität Dresden DFG-Center for Regenerative therapies Dresden Cluster of Excellence, Dresden, Germany

Humanized mouse models represent a powerful tool for basic research and preclinical studies, as has been shown for human hematopoietic stem cells, hepatic and cancer cells.Within the eye, the subretinal space between the photoreceptors and the retinal pigment epithelium has a long-standing history as an exceptional niche where transplanted cells can survive and mature beyond levels achieved ex vivo.Thus, we would like to develop a humanized retinal disease mouse model to monitor and study human disease processes in an in vivo setting. Here we show that hiPSC-derived retinal organoids can be successfully generated using our modified protocol of the pioneering methodology from the Sasai laboratory. Analysis of hiPSC-derived retinal organoids up to day 135 of differentiation shows development of stratified tissues. Immunostaining analyses suggest that retinal organoids contain retinal ganglion cells [BRN3, ELAVL3/4], amacrines [CALB2], cone [CRX, RCVRN, PNA, ARR3] and rod [CRX, RCVRN, NRL] photoreceptors and potentially retinal pigment epithelium. 96 days old hiPSC-derived retinal organoids were dissociated and a single cell suspension was transplanted into the subretinal space of wildtype mice. We found that grafted cells survived at least up to 3 weeks after transplantation and expressed photoreceptor markers such as CRX and RCVRN. Our results open up the possibility to study the mouse subretinal space as a permissive niche for human photoreceptor and retinal disease development in situ and in vivo.

T2012

ORAL MUCOSAL EPITHELIAL CELLS GROWN ON POROUS SILICON MEMBRANES: A MODEL STEM CELL NICHE FOR OCULAR SURFACE REPAIR IN THE RAT

Irani, Yazad D.[1], Klebe, Sonja[1], McInnes, Steven, JP[2], Jasieniak, Marek[2], Voelcker, Nicolas[2] and Williams, Keryn A[1], [1]Flinders University, Bedford Park, Australia, [2]University of South Australia, Mawson Lakes, Australia

In humans, the adult stem cells that maintain the corneal epithelium reside at the limbus. Dysfunction of stem cells or their niche can result in painful, potentially sight-threatening ocular surface disease. In an attempt to recapitulate the stem cell niche, we coated porous silicon (pSi) membranes with collagen IV and vitronectin, proteins expressed at the limbus. Oral mucosal tissue, biopsied from juvenile (4-8 week old) male Sprague-Dawley rats, was treated with dispase and the epithelium detached from underlying tissue. Epithelial tissue was disaggregated with trypsin and the result single cell suspension was cultured on pSi membranes. Approximately 75% of rat oral mucosal epithelial cells (OMECs) cultured on these scaffolds expressed the transient amplifying cell marker p63. Expression of corneal epithelial cell markers CK3/12 and CK19 was observed in 20% of OMECs. Furthermore, a small population of cells (less than 5%) expressed the putative stem cell marker ABCG2. Scaffolds carrying rat oral mucosal cells labelled with the cell tracker PKH26 were implanted under the bulbar conjunctiva of female Sprague-Dawley rats in which mild ocular surface disease had been induced by n-heptanol debridement, to stimulate migration of transplanted cells. Ocular surface disease was confirmed by presence of goblet cells, normally only present in the conjunctival epithelium, in the central corneal epithelium. Immunohistochemistry for pan-cytokeratin detected transplanted epithelial cells on pSi membranes at 8 weeks post-implant. Transplanted cells migrated locally off the coated porous silicon scaffolds over 8 weeks, as evidenced by PKH26 staining, but were not detected in the central cornea, by PCR for the male specific sry gene. Further optimisation will be required to promote migration of daughter cells over the ocular surface, but the existing scaffold proved to be biocompatible and shows some promise as an artificial adult stem cell niche.

Funding Source: This work was supported by the National Health and Medical Research Council of Australia, the Ophthalmic Research Institute of Australia and the Flinders Medical Centre Foundation.

T2014

INVESTIGATING THE MOLECULAR MECHANISMS OF LATE-ONSET RETINAL DEGENERATION USING PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

Miyagishima, Kiyoharu Joshua[1], Sharma, Ruchi[2], Qureshy, Zoya[1], Clore-Gronenborn, Katharina[1], Zhang, Congxiao[1], Khristov, Vladimir[3], Rajan, Vaishakh[1], Silver, Jason[1], Sieving, Paul[1], Cukras, Catherine[1], Miller, Sheldon S[4] and Bharti, Kapil[1], [1]National Institutes of Health, Bethesda, MD, U.S., [2]NEI/NIH, bethesda, MD, U.S., [3]National Eye Institute, National Institutes of Health, Bethesda, MD, U.S., [4]National Eye Inst, National Inst of Hlth, Bethesda, MD, U.S.

Late-Onset Retinal Degeneration (L-ORD) is a rare autosomal dominant disorder that shares clinical similarities to more prevalent retinal degenerations, such as age-related macular degeneration. L-ORD is caused by a single S163R amino acid substitution in the globular domain of the CTRP5 protein. The CTRP5 gene is contained within the 3'-untranslated region of another gene, which encodes the membrane-frizzled related protein (MFRP). Investigating this genetically simple disease will provide us with valuable insight into the shared mechanisms of other retinal degenerative diseases and help us identify novel targets for therapy. We generated induced pluripotent stem cells (iPSCs) from fibroblasts of two L-ORD affected and two unaffected siblings. iPSCs were characterized by the expression of pluripotent markers: SSEA4, SOX2, OCT4, and NANOG. Karyotype analysis revealed no aberrations. Sequencing confirmed that the patients' cells retained the S163R point mutation characteristic of L-ORD. iPSCs were differentiated into RPE using a directed differentiation protocol. The mRNA expression levels of CTRP5 and MFRP were assessed by qPCR. The amounts of CTRP5 protein released apically and basally were measured by western blot in iPSC-RPE derived from patients and healthy siblings. Localization and relative expression of MFRP was determined by immunofluorsescence. Intracellular calcium levels were measured with ratiometric calcium dye, Fura-2. RPE differentiated from iPSCs of L-ORD patients and their healthy sibling express similar amounts of CTRP5 protein. mRNA expression of MFRP is also not significantly different between patients and healthy cells. However, patient's cells show characteristic deposits that are reminiscent of deposits seen in the eyes of L-ORD patients. Calcium imaging results demonstrate lower baseline intracellular calcium levels in patient cells as compared to their healthy siblings. L-ORD patient RPE cells show cellular endophenotypes that are reminiscent of phenotype seen in the eyes of L-ORD patients. Reduced baseline calcium in L-ORD patient cells suggests defective calcium homeostasis that might contribute to accumulation of intra and sub-cellular deposits.

Funding Source: NIH NEI Intramural Funds

T2016

BIOMECHANICAL FINGERPRINTING OF ROD PHOTORECEPTOR DEVELOPMENT

Santos-Ferreira, Tiago[1], Herbig, Maik[2], Otto, Oliver[2], Guck, Jochen[2] and Ader, Marius[3], [1]DFG-Center for Regenerative Therapies Dresden (CRTD)/TU Dresden, Dresden, Germany, [2]BIOTEC, TU Dresden, Dresden, Germany, [3]Technische Universität Dresden/CRTD, Dresden, Germany

Retinal degeneration affecting photoreceptors are amenable to cell replacement therapy and remarkable progress was achieved using primary mouse photoreceptors for visual repair in pre-clinical studies. Transplantation success was significantly increased following enrichment of donor photoreceptors using fluorescent reporters or cell surface markers. Mechanical phenotyping might be used as an alternative marker-free methodology to identify and enrich distinct cell populations for transplantation. Recently, real-time deformability cytometry (RT-DC) was developed allowing the high-throughput characterization of mechanical properties of cells. Briefly, cell suspensions of interest are flowed through a microfluidic constriction and cells acquire a deformed shape. These changes are recorded, processed by an algorithm that measures cell size, deformation and calculates cells' elasticity independently of its size. Indeed, RT-DC has been successfully applied for the identification of different cell populations in whole blood samples and for tracking stem cell differentiation into distinct lineages. Here, we show for the first time the biomechanical fingerprinting of rod photoreceptor development using RT-DC. Retinas isolated from GFP reporter mouse lines at different developmental stages (embryonic day (E) 15.5, postnatal day (P) 4, P10 and P20) were dissociated to a single-cell suspension and FAC-Sorted. Unsorted, GFP-positive and GFP-negative fractions were analyzed by RT-DC. Retinal progenitors were collected at E15.5 from Hes5-GFP mice while rods were isolated from the Nrl-GFP mouse line at all developmental stages. At E15.5, retinal progenitors (Hes5-GFP$^+$) displayed bigger cell size and deformation when compared to Hes5-GFP$^-$ cells while elasticity remained unchanged. Rod photoreceptors (Nrl-GFP$^+$) displayed a reduction of cell size and deformation as development proceeds and were slightly softer at P4 than other developmental stages. Interestingly, rods present significant differences at P10 in cell size and elasticity when compared to GFP-negative cell populations. Such biomechanical differences could be explored to enrich transplantable rod photoreceptors for cell replacement studies in the retina.

T2018

MODELLING REACTIVE GLIOSIS IN MOUSE ESC-DERIVED RETINA ORGANOIDS

Voelkner, Manuela[1], Kavak, Cagri[1] and Karl, Mike O.[1,2], [1]German Center for Neurogenerative Diseases (DZNE), Dresden, Germany, Dresden, Germany, [2]Center for Regenerative Therapies Dresden, Technische Universitat Dresden, Germany, Dresden, Germany

Pluripotent stem cell derived retinal organoids are a new experimental model system for studies on retinal degeneration and regeneration. Here we started to develop a retinal organoid-based model to study reactive gliosis, a glial response frequently associated with neuronal damage and disease in mammals and potentially related with the regenerative capacity in other species. We first characterized the development and molecular state of radial Müller glia (MG), the major type of glia in the retina, in the organoid system. We observed the first MG in developing retina organoids on day 18 and postmitotic MG by day 22. Next, we characterized cell death in the mESC-derived retina organoid system using TUNEL assay. Cell death increases with ongoing neurogenesis, and photoreceptors and bipolar cells (8% ± 5 SEM) are less affected compared to amacrine and ganglion cells (25% ± 1 SEM). Strikingly, MG in retina organoids do not express glial fibrillary acidic protein (GFAP), a hallmark of reactive gliosis, or other known gliosis associated markers in response to the organoid system culture conditions and organoid immanent cell death. Reactive gliosis is well-known to be strongly induced in retina and brain organotypic tissue and cell culture. We hypothesized that the organoid system offers a unique opportunity to study reactive gliosis ex vivo. Thus, we asked if application of neuronal damage-related signaling factors, i.e. cytokines, are sufficient to induce reactive gliosis in the retina organoid system. We observed that application of TNF on day 20 induced GFAP expression in MG, increased cell proliferation, as well as a loss of retinal stratification within 5 days. In sum, our data suggests that MG develop in the retinal organoid system, become quiescent and remain non-reactive at the end of retinogenesis. Experimental stimulation of defined signaling pathways is sufficient to induce MG reactive gliosis and a pathologic phenotype in retinal organoids. We conclude, the organoid system offers a new model system and unique opportunity to study the mechanisms of reactive gliosis ex vivo.

NEURAL CELLS

T2020

THE LONG NONCODING RNA PNKY REGULATES MOUSE V-SVZ NEURAL STEM CELLS IN VIVO

Andersen, Rebecca Ellen, Ramos, Alexander and Lim, Daniel, UCSF, San Francisco, CA, U.S.

While there has been an increasing number of reports describing long noncoding RNAs (lncRNAs) with important roles in cell culture studies, our understanding of lncRNA function in vivo is limited. Furthermore, certain aspects of stem cell biology cannot always be fully assessed in culture. The ventricular-subventricular zone (V-SVZ) of the adult mouse brain harbors a population of neural stem cells (NSCs) that generates a large number of new neurons for the olfactory bulb throughout life. We have previously shown that the neural-specific lncRNA Pnky regulates neurogenesis from V-SVZ NSC cultures. Here, we have generated a Pnky conditional knockout mouse to study the in vivo function of this lncRNA. The Pnky conditional allele has loxP sites closely flanking the 5' and 3' ends of the Pnky gene, and Cre-mediated recombination resulted in ablation of Pnky expression. Importantly, Pnky deletion did not disrupt expression of the neighboring gene Pou3f2. Consistent with our previous results using shRNA-mediated Pnky knockdown, genetic deletion of Pnky in NSC cultures resulted in a several-fold increase in neuron production. Using various constitutive and inducible Cre-drivers, we have been studying adult V-SVZ neurogenesis in mice with Pnky conditional knockout (Pnky-cKO). These mice exhibited large accumulations of neuroblasts, consistent with increased neurogenesis. Because V-SVZ neurogenesis occurs over the lifespan of the mouse, we have also been able to interrogate the role of Pnky in the long-term function of the V-SVZ NSCs. Analyses of Pnky-cKO mice suggest a critical role for Pnky in the long-term maintenance of this NSC population, which is an aspect of stem cell biology that cannot be easily addressed in vitro. By generating a novel Pnky-cKO mouse and studying a well defined, adult NSC population in vivo, we have gained new insights into the function of a neural specific lncRNA in neurogenesis and tissue homeostasis.

T2022

IDENTIFICATION OF A NOVEL NEURAL STEM CELL POPULATION IN THE HYPOTHALAMUS OF MAMMALIAN BRAIN

Bekki, Goezde[1], Roth, Lena[2], Grinevich, Valery[2] and Liu, Haikun[2], [1]DKFZ, Heidelberg, Denmark, [2]DKFZ, Heidelberg, Germany

In the adult mammalian brain, active neurogenesis has been found in two neurogenic regions, which are the sub-

granular zone (SGZ) of the dentate gyrus in the hippocampus and the subventricular zone (SVZ) of the lateral ventricles. Despite being limited under physiological conditions, neurogenesis was suggested to exist in the hypothalamus, the region in the brain, which is responsible for many vital events for survival and reproduction of the organism. However, the identity of hypothalamic neural stem cell (NSC) populations remains poorly characterized. Consequently, molecular regulation of the hypothalamic neurogenesis in the postnatal and adult mammalian brain still keeps its mystery.

The nuclear receptor tailless (Tlx, NR2E1) is a very specific marker and an important regulator for adult NSCs. Here we demonstrate that Tlx is also expressed by a subpopulation of hypothalamic tanycytes that reside in the third ventricle (3rdV) of the adult mouse brain. These cells are active during early postnatal development but become quiescent in adulthood. Nevertheless, FGF2 infusion activates these cells *i*n vivo. Whereas loss of Tlx leads to the depletion of FGF2 induced neurogenesis in the adult hypothalamus, overexpression of Tlx results in an increase of FGF2 induced neurogenesis in vivo. Besides, Tlx expressing hypothalamic cells can be cultured in vitro. Moreover, hypothalamic Tlx positive cells that are isolated postnatally, are transplantable into the adult mouse brain. Transplanted cells are able to integrate into the existing network and differentiate into both glial and neuronal lineages. Examination of postnatal human sections indicates the existence of active neurogenesis in the human 3rdV at postnatal stage. Moreover, our in situ hybridization (ISH) data demonstrates the expression of Tlx in the 3rdV region of the postnatal human brain. This suggests that similar to mice, Tlx dependent neurogenesis mechanism may exist in the human hypothalamus. This study identifies a novel population of hypothalamic NSCs and provides insight into the molecular regulation of hypothalamic neurogenesis.

T2024

PEROXYNITRITE ENHANCES SELF-RENEWAL AND NEURONAL DIFFERENTIATION OF NEURAL STEM/PROGENITOR CELLS

Chen, Xingmiao, Yan, Tingting and Shen, Jiangang, The University of Hong Kong, Hong Kong

Neural stem/progenitor cells (NSCs) have limited capacities of growth and differentiation, offering possible regeneration therapy for stroke and neurodegenerative diseases. Hypoxic/ischemic stimulation mediates the growth and differentiation of NSCs into mature neurons. In hypoxic/ischemic brain, nitric oxide and superoxide are simultaneously produced and they rapidly react to form peroxynitrite. Peroxynitrite has been considered to be responsible for neurotoxicity after stroke for long time. However, recent studies indicate low concentrations of peroxynitrite promote endothelial cell growth for angiogenesis and contribute to hypoxia-induced muscle cell proliferation. Here, we investigated peroxynitrite function in neural stem/progenitors cells. Somewhat surprisingly, with our new sensitive and specific peroxynitrite probe HKYellow-AM, we found that cells maintained an endogenous relative high peroxynitrite level showed the more potent capacity of proliferation and self-renewal. Low concentrations of exogenous peroxynitrite promoted NSCs proliferation, self-renewal and neuronal differentiation. Increased peroxynitrite during hypoxia was visualized HKYellow-AM staining. Meanwhile, peroxynitrite decomposition catalysts (PDCs, FeTMPyP and FeTPPS) treatment reduced hypoxia-induced peroxynitrite formation, NSCs proliferation, self-renewal and neuronal differentiation. Moreover, effects of peroxynitrite on neurogenesis were partly through activating HIF-1α correlated with enhanced Wnt/β-catenin signaling pathway. These results suggest that peroxynitrite may serves as a cellular signal for promoting NSCs proliferation, self-renewal and neuronal differentiation.

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T2026

CELL CYCLE REGULATION AND MICRORNA IN CLINICALLY-RELEVANT HUMAN EMBRYONIC STEM CELL-DERIVED NEURAL STEM CELLS.

Dolezalova, Dasa, Urbanovsky, Ondrej, Fedorova, Veronika, Peterkova, Miroslava, Valouchova, Veronika and Hampl, Ales, Masaryk University, Brno, Czech Republic

Regeneration and replacement of neurons and glia that undergo cell death are the main goals of stem cell-based therapies. Clinically-relevant methods for generation of neural stem cells (NSCs) from human embryonic stem cells (hESCs) only begin to emerge. We have previously derived such self-renewing NSC lines with the ability to terminally differentiate into functional neurons and glia in vivo. Importantly no tumor formation was noted, therefore studying self-renewing mechanisms of NSCs with “non-tumorigenic nature” might shed a light on deregulated proliferation mechanisms often found in CNS tumors. Thus, the aim of the present study was to define molecular mechanisms responsible for the maintenance of unlimited self-renewal of NSCs. Since self-renewal and differentiation has been previously connected with the regulation of cell cycle partially via microRNA in hESCs, we hypothesize that differentiation-associated miRNAs contribute to cell cycle regulation in self-renewing NSCs as well. We initiated our experiments by analyzing high throughput gene expression data in undifferentiated hESCs, self-renewing NSCs, and non-self-renewing neural progenitor cells (NPCs). Results show that only 16 genes

are significantly upregulated in NSCs in comparison to NPCs. These include LIN28B, which has been associated with self-renewal of NSCs before, thus confirming our data. However other proteins, such as SALL4, have been connected to pluripotency of hESCs but never studied in the context of NSC-self renewal. Moreover, the expression of selected cell cycle regulatory molecules (most prominently p16/CDKN2A) is low in self-renewing hESCs and NSCs, and only increases in NPCs, and has not been described before. Altogether, our data point to several candidate genes responsible for maintenance of self-renewal of NSCs and functional studies are ongoing to reveal underlying regulatory pathways. Curiously, results from qPCR screen did not identify miRNAs which are specifically expressed in NSCs and could thus be implicated in the maintenance of NSC phenotype and/or control of self-renewal. Therefore, we are now initiating a complex study of miRNA expression by RNA deep sequencing. This analysis will help to identify miRNAs with putative role in NSC maintenance and self-renewal.

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T2028

A PERSISTENT ENTEROVIRAL INFECTION LEADS TO LASTING ALTERATIONS IN AUTOPHAGIC FLUX IN NEURAL PROGENITOR CELLS AND INCREASED VIRULENCE

Feuer, Ralph[1], Rhoades, Ross[1], Nguyen, David P[1], Deline, Steven[1], Montes de Oca, Alicia V.Z.[1], Gurney, Michael A.[1], Gottlieb, Roberta A.[2] and Tsueng, Ginger[1], [1]San Diego State University, San Diego, CA, U.S., [2]Cedars-Sinai Heart Institute, Los Angeles, CA, U.S.

Coxsackievirus, an enterovirus, frequently targets the central nervous system (CNS) in newborn infants causing serious disease, such as meningitis and encephalitis. We previously described the ability of coxsackievirus B (CVB) to preferentially infect and deplete neural progenitor cells (NPCs), persist in the CNS, and cause developmental defects and chronic immunopathology in our juvenile model of infection. Utilizing a recombinant CVB expressing "fluorescent timer" protein which undergoes slow conversion of fluorescence from green to red, we determined that this virus commandeered the autophagy pathway in progenitor cells in order to manufacture double-membrane virus replication organelles which were eventually ejected from the host cell as single membrane $LC3^+$ microvesicles harboring infectious virus. Nevertheless, a small percentage of NPCs survived acute infection and established a carrier-state infection. Infected NPCs induced low levels of interferon-beta, showed early signs of cytopathic effects followed by partial recovery upon passage in culture, and expressed detectable levels of viral protein for over 50 days post-infection (PI). Viral titers and viral plaque sizes initially increased at day 6 PI and then decreased over time, suggesting that strong selection pressures in these dynamic NPC cultures contributed to the generation of a highly virulent strain, followed eventually by viral attenuation at later time points. Plaque-purified viral isolates obtained from infected NPCs at day 6, 27, and 48 PI consistently maintained their temporally-dependent virulent or attenuated phenotype. Viral isolates from varying time points were sequenced and unique nucleotide mutations were identified, suggesting these mutations may be responsible for virulence and eventual attenuation during persistent infection. Persistently-infected NPCs retained their ability to differentiate into neurons, astrocytes, and oligodendrocytes. Nevertheless, autophagic flux was altered in surviving NPCs, and these cells exhibited an accelerated propensity to differentiate. These results suggest that CVB undergoes dramatic changes in virulence as the virus establishes a persistent infection, and surviving NPCs in the CNS may be altered in their function.

Funding Source: This work was supported by National Institutes of Health (NIH) R01 Award NS054108 (to RF)

T2030

INSULIN-LIKE GROWTH FACTOR-1 RECEPTOR SIGNALING DETERMINES THE SURVIVAL AND MIGRATION OF GRAFTED NEURAL STEM CELLS IN THE LESIONED SPINAL CORD

Hwang, Dong Hoon[1], Shin, Hae Young[2], Suh-Kim, Haeyoung[2] and Kim, Byung Gon[2], [1]Ajou University School of Medicine, Suwon, Korea, South, [2]Ajou University School of Medicine, Suwon, Korea

Therapeutic impact of neural stem cell (NSC) transplantation for spinal cord injury is limited by a huge loss of grafted NSCs. We have previously demonstrated that survival of neural stem cell (NSC) grafts rapidly declines after transplantation in the lesioned spinal cord and that combined treadmill locomotor training (TMT) increased the NSC survival in part via insulin-like growth factor-1 (IGF-1). The current study aimed to provide genetic evidence that IGF-1 receptor (IGF-1R) signaling in NSCs may critically regulate graft survival following transplantation into the injured spinal cord in mice. The neurospheres obtained from E14 IGF-1R (+/-) mice showed similar size and proliferation rate to those from littermate wild type (WT) embryos. However, IGF-1R (+/-) NSCs were more susceptible to the cellular stresses induced by reactive oxygen or nitrogen species that those from WT embryos. IGF-1R (+/-) NSC grafts showed significantly attenuated survival compared WT NSC grafts. In addition, whereas most NSCs derived from IGF-1R (+/-) were confined around the injection site, WT NSCs exhibited more extensive migration from the injection site and consequently interacted with host spinal cord tissue in animals subjected to TMT. These results indicate that the IGF-1 receptor signaling is

a central regulator of the survival and migration of NSC grafts after SCI. Our study may lead to development of novel strategies to enhance survival of NSC grafts for spinal cord regeneration.

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T2032

NEURAL CELLS THAT CARRY A GENETIC VARIANT ASSOCIATED WITH SERIOUS MENTAL ILLNESS DISPLAY DECREASED EXPRESSION OF TRANK1 THAT IS RESCUED BY SODIUM VALPROATE

Jiang, Xueying, NIMH/NIH, Bethesda, MD, U.S.

Biological characterization of genetic variants identified in genome-wide association studies (GWAS) of neuropsychiatric disorders remains a substantial challenge. Here we used human induced pluripotent stem cells (hiPSC) and their neural derivatives to characterize a common variant associated by GWAS with both bipolar disorder (BD) and schizophrenia. We measured the relationship between genotype and expression of the nearby gene, TRANK1, tested the impact of commonly used therapeutic agents, and assessed expression of correlated genes and gene networks. HiPSC-derived neural progenitor cells carrying the risk allele of the SNP, rs9834970, displayed lower baseline TRANK1 mRNA expression that was rescued by chronic treatment with therapeutic dosages of valproic acid (VPA). VPA had no impact on TRANK1 expression in cells carrying no risk alleles. Both VPA treatment and TRANK1 knockdown by shRNA suggested reciprocal regulation by histone deacetylases. This study illustrates an efficient strategy to characterize GWAS findings functionally and shed light on therapeutic mechanisms.

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T2034

THE THERAPEUTIC IMPACT OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURAL PRECURSOR CELLS IN CERVICAL SPINAL CORD INJURY

Khazaei, Mohamad[1], Nakashima, Hiroaki[2], Nagoshi, Narihito[2], Satkunendrarajah, Kajana[2], Badner, Anna[2] and Fehlings, Michael G.[2], [1]Toronto Western Hospital, Toronto, ON, Canada, [2]University Health Network, Toronto, ON, Canada

Transplantation of induced pluripotent stem cell derived neural precursor cells (iPSC-NPCs) is a promising therapeutic strategy for spinal cord injury (SCI), but significant challenges remain regarding neuronal integration, survival and functional connectivity. GDNF is a potent neurotrophic factor that promotes survival and integration of graft cells. To increase the survival and integration of graft cells, we have engineered hiPSC-NPCs using piggyback vectors to express GDNF. GDNF-expressing hiPSC-NPCs were transplanted into the spinal cords of a rodent model of moderate contusion SCI at the cervical level 6/7 two weeks after injury while GFP-expressing hiPSC-NPCs transplanted as control. Cell grafts in both groups were localized rostrocaudally surrounding the lesion through white and grey matter with significantly better survival and integration of GDNF expressing cells compared to control (21.3±2% vs. 12.23±3%) at 8 weeks after transplantation. Although a considerable subset of transplanted cells in both groups remained undifferentiated (Pax6$^+$ and Nestin$^+$; 36% in GFP group vs 27% in GDNF group) the majority of cells differentiated to all three neuro-glial lineages with grater oligodendroglial profile (APC$^+$, Olig2$^+$) in GDNF group (56% vs 52%). In GDNF group, transplanted cells formed longer NF-H$^+$ fibers than in GFP-hiPSC-NPCs. Axonal tracing showed a significant increase of biotin dextran amine positive corticospinal tract fibers in GDNG-cell transplanted animals caudally to the lesion site. Furthermore, transplantation of GDNF expressing hiPSC-NPCs resulted in increased survival of host motor-neurons, reduced cavity size, better axonal conduction (by *in vivo* electrophysiological), improved forelimb grip strength and fine motor control during locomotion compared to GFP expressing hiPSC-NPCs. These results mark an important step forward to improve hiPSC-NPC transplantation outcomes by optimizing transplanted cell survival and fate.

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T2036

PSA-NCAM-POSITIVE NEURAL PRECURSOR CELLS ENHANCE MOTOR FUNCTION AND PROMOTE NEURAL TISSUE REPAIR IN RAT MODELS OF NEUROLOGICAL DISEASES

Yang, Wonsuk[1], **Lee, Dongjin R.**[1,2], Ji, Eunhyun[1] and Kim, Dong-Wook[1,3], [1]Yonsei University College of Medicine, Seoul, Korea, South, [2]Brain Korea 21 PLUS Project for Medical Science, Seoul, Korea, South, [3]Department of Physiology, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-g, Seoul, Korea, South

While no effective cure exists for treating ischemic brain damage and spinal cord injury (SCI), homogeneous culture of neural precursor cells (NPCs) are being considered for a promising therapeutic alternative. Despite many ef-

forts, human pluripotent stem cells (hPSCs) derived NPCs have repeatedly produced tumors in animal models of neurological diseases even though pluripotent cells were not detected. We found that polysialic acid-neural cell adhesion molecule (PSA-NCAM)-negative cells among the early neural rosette derived NPCs are tumorigenic, whereas PSAN-CAM-positive NPCs are non-tumorigenic. It prompted us to investigate the therapeutic potential of PSA-NCAM-positive NPCs expressing neural markers SOX1, SOX2, NESTIN, and MSI1 in a rat stroke model or a rat SCI contusion model, respectively. Interestingly, transplanted PSA-NCAM-positive NPCs principally differentiated into TUJ1$^+$ or MAP2$^+$ neuronal cells, which incorporated well into host tissue. Therapeutically, transplanted PSANCAM-positive NPCs proved to improve locomotor activity, as compared to mesenchymal stem cells (MSCs) or phosphate buffered saline (PBS). In addition, glial activation decreased, whereas α-SMA$^+$ and ANGPT1$^+$ angiogenic activity increased, suggesting the formation of micro-milieu favorable for neural tissue repair following PSA-NCAM-positive NPCs transplantation. Taken together, the present findings support the idea that PSA-NCAM-positive NPCs can be therapeutically applied to treat neurological diseases encompassing ischemic stroke and SCI.

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T2038

DIRECTED DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO ASTROCYTES FOR THE STUDY OF SPINAL MUSCULAR ATROPHY

Loh, Sharon, Genome Institute of Singapore, , Singapore and Stanton, Lawrence, Genome Institute of Singapore, Singapore

Spinal Muscular Atrophy (SMA) is a neuromuscular childhood disease. SMA arises due to the mutation of *SMN1*. Motor Neurons (MNs) are particularly sensitive to the low levels of functional SMN protein and they selectively degenerate. In SMA mice models, overexpression of the SMN protein in MNs alone did not confer extended survival or increase muscle mass. However, when SMA protein levels were increased in both MNs and astrocytes, SMA mice displayed improved survivability and muscle mass, indicating the importance of astrocytes in SMA pathology. To study the roles of astrocytes in SMA pathology in human genetic background, we established an efficient method of generating SMA and WT astrocytes from induced pluripotent stem cells (iPSCs). We are performing studies such as glutamate uptake tests and calcium imaging on the iPSCs-derived astrocytes to uncover functional differences between WT and SMA conditions. We are also examining the levels of intracellular reactive oxygen species to determine the presence of cellular oxidative stress in iPSCs-derived astrocytes, which is an important indicator of astrocytes pathology. We hypothesize that iPSCs-derived SMA astrocytes will exhibit functional abnormalities and have the propensity of inducing cell death of the surrounding MNs in SMA conditions.

T2040

A HIGH THROUGHPUT APPROACH TO DRUG DISCOVERY FOR THE TREATMENT OF MULTIPLE SCLEROSIS

Moore, Katharine Cynthia, Vergani, Naja, Beyer, Brittney, Plaisted, Warren, Lairson, Luke and Deshmukh, Vishal, California Institute for Biomedical Research, La Jolla, CA, U.S.

Multiple sclerosis (MS) is a chronic autoimmune disease characterized by degeneration of the myelin sheath and loss of oligodendrocytes, the myelinating cell type in the central nervous system (CNS). Current FDA-approved therapies for MS address only the immune component of the disease, and the majority of patients progress to a stage of accumulating disability despite treatment. Oligodendrocyte progenitor cells (OPCs) are a type of stem cell in the CNS with the ability to differentiate to oligodendrocytes. However, in patients with MS, OPCs fail to differentiate and do not remyelinate damaged axons, leading to a number of debilitating neurological deficits. Thus, there is an unmet clinical need for a therapeutic strategy that addresses progressive demyelination by increasing the number of myelin-competent oligodendrocytes in the CNS. The goal of this project is to identify drug-like small molecules that selectively induce differentiation of OPCs to mature, myelinating oligodendrocytes. To that end, we have utilized a high content imaging-based phenotypic assay to screen a collection of 80,000 novel and structurally diverse compounds. Increased levels of myelin basic protein (MBP), a main component of the myelin sheath, were used as a surrogate marker for differentiation in cultured primary rat optic nerve-derived OPCs. Our primary screen produced a hit rate of .42% and secondary screening to validate potential hits is currently underway. Confirmed hits will be used in an *in vitro* co-culture assay to demonstrate bona fide myelination of neurons. Cumulatively, these results will serve as a starting point for drug design while providing new biological targets for treating MS.

T2042

MODELING HAND-ASSOCIATED NEUROTOXICITY WITH HIPSC-DERIVED NEURONS

Nerenberg, Maxwell James, Sanchez, Ana and Kaul, Marcus, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, U.S.

HIV-1 infection has been linked to neuronal death which causes mental decline, loss of brain functions, and HIV-associated neurocognitive disorders (HAND). It has been shown that the presence of HIV in the central nervous system is caused in part by migration of peripheral monocyte-derived macrophages (MDM) into the brain. Infected or activated MDM produce pro-inflammatory molecules that cause neural injury and eventually lead neurons to undergo apoptosis likely causing HAND. This study aims to assess toxic effects in human iPSC-derived neurons in the contexts of HIV infection. For this study, the iCell Neurons (Cellular Dynamics) were plated on a 384 well plate using Complete Maintenance Medium. The medium was changed 24 after and then replaced with 40ul/well every 3-5 days. The survival and death of the cells was determined through immunocytochemistry staining for neuronal dendrites with rabbit MAP-2 at 1:200 and mouse Tuj-1 at 1:100 in combination with Hoechst at 1:100 for nuclei staining. After incubation over night at 4°C, cells were incubated with fluorescence antibodies using Rhodamine Red X (Jackson 711295152) and Alexa 488 (Invitrogen A11029) both at a concentration of 1:1000. To calculate cell death and overall neurotoxicity, the DNA numbers were compared side by side to the cell body numbers. This test was performed using supernatants from infected and un-infected MDM of four different donors at two concentrations of supernatant; 10% sups and 2.5% sups. Fluorescence imaging and cell counting suggested that the supernatant at 2.5% proved to be more neurotoxic and caused more neuronal cell death than the supernatant at 10%. When viewing the images of the 2.5% supernatant test, there were a lot of visible broken axons and vacuoles in the cell body and dendrites, modelling the neurotoxic effect of the viral infection in the brain. Future goals are to analyze the response of the human neurons to toxic effects and identify factors that allow for neural resistance to the toxicity from HIV infection.

T2044

CELLULAR AND GENETIC MECHANISMS UNDERLYING VARIATIONS IN PRIMATE CEREBRAL CORTEX SIZE

Otani, Tomoki[1], Marchetto, Maria Carolina[2], Gage, Fred H.[3], Simons, Benjamin David[1,4] and Livesey, Frederick J.[1], [1]Wellcome Trust /CRUK Gurdon Institute, Cambridge, U.K., [2]Salk Institute, La Jolla, CA, U.S., [3]Salk Institute for Biological Studies, La Jolla, CA, U.S., [4]University of Cambridge, Cambridge, U.K.

The cerebral cortex is the integrative and executive centre of the central nervous system, making up over three-quarters of the human brain by volume. The drastic increase in cerebral cortex size is thought to underlie the difference in cognitive ability between humans and non-human primates. However, the cellular and genetic mechanisms that have allowed this expansion during evolution are poorly understood. In this study, we used differentiated human and non-human primate pluripotent stem cells (PSCs) in adherent 2D and organoid 3D cultures to replay and compare key developmental stages of cerebral corticogenesis between species. We identified proliferative behaviours unique to human cortical progenitors that contribute to the enlargement of the human cerebral cortex. Compared to cortical progenitors derived from macaque PSCs, human progenitors balanced extra rounds of progenitor expansion with early neurogenesis to generate much larger clonal output. We co-cultured progenitors from different species in chimera experiments and demonstrated that this mechanism is regulated in a cell autonomous manner. In order to identify differentially expressed genes (DEGs) that could explain this difference in proliferative behavior, we have generated and compared transcriptome data from human and macaque cortical progenitors. Preliminary results from functional studies overexpressing candidate human DEGs in macaque progenitors are starting to unravel key genetic mechanisms regulating the evolutionary enlargement of the human cerebral cortex.

T2046

GDAP1 AFFECTS MITOCHONDRIAL DYNAMICS AND FUNCTIONS STUDIED IN MOTONEURONS DERIVED FROM PLURIPOTENT STEM CELLS

Pouya, Alireza[1] and Methner, Axel[1,2], [1]Focus Program Translational Neurosciences (FTN), Johannes Gutenberg University Mainz/ University Medical Center, Mainz, Germany, [2]Rhein-Main-Neuro-Zentrum (rmn2), Universitätsmedizin der Johannes Gutenberg-Universität, Klinik und Poliklinik für Neurologie, Mainz, Germany

Mutations in the gene *GDAP1* (Ganglioside Induced Differentiation Associated Protein 1) are responsible for recessive (CMT4A) and dominant (CMT2K) Charcot-Ma-

rie-Tooth disease, a hereditary peripheral neuropathy disease affecting motor and sensory neurons, indicating that GDAP1 is essential for the viability of cells in the peripheral nervous system. The aim of this project was to study the role of GDAP1 in more relevant cellular model systems; in motoneurons derived from mouse embryonic and patient-derived induced pluripotent stem cells and in human neuroblastoma SH-SY5Y cells. Motoneurons were generated from pluripotent stem cells by over expression of the transcription factors, Ngn2, Isl1 and Lhx3 (the NIL factors) that are sufficient to rapidly and efficiently achieve a spinal motor neuron identity. We over-expressed wildtype and mutant GDAP1 together with genetically-encoded reporters of the cellular metabolic state like ATP, lactate, and glutathione as well as reporters for mitochondrial [Ca2^{+}], age and mitophagy. Mitochondrial morphology, membrane êψ and superoxide production were quantified by specific dyes. Gdap1 knockdown led to an elongated mitochondrial whereas overexpression significantly increased the lactate production in the pluripotent stem cells derived motoneurons. Our results suggest that GDAP1 is a sensor protein, which transmits information about the cytosolic redox state to the mitochondria by altering the mitochondrial fission/fusion-equilibrium and shifts energy production away from oxidative phosphorylation towards glycolysis or the hexose monophosphate shunt.

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T2048

DIRECT CONVERSION OF HUMAN PERIPHERAL BLOOD CELLS INTO STABLY EXPANDABLE MULTIPOTENT INDUCED NEURAL STEM CELLS (INSCS)

Sheng, Chao[1,2], Wiethoff, Hendrik[1], Jungverdorben, Johannes[1,3], Kesavan, Jaideep[1], Lin, Qiong[4], Fischer, Julia[1], Hebisch, Matthias[1,2], Wagner, Wolfgang[4], Peitz, Michael[1,2] and Brüstle, Oliver[1,2], [1]Institute of Reconstructive Neurobiology, University of Bonn, 53127 Bonn, Germany, [2]German Center for Neurodegenerative Diseases (DZNE), 53175 Bonn, Germany, [3]Institute of Reconstructive Neurobiology, Bonn, Germany, [4]Helmholtz-Institute for Biomedical Engineering, RWTH Aachen University Medical School, 52074 Aachen, Germany

Reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) represents an attractive approach to generate donor cells for disease modelling, drug discovery, and neuro-restorative approaches. However, generation and subsequent differentiation of human iPSCs is time-consuming, and the extended cultivation periods may result in the acquisition of mutations. Here, we present a robust approach for the direct conversion of human peripheral blood cells into homogenous and transgene-free induced neural stem cells (iNSCs). After transduction of the donor cells with non-integrating Sendai viruses expressing Sox2 and c-MYC, neuroepithelium-like iNSC colonies emerge within a week in chemically defined conditions. iNSCs express early neuroectodermal markers including PAX6, SOX2, NESTIN, PLZF and ZO-1 as well as the neural crest markers AP2α and HNK1. They are stably self-renewing and multipotent at single cell level and respond to instructive patterning and differentiation cues promoting, e.g., specification of neuronal and glial subtypes including spinal motoneurons, midbrain dopamine-like neurons and oligodendrocytes. iNSC-derived neuronal cultures exhibit mature electrophysiological properties including spontaneous postsynaptic currents indicating functional synapse formation. iNSCs display global gene expression and DNA methylation profiles similar to those of PSC-derived neural cells. However, unlike the embryonic-like age signatures in PSC-derived neural cells, iNSCs at least partially preserve age-associated epigenetic signatures and can be used for modelling pathological protein aggregation in late-onset neurodegenerative diseases such as spinocerebellar ataxia type 3 (SCA3; Machado–Joseph disease). Human peripheral blood cell-derived iNSCs thus represent a promising alternative patient-specific cellular resource for disease modelling, regenerative medicine and other biomedical applications.

T2050

ANTI-APOPTOSIS PREVAILS IN EARLY DIFFERENTIATION OF HUMAN NEURAL STEM CELLS

Tong, Zhi-Bin and Gerhold, David L., NCATS/NIH, Rockville, MD, U.S.

Apoptosis is known to play a role in neurogenesis during brain formation in mammals. Neurogenesis and apoptosis are highly regulated cellular processes that interact to shape brain development. However, elucidating the regulation of apoptosis during neurogenesis is extremely difficult since there are so many proteins to regulate these cellular events independently and by cross-talk. In this study, we cultured human neural stem cells (hNSC) and induced a spontaneous differentiation for 7 days as a cellular model to investigate a hypothesis that apoptosis is down-regulated during early hNSC differentiation. In a gene array for human neurogenesis, most of the 84 genes demonstrated a progressive increase in gene expression during the first week of the hNSC differentiation, suggesting an active participation of genome activation in neural differentiation. In the array for apoptotic gene expression, we showed that several pro-apoptotic genes decreased their expression significantly such as *BAX, BFAR, BID, BIRC5* and *FAS,* while anti-apoptotic genes

such as *BCL2* and *NAIP* increased their expression after one week of hNSC differentiation. This suggests that neural development is protected from apoptosis early during the hNSC differentiation. Indeed, we confirmed the intracellular caspase 3/7, a biomarker for apoptotic activity, was much lower (<50%) in the differentiating hNSC than the undifferentiated hNSC cells after they were treated with chemical compounds. The differentiating hNSC cells became more resistant to 6-hydroxydopamine (6-OHDA) and hexachlorophene (HCP) to induce apoptotic cell death, although the sensitivities to most of the tested 32 compounds for inducing cell death were comparable between undifferentiated and differentiating hNSC cells. Using the apoptosis-inducing chemicals to treat the cells for 24 hours at a threshold dosage that did not cause obvious cytotoxicity, we demonstrated that the onset of neurogenic gene expression was significantly suppressed by cytosine arabinoside (Ara-C, 50 nM) and malachite green oxalate (MGO, 50 nM), further indicating that threshold levels of these apoptotic-toxicants had a negative impact on neurogenesis in this hNSC model. Taken together, this study suggests that the apoptotic pathways are down-regulated to protect the hNSC early differentiation.

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T2052

IDENTIFICATION OF A NEW NEURONAL LINEAGE SPECIFIER, GLYPICAN 4, USING HIGH-CONTENT IMAGING OF STEM CELL-DERIVED NEURONS

Weiss, Stefan[1], Desbordes, Sabrina Catherine[1,2], Schorpp, Kenji[1], Hadian, Kamyar[1], Sandholzer, Michael[1] and Irmler, Martin[1], [1]Helmholtz Zentrum Munich, Neuherberg, Germany, [2]Institute of Developmental Genetics, Helmholtz Zentrum Munchen, Neuherberg, Germany

Autologous transplantation of stem cell-derived neurons might be a powerful approach for the future treatment of neurodegenerative diseases. Current protocols of pluripotent stem cell neuronal differentiation mimic neurodevelopment in a dish using either activation or inhibition of various developmental signaling pathways. Although sophisticated protocols lead to desired cell types, a main limitation is the low differentiation efficiency to specific neural subtypes. It is therefore desired to identify new members involved in neuronal subtype specification to increase differentiation efficiency. In order to identify new neural lineage specifiers, we developed a high-content imaging assay to quantify pluripotent stem cell-derived dopaminergic (TH+) and cortical (CTIP2+) neural subpopulations. We then targeted candidate genes by RNA interference and analyzed their effect on the differentiation efficiency to these subpopulations. Our highly sensitive assay allowed us to identify Glypican 4, a membrane-anchored heparan sulphate proteoglycan, as a major regulator of cortical lineage specification. Deeper analysis demonstrated that the decrease of cortical neurons in Glypican 4-knockdown cells is accompanied by an increase of the dopaminergic neuron subpopulation. We showed further, that Gpc4 mRNA is upregulated during cortical but not during dopaminergic neuron differentiation. These results gave evidence that Glypican 4 is a neuronal subtype decision gene and is required for cortical neuron differentiation. Additionally, we demonstrated that Glypican 4 regulates early mouse neurodevelopment with a specific alteration of the Six gene family expression. Finally, our in vivo data confirmed its role as a regulator of mammalian forebrain development, through the regulation of the Hh/Wnt pathways. High resolution 3D imaging allowed us to study in detail the cyclopic phenotype of the Glypican 4 KO embryos and to demonstrate the reminiscence of this KO embryos phenotype to human holoprosencephaly. Taken together, our results strongly support a role of Glypican 4 in neuronal subtype specification and open doors for novel therapeutic applications in neurodegenerative diseases.

T2054

BRAF MUTATION LEADS TO DISTINCT PHENOTYPES IN CFC PATIENT-DERIVED IPSCS DIFFERENTIATED INTO GLUTAMATERGIC CORTICAL NEURONS AND GABAERGIC INTERNEURONS

Yeh, Erika[1], Wu, Zhi Yong[1], Mendoza-Camacho, Federico[1], Santoshi, Kandalam M[1], Rauen, Katherine A[2] and Weiss, Lauren A[1], [1]University of California, San Francisco, San Francisco, CA, U.S., [2]University of California Davis, CA, U.S.

Induced pluripotent stem cells (iPSC) are a powerful tool to investigate disease mechanisms, especially when the affected tissue is difficult to access, e.g. brain. Cardiofaciocutaneous syndrome (CFC) is a rare monogenic disorder caused mainly by mutations in *BRAF* with several neurological/ behavioural findings: intellectual disability (>80%), autism (60%), and seizures (45%). We hypothesized that CFC mutation *BRAF* Q257R affects excitatory-inhibitory imbalance, linked to autism and epilepsy. We differentiated iPSC lines derived from 4 CFC patients and compared them with 3 sex- and age-matched controls, following three established protocols: 1) differentiation into a mixed neural population, 2) directed differentiation into glutamatergic cortical excitatory neurons, and 3) directed differentiation into GABAergic inhibitory interneurons. In the first mixed population protocol, we observed imbalance in the neuronal types generated in CFC: they had a 3.3-fold increase in deep layer glutamatergic neu-

rons (TBR1+, FOXP2+; p<0.0001) and a 2-fold decrease in upper layer glutamatergic neurons (CUX1; p=0.003) compared to controls. Moreover, CFC had a 3.2-fold increase in interneurons (GABA+; p=0.03). However, the glutamatergic neuron directed differentiation protocol did not show a difference in the proportion of deep layer (TBR1+, FOXP2+) or upper layer neurons (CUX1+) between CFC and controls. Under this protocol, morphologically, CFC glutamatergic neurons had more neurites (p=0.003) and branching (p=0.014). The interneuron directed differentiation protocol also did not show difference in the percentages of GAD67+, NKX2.1+ or GABA+ cells between CFC and control lines; yet CFC interneurons had a decrease in the number of neurites per cell (p=0.014). Our results show that neural induction of patient-derived iPSC lines produces imbalance of the different neuronal types in vitro; but not when exposed to specific cues towards a defined neuronal fate. However, directed differentiation into specific neuronal lineages highlighted the morphological differences of CFC neurons, which are cell type dependent. Thus, we show that different protocols highlight different aspects of the cellular phenotype of patient-derived iPSC cultures and must be carefully selected for the intended experimental design.

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T2056

HUMAN CEREBRAL ORGANOIDS AS A MODEL SYSTEM FOR NEURAL DEVELOPMENT

Watanabe, Momoko[1], Buth, Jessie E.[2], Vishlaghi, Neda[3], Taxidis, Jiannis[2], Torre-Ubieta, Luis de la[4], Pearson, Caroline[2], Golshani, Peyman[2], Geschwind, Daniel[4] and Novitch, Bennett G.[1], [1]University of California, Los Angeles, /Broad Stem Cell Research Center, Los Angeles, CA, U.S., [2]University of California, Los Angeles, Los Angeles, CA, U.S., [3]University of California, Los Angeles, CA, U.S., [4]University of California, Los Angeles, Neurogenetics Program, Los Angeles, CA, U.S.

The neocortex is a highly organized tissue, which enables complex sensory activities and higher cognitive functions. Marked enlargement of the neocortex is one of the factors that endow humans and other primates with unique intellectual capacities. Recent studies have proposed that a key contributor of neocortical growth in primates is a prominent expansion in the production of basal progenitors (BPs) in the subventricular zone (SVZ). However, little is known about molecular mechanisms through which BP expansion is achieved. To model this aspect of human development, we created a three-dimensional "organoid" cell culture system from human pluripotent stem cells (hPSCs) that exhibit features of the developing human cortex in vivo. Our established organoid technique was efficient and reproducible from couple lines e.g. H9 and Hips2 with specific maintenance conditions, with >80% of structures expressing the forebrain marker FOXG1 and exhibiting apicobasal neuroepithelial architecture, recapitulating the laminar organization of the developing neocortex. We also examined the similarity of the organoid transcriptome to bone fide human fetal brain reference sets using the Classification of Neuroanatomical and Temporal Expression via Transcriptomics (CoNTExT) analytic algorithm. An expansion in BP-like cells and expression of lower and upper layer neuronal markers were also observed in this cell culture system. Furthermore, using optogenetic sensors of neuronal activity, we find signs of network activities. Lastly, we demonstrate that gene expression can be manipulated in organoid structures using DNA electroporation. Together, these data define neocortical organoid culture methods that can faithfully and reliably recapitulate salient features of human cortical development and serve a convenient experimental platform for investigating the mechanistic details behind human brain development and disease.

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T2058

A COMPARATIVE STUDY OF NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS ON ALIGNED AND RANDOMIZED ELECTROSPUN POLY-LACTIC CO-GLYCOLIC ACID SCAFFOLDS

Sperling, Laura Elena[1], Reis, Karina[1], Pozzobon, Laura[1], Girardi, Carolina[1] and Pranke, Patricia[1,2], [1]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Stem Cell Laboratory, Fundamental Health Science Institute, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [2]Institute for Research with Stem Cells, Porto Alegre, Brazil

Tissue engineering applications for nervous tissue require a reliable source of cells and an appropriate supportive three dimensional (3D) matrix. Embryonic stem cells (ESCs) have gained considerable attention because of their potential use in cell-based transplantation therapy in many neurodegenerative diseases and nervous system injuries. In this study, neural differentiation of mouse embryonic stem cells (mESCs) was investigated in combination with 3D electrospun nanofibers as a substitute for the extracellular matrix (ECM). The main objective of this study was to compare the differentiation of mESCs seeded on randomly and aligned nanofibers. Scaffolds of random and aligned nanofibers of poly lactic co-glicolic acid (PLGA) were produced by the electrospinning technique using an 18% polymer solution. The mESCs were induced to differentiate for 10 days by the formation of embryoid bodies (EBs) and the selection of neural precursors with

a neural specific media The EBs were cultivated either on gelatin coated plates or on PLGA fibers in order to study the effect of the polymer and fiber orientation on cell survival, morphology and differentiation. Cell survival was analyzed by live dead assay and confocal microscopy. The morphology was analyzed by scanning electron microscopy and neural specific protein expression was evaluated by flow cytometry and immunocytochemistry. Fibers with an average diameter of 1.7 μm were produced by electrospinning using a solution of 18% PLGA. The cells cultivated on the PLGA scaffolds showed a better survival capacity than the cells on gelatin. On the random fibers the cells stretched across multiple directions, while the cells cultivated on the aligned fiber exhibited growth along the fibers, leading to better cell migration. The cells on the two types of scaffolds showed neural marker expression, as seen by the analyzing expression of nestin, beta 3 tubulin, O4 and neurofilament M. The cells on the PLGA scaffolds were successfully differentiated into neural phenotype; however, the random electrospun fibers downregulated the precursor neural marker nestin and neuronal marker TUJ when compared with the othr two fiber groups. In conclusion, aligned electrospun fiber scaffolds are better candidates for promoting and supporting ESC differentiation into neural progenitor cells.

Funding Source: CNPq, CAPES, FAPERGS, Stem Cell Research Institute

REPROGRAMMING

T2060

CONVERSION OF HUMAN FIBROBLASTS TO STABLY SELF-RENEWING NEURAL STEM CELLS WITH A SINGLE ZINC-FINGER TRANSCRIPTION FACTOR

Shahbazi, Ebrahim[1], Moradi, Sharif[2], Nemati, Shiva[2], Satarian, Leila[2], Basiri, Mohsen[2], Gourabi, Hamid[2], Zare Mehrjardi, Narges[3], Günther, Patrick[4], Lampert, Angelika[5], Händler, Kristian[4], Hatay, Fulya[6], Schmidt, Diana[7], Molcanyi, Marek[8], Hescheler, Jürgen[6], L. Schultze, Joachim[4], Saric, Tomo[6] and **Baharvand, Hossein**[2], [1]Royan Institute for Stem Cell Biology and Technology, Tehran, Iran, [2]Royan Institute, Tehran, Iran, [3]institute for neurophysiology, Cologne, Germany, [4]Life and Medical Sciences Institute, Department for Genomics and Immunoregulation, University of Bonn, Bonn, Germany, [5]Institute of Physiology, RWTH, Aachen University, Aachen, Germany, [6]Institute for Neurophysiology, University of Cologne, Cologne, Germany, [7]Institute of Physiology and Pathophysiology, Friedrich-Alexander-University of Erlangen- Nürnberg, Erlangen, Germany, [8]Center for physiology and pathophysiology, Cologne, Germany

Direct conversion of somatic cells into neural stem cells (NSCs) by defined factors holds great promise for mechanistic studies, drug screening, and potential cell therapies for different neurodegenerative diseases. Here, we report that a single zinc-finger transcription factor, Zfp521, is sufficient for direct conversion of human fibroblasts into long-term self-renewable and multipotent NSCs. In vitro, Zfp521-induced NSCs maintained their characteristics in the absence of exogenous factor expression and exhibited morphological, molecular, developmental, and functional properties that were similar to control NSCs. Additionally, the single seeded induced NSCs were able to form NSC colonies with efficiency comparable to control NSCs and expressed NSC markers. The converted cells were capable of surviving, migrating and attaining neural phenotypes after transplantation into neonatal mouse- and adult rat brains, without forming tumors. Moreover, the Zfp521-induced NSCs predominantly expressed rostral genes. Our results suggest a facilitated approach for establishing human NSCs through Zfp521-driven conversion of fibroblasts.

T2062

DISSECTING THE UNDERLYING MECHANISM OF RESETTING PRIMED PLURIPOTENCY TO NAIVE PLURIPOTENCY

Dunn, Sara-Jane[1], Li, Meng Amy[2], Martello, Graziano[3] and Smith, Austin G.[2], [1]Microsoft Research, Cambridge, U.K., [2]Wellcome Trust - Medical Research Council Cambridge Stem Cell Institute, Cambridge, U.K., [3]University of Padova, PADOVA, Italy

Reprogramming to induced pluripotency is a process that can be achieved from diverse cell types in multiple steps. The final step of all such conversions is the establishment of an embryonic stem cell (ESC) like state, which is safeguarded by a network of transcription factors. Activation of this network is required to attain the naïve, pluripotent state, but how this is achieved during reprogramming remains elusive. Epiblast-derived stem cells (EpiSC) are the primed counterparts of naïve ESC, expressing key pluripotency factors and capable of multi-lineage differentiation. They can be reset to the naïve state simply through exposure to the ground state culture conditions, 2i+LIF. However, the efficiency of this transition is limited, and while the enhancement effect of a few transcription factors is known, the molecular process by which conversion occurs has not been characterised. As such, these cells provide a useful experimental model to examine the reprogramming phenomenon, being well characterised, functionally distinct, yet developmentally close to the naïve state. Using EpiSC to ESC reprogramming as a cellular model, we sought to derive mechanistic insight into the process of naïve transcriptional network activation. Our previous work enabled us to characterise the transcriptional program governing naïve pluripotency. We utilised this understanding, and exploited our formal reasoning methodology encapsulated in the tool RE:IN*, to investigate the dynamics of the activation of this network. Our approach allowed us to predict accurately the relative impact of single and double factor overexpression on conversion efficiency, in addition to the requirement of specific factors for the conversion to be possible. Most importantly, we derived a mechanistic explanation of these results, and ultimately the process by which resetting proceeds, which is amenable to analysis and testing. The understanding and methodology we bring to this domain is anticipated to have impact beyond this specific transition and could be further applied to the study of reprogramming and direct conversion of murine and human cells. *RE:IN can be accessed at www.research.microsoft.com/rein.

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T2064

OPTIMIZING HUMAN PLURIPOTENT CELL CULTURE, DIFFERENTIATION AND REPROGRAMMING BY USING CALLISTO, AN AUTOMATED CELL CULTURE SYSTEM

Guo, Tingxia[1], Boutet, Stephane[1], Gibson, Jason[2], Watson, Michael[1], Devaraju, Naga Gopi[1], Szpankowski, Lukasz[1], Fowler, Brian[1], Norris, Michael[1], Thu, Myo[1], Wong, Michael[1], Harris, Greg[1], Lu, Ying-Wei[1], Johnson, Christian[1], Leyrat, Anne[1], Wang, Xiaohui[1], Sun, Gang[1], West, Jay[1], Unger, Marc[1], Jones, Robert[1], Nelson, Craig[2] and Li, Nianzhen[1], [1]Fluidigm Corporation, South San Francisco, CA, U.S., [2]University of Connecticut, Storrs, CT, U.S.

In recent years, advances in the stem cell field opened many opportunities for disease modeling and cell-based therapies. However, to define the optimal condition for cell expansion, differentiation, and reprogramming remains labor-intensive and costly. To facilitate the exploratory processes, we have developed Callisto™, an automated cell culture system for cell manipulation with environmental control. The system consists of an integrated fluidic circuit (IFC), an electropneumatic controller instrument, experimental designer software and automated run time control software. Each IFC has 32 culture microchambers and 16 reagent inlets. Each microchamber can be dosed separately with different combinations and ratios of up to 16 reagents at various predefined time points. Using this system, we have developed a protocol for reprogramming human somatic cells into pluripotent cells by viral infection. Analysis of pluripotency gene expression demonstrates an efficient reprogramming of human fibroblasts on an IFC, similar to the results on a standard well plate. We are also exploring reprogramming through combinations of different mRNAs, including self-replicating RNA and miRNAs. Reprogrammed cells can be exported live from individual chambers and replated onto standard plates for expansion. Furthermore, on-IFC differentiation assays will be used to confirm pluripotency of the reprogrammed cells. We have demonstrated that human induced pluripotent stem cells (iPSCs) can be directed into primitive lineages of all three germ layers on one IFC within four days by using combinations of small molecules and proteins in chemically defined media. Multiple commercially available differentiation kits are also tested on-IFC. In summary, the automated microfluidic platform employs precise control of the microenvironment of cells, facilitates studies of multifactorial combinations, and enables development of robust, reproducible, and chemically defined cell culture and manipulation.

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T2066

A SIMPLE FUNCTIONAL ASSAY DEMONSTRATING PLURIPOTENCY USING DIRECTED DIFFERENTIATION TO ALL THREE GERM LAYERS IN MONOLAYER CULTURE

Kardel, Melanie Dawn[1], Noort, Rebecca[1], Hadley, Erik[1], Riedel, Michael J.[1], Luu, Yvonne[1], Lee, Vivian[1], Thomas, Terry E.[1], Eaves, Allen C.[1,2] and Louis, Sharon A.[1], [1]STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Assessing the pluripotency of human pluripotent stem cell (hPSC) lines requires a functional demonstration of differentiation into ectoderm, mesoderm and endoderm lineages representing the three germ layers. The current gold standard in vivo teratoma assay is time consuming, costly and requires access to animals and specialized resources. Alternately, spontaneous in vitro differentiation of embryoid bodies (EBs) in serum-containing medium is inefficient and unpredictable in terms of differentiation kinetics and lineages produced, and thus not used as a replacement for the teratoma assay. We have developed a simple kit comprising a monolayer-based culture assay to demonstrate pluripotency using STEMdiff™ Trilineage Ectoderm, Mesoderm and Endoderm Media and protocols specific for each germ layer. Briefly, undifferentiated hPSCs were plated as single cells at 0.5 - 2 x 10^5 cells/cm^2 on Corning® Matrigel® overnight in either mTeSR™1 (for mesoderm, endoderm protocols) or STEMdiff™ Trilineage Ectoderm Medium containing 10 µM Y-27632. Starting the next day, each lineage-specific differentiation medium was fully exchanged daily for the duration of the assay (5 - 7 days). In this new monolayer assay, differentiation was primarily assessed using flow cytometry, achieving high proportions of $PAX6^+Nestin^+$ ectoderm (84.6 ± 3.7%; mean ± SEM; n=12), T^+ mesoderm (93.4 ± 1.4%), and $SOX17^+CXCR4^+$ endoderm (88.1 ± 1.6%). Qualitatively, similar proportions of the respective differentiated cells were observed by immunocytochemistry. Microarray analysis of cells from the separate differentiation cultures showed specifically upregulated markers of the anticipated lineage and decreased markers of undifferentiated cells. Similar analysis of EBs generated from the same hPSCs and differentiated in serum-containing medium primarily showed upregulation of ectoderm markers, indicating EB-based spontaneous differentiation protocols are lineage-biased and inaccurate for measuring full hPSC differentiation capacity. In summary, STEMdiff™ Trilineage Differentiation Kit can be used to rapidly assess pluripotency of established hPSC lines and screen potentially large numbers of putative hPSC lines in a more cost-effective and reproducible manner than teratoma assays or spontaneous differentiation protocols.

T2068

LIF, VITAMIN C, BMP4, AND MODIFIED SERUM-FREE ES MEDIA FACILITATE RAPID ACTIVATION OF NAIVE CELL PROGRAMS FROM PRIMED AND SOMATIC CELLS

Kime, Cody, Kyoto University of Medicine, CDB/RIKEN, Kobe, Japan

Early embryonic cell development loses homogenous definition as inner and outer cell lineages exit the totipotent 8-cell stage. Inner cells assemble the naïve state inner cell mass embryonic lineage, from which naive pluripotent embryonic stem cells (ESCs) are derived, and progress forward toward a later stage implanted blastocyst's flattened epiblast stem cell 'primed' state, from which primed pluripotent epiblast stem cells (EpiSCs) are derived. EpiSCs cannot form chimeras in preimplantation embryos as they hold disparate intrinsic epigenetics, reduced plasticity pluripotency, and require different cytokines. As in-utero animals develop, cells diverge through germ layers toward somatic fates such as fibroblasts, and further establish analogous disparate intrinsic epigenetics and environmental requirements. Although primed pluripotent cell state is proximal to naive state, conversion (reversion) of primed EpiSCs to naive ESC-like cells is as inefficient as reprogramming of somatic differentiated fibroblasts to pluripotent stem cells with the transcription factors OCT3/4, SOX2, KLF4 and cMYC. Here, we have identified combinations of cytokines and bioactive compounds that support efficient and rapid activation of naïve cell programs from primed EpiSCs and from fibroblasts. Some cytokines and bioactive compounds used are the same between the conversion and reprogramming experiments. These molecules have been previously associated with naïve-state maintenance and implicated in low efficiency conversions and reprogramming toward naivety with chemical inhibitors and/or gene manipulation. The efficiencies both from EpiSCs and from fibroblasts further increased by modifying basal cell medium. We observed a cooperative activity among these molecules and media that intersect key gene regulatory networks that establish definitive naivety. We will discuss differences and similarities in the combination of the molecules, and importance of basal medium for conversion and reprogramming.

T2070

STIFFNESS OF HYDROGELS REGULATES EFFICIENCY OF CELLULAR REPROGRAMMING THROUGH MESENCHYMAL-TO-EPITHELIAL TRANSITION AND PLURIPOTENCY MARKERS

Choi, Bogyu[1], Park, Kwang-Sook[1,2], Kim, Ji-Ho[1], Ko, Kyoung-Won[1], Kim, Jin-Su[1], Han, Dong Keun[2] and **Lee, Soo-Hong**[1], [1]CHA University, Seongnam-si, Gyeonggi-do, Korea, [2]Korea Institute of Science and Technology, Seoul, Korea

The stiffness of hydrogels has been reported to direct cell fate. Here, we found that the stiffness of hydrogels promotes the reprogramming of mouse embryonic fibroblasts into induced pluripotent stem cells (iPSCs). We prepared cell culture substrates of various stiffnesses (0.1, 1, 4, 10, and 20 kPa) using a polyacrylamide hydrogel. We found that culture on a soft hydrogel plays an important role in inducing cellular reprogramming into iPSCs via activation of mesenchymal-to-epithelial transition and enhancement of stemness marker expression. These results suggest that physical signals at the interface between cell and substrate can be used as a potent regulator to promote cell fate changes associated with reprogramming into iPSCs, which may lead to effective and reproducible iPSC-production.

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T2072

IN VIVO LINEAGE REPROGRAMMING OF CELLS ACROSS GERM LAYERS

Campbell, Clyde[1], Lancman, Joseph[1], **Monroe, Cambria Rand**[2] and Dong, Duc Si[1], [1]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, U.S., [2]Sanford Burnham Prebys Medical Discovery Institute, San Diego, CA, U.S.

Recent studies using in vivo cellular reprogramming have shown that through the ectopic expression of certain transcription factors, somatic cells can be induced into a pluripotent state (iPS cells). In a similar fashion, fully differentiated cells can be directly transformed from one cell lineage to another. This transdifferentiation has been accomplished in producing cardio myocytes from cardio fibroblasts, macrophages from B-Cells, and pancreatic Beta-cells from exocrine cells. Despite these advances in cell-fate alterations, in vivo reprogramming of cells has been restricted to those derived from the same germ-layer. The ability to break this barrier and transdifferentiate cells across germ layers will not only help to illuminate the processes governing cell fate, but will also expand the potential applications of in vivo reprogramming, and ultimately provide a powerful tool for the creation of new therapeutic applications. This work describes the initial results of the first successful reprogramming of a mesoderm-derived cell within a live vertebrate model into an endoderm cell. Reprogramming of differentiated mesoderm-derived muscle cells into endoderm cells was accomplished through the forced expression of a specific combination of transcription factors. This was done in transgenic lines to visualize endoderm cells within a live zebrafish model, Danio rerio. By utilizing immunofluorescent imaging, whole mount in situ hybridization, and qPCR data, we can observe the beginnings of direct in vivo transdifferentiation, based on the simultaneous expression of muscle and endodermal markers. This is the first demonstration of in vivo cell fate reprogramming of cells derived from germ layer to that of another, thereby suggesting that somatic cell fate within animals can be altered without limitations.

T2074

MECHANISMS OF TRANSCRIPTION FACTOR-MEDIATED DIRECT REPROGRAMMING OF MOUSE EMBRYONIC STEM CELLS TO TROPHOBLAST STEM-LIKE CELLS

Rhee, Catherine, Beck, Samuel, Lee, Bum-Kyu, Tucker, Haley and Kim, Jonghwan, The University of Texas at Austin, Austin, TX, U.S.

Direct reprogramming, the process of converting one cell type to another by overexpression of defined transcription factors, has remarkable potential for future cell therapies. While a recently proposed concept of pioneer factors provides an understanding of how reprogramming factors activate cell-type specific genes by binding to closed chromatin, it is still elusive if the model is generally applicable to explain various reprogramming systems. Recently, we reported a direct reprogramming factor, Arid3a, which induces mouse embryonic stem (ES) to trophoblast stem (TS)-like cell transition. Arid3a promotes down-regulation of pluripotency genes while up-regulating TS-specific genes during reprogramming. Similar ES to TS-like cell conversions by Cdx2 and Gata3 have been also reported. Since global mapping of these reprogramming factors showed their target occupancy nearby the regulatory elements of both ES- and TS-specific genes, it is plausible that these factors have dual functions mediating selective repression and activation of targets during the reprogramming. However, until now, little has been known about the mechanisms of ES to TS-like cell transition, especially how reprogramming factors repress original cell-type specific genes. Through time-course expression profiling during reprogramming of ES to TS-like cells, we found gradual deactivation of pluripotency factors followed by activation of TS-specific regulators. By mapping

chromosomal targets of Arid3a, Cdx2, and Gata3 during the initial phase of reprogramming, we additionally observed three remarkable aspects of the ES to TS-like cell conversion. First, reprogramming factors strongly occupy open chromatin regions, such as enhancers of active ES cell-specific genes, resulting in repression. Second, these factors also show pioneer factor-like activity as they occupy closed chromatin-associated inactive TS-specific genes, facilitate chromatin opening, and subsequently activate TS-specific genes. Lastly, further analyses of the target occupancy patterns revealed multiple classes of common and unique targets with distinct cellular functions. Taken together, our results illuminate the initial regulatory events mediated by reprogramming factors, and will provide novel insights into the means of cell fate manipulation.

T2076

ENGINEERING SOX FACTORS FOR OCT4-INDEPENDENT REPROGRAMMING

Velychko, Sergiy, MacCarthy, Caitlin, Cojocaru, Vlad and Schöler, Hans R., Max Planck Institute for Molecular Biomedicine, Muenster, Germany

Induced pluripotent stem (iPS) cell technology holds great potential for regenerative medicine and drug discovery. Of the four factors capable of reprogramming somatic cells to pluripotency—Oct4, Sox2, Klf4, and cMyc—Oct4 is the only factor that cannot be replaced by members of its family. Based on structural data, the single residue mutation E57K was found to confer reprogramming ability to the endoderm-specific factor Sox17. In this study, we showed that replacing Sox2 with the Sox17-E57K mutant in the reprogramming cocktail rescues otherwise detrimental Oct4-linker mutations as well as allows reprogramming with other POU factors, such as Brn4 and Oct2. We generated a library of Sox2 and Sox17 chimera proteins and found that a single residue is responsible for the rescuing phenomenon. The residue is located at the Oct-Sox interface and likely modifies the heterodimerization capability of the factors. In addition, we discovered a Sox2-Sox17 chimera that when combined with Oct4, Klf4, with or without cMyc enhances the reprogramming efficiency by up to 30 times comparing to wild type Sox2. Remarkably, this chimeric protein when combined with Klf4 only—i.e. in the absence of any exogenous POU factor—was also able to reprogram fibroblasts into iPS cells. We are currently using in vitro and in silico approaches to study the molecular activity of the key mutation. Furthermore, we are in the process of generating a knock-in mouse to enhance our understanding of the role Sox and POU factors play during mammalian development.

T2078

REGULATION OF SMAD SIGNALING BY SUBSTRATE STIFFNESS IN DIRECT NEURON REPROGRAMMING

Wong, Sze Yue, Soto, Jennifer, Chu, Julia, Park, Hyungju, Poo, Mu-Ming and Li, Song, University of California, Berkeley, CA, U.S.

Direct reprogramming is the conversion of one cell type into a completely different cell type. Although the role of topography has been studied in neuron reprogramming, there currently exists a knowledge gap with regard to the effect of substrate stiffness on the direct conversion of fibroblasts to neurons. Thus, we set out to investigate the effects of substrate stiffness on this process. Within the range of stiffness tested, we were surprised to observe a biphasic trend where an intermediate stiffness was optimal for direct reprogramming into neurons, which was independent of the different ECM protein coatings. The induced neurons reprogrammed on gels were mature and functional. We further observed that both the expression and the phosphorylation of R-SMADs (p-SMADs) were modulated by stiffness. Indeed, inhibition of SMAD signaling via addition of small molecule inhibitors of the ALK receptors enhanced the reprogramming efficiency on substrates expressing high levels of pSMADs, suggesting the involvement of SMAD signaling. Interestingly, addition of siRNA against SMAD ubiquitination regulatory factor (SMURFs), which suppresses R-SMAD degradation, attenuated the reprogramming efficiency on the intermediate stiffness to the basal level. Altogether, our results suggest that substrate stiffness regulates R-SMAD degradation and phosphorylation, which in turn may regulate direct neuron reprogramming, thus providing insights into the role of biophysical factors in this process.

T2080

EFFICIENT GENERATION OF IPS CELLS FROM ADULT HUMAN FIBROBLASTS WITH 5 FACTORS SELF-REPLICATING RNA (5F SRRNA) CONTAINING OCT-4, SOX2, KLF4, GLIS1 AND CMYC

Yoshioka, Naohisa[1], Lu, Min[1], **Chu, Vi T.**[1] and Dowdy, Steven F.[2], [1]MilliporeSigma, Temecula, CA, U.S., [2]University of California, San Diego School of Medicine, La Jolla, CA, U.S.

The generation of induced Pluripotent Stem Cells (iPSCs) has the potential to develop regenerative medicine therapies to treat diseases. We previously devised a RNA-based approach to generate iPSCs that uses a polycistronic synthetic, self-replicating RNA (srRNA) that simultaneously expresses four reprogramming factors, including OCT4, KLF4, SOX2, and GLIS1 or c-MYC (OKS-iG or OKS-iM). We observed in some adult human fibro-

blasts that the four factor (4F) srRNAs, especially OKS-iM, resulted in low yield of iPSCs. To overcome this limitation, we engineered a five factors (5F) srRNA that included OCT4, KLF4, SOX2, GLIS1 and c-MYC (5F srRNA). The 5F srRNA efficiently generated iPSCs from adult human fibroblasts, including a donor from cardiomyopathy patient. Interestingly, 5F srRNA induced LIN28, which was originally used for human iPSC generation with OCT4, SOX2 and NANOG, and frequently used for increasing iPSC generation with Yamanaka Factors (OCT4, SOX2, KLF4 and cMYC). We also observed that 5F srRNA accelerated reprogramming by a week compared to 4F srRNAs. In summary, the 5F srRNA has a significantly greater potential for iPSC generation from difficult and slow growing adult human cells from patient's cells and for cell-based therapy applications.

IPS CELLS

T2082

ELECTROMAGNETIC FIELDS MEDIATE EFFICIENT CELL REPROGRAMMING INTO A PLURIPOTENT STATE

Soon Won Choi[1] and Jongpil Kim[2], [1]Seoul National University, Seoul, Korea, South, [2]Dongguk University, Seoul, Korea, South

Life on earth is constantly exposed to natural electromagnetic fields (EMF) and it is generally accepted that EMF may exert a variety of effects on biological systems. Particularly, extremely low frequency electromagnetic fields (EL-EMFs) affect biological processes such as cell development and differentiation, however, the fundamental mechanisms by which EMF influences these processes remain unclear. Here we show that that EMF exposure induces epigenetic changes that promote efficient somatic cell reprogramming to pluripotency. These epigenetic changes resulted from EMF-induced activation of the histone lysine methyltransferase Mll2. Remarkably, an EMF-free system that eliminates earth's naturally occurring magnetic field abrogates these epigenetic changes, resulting in a failure to undergo reprogramming. Therefore, our results reveal that EMF directly regulates dynamic epigenetic changes through Mll2, providing efficient tool for epigenetic reprogramming including the acquisition of pluripotency.

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T2084

GENERATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS FOR MODELLING NOVEL MUTATIONS OF LONG QT SYNDROME

Cagavi, Esra[1,2], Goktas, Sevilay Sahoglu[1,2], Yildirim, Arzuhan Koc[1,2], Akgul, Tuba[1,2], Gorgulu, Ilayda G.[1,2], Tuzcu, Elif N.[3] and Tuzcu, Volkan[3], [1]Istanbul Medipol University, Regenerative and Restartive Research Centre, Istanbul, Turkey, [2]Istanbul Medipol University, Department of Medical Biology, International School of Medicine, Istanbul, Turkey, [3]Istanbul Medipol University, Department of Pediatric Cardiology, School of Medicine, Istanbul, Turkey

One of the major reasons for mortality in heart disease is due to cardiac arrhythmia. Long QT Syndrome (LQTS) is the most common form of arrhythmia and is seen in 1:2000 people. The underlying mechansim for LQTS is the mutations on ion channels in cardiomyocytes (CM) that result in the prolongation of the QT interval analysed on an electrocardiogram. From 13 different sub-types seen in the clinic, about 40-50% of all LQTS patients fall in Type 1 (T1) and are caused by mutations in KvLQT1 (KCNQ1) K^+ channel protein. In most cases, LQTS is a monogenic disease, however, various mutations in a single ion channel for T1 has been reported to cause genetic and phenotypic variation which also affects efficacy of the treatment. Based on this fact, we generated LQTS-T1 patient-specific CM from induced pluripotent stem cells (iPSC) in order to explore molecular mechanisms of novel mutations. We first performed whole genome and/or exome sequencing of LQTS-T1 patients and healthy relatives for identifying their disease related genetic variants. Next, we collected the peripheral blood mononuclear cells from individuals and transduced via Sendai virus containing four reprogramming vectors. We have established iPSC lines from LQTS-T1 Patient-1 carrying the mutation of KvLQT1 channel protein at p.T322M (c.940C>T), that coincides with the pore region; Patient-2 carrying a novel mutation at the third transmembrane segment and Patient-3 carrying a novel splice site mutation at the intervening sequences. Additionally, we have established two iPSC line from the healthy siblings, who do not carry any mutation in the LQTS-defined genes. We have characterised iPSCs immunocytochemically for pluripotency markers; TRA1-60, SSEA-4, Sox-2 and Oct-4. Next, we have confirmed genomic stability of iPSC clones by karyotype analysis and the developmental potential of clones by teratoma formation. We have generated iPSC-derived CM and currently characterizing electrophysiological and pharmacological properties of patient-specific cardiomyocytes and correcting the mutations by CRISPR/Cas9 strategy. Understanding the molecular basis of these different LQTS mutations would be critical for screening and developing

new therapeutic options and reveal patient to patient clinical and pharmacological variation.

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T2086

SMOOTH MUSCLE CELL PRECURSORS DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS CAN RESTORE URETHRAL FUNCTION IN A URINARY INCONTINENCE RAT MODEL

Chen, Bertha[1], Wen, Yan[2], Wang, Zhe[2,3], Li, Yanhui[2,4], Wani, Prachi[2], Green, Morgaine[2], Wei, Yi[2], Ramamurthi, Anand[5], Swaminathan, Ganesh[5] and Reijo Pera, Renee A.[6], [1]Stanford University School of Medicine, Stanford, CA, U.S., [2]Stanford University School of Medicine, Department Obstetrics & Gynecology, Stanford, CA, U.S., [3]Southern Medical University, Guangzhou, Guangdong, China, [4]Huazhong University of Science and Technology, Wuhan, China, [5]Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH, U.S., [6]Montana State University, Bozeman, MT, U.S.

Stem cell (SC)-based therapies for urinary incontinence (UI) are currently derived from adult stem cell sources. It is thought that muscle- or adipose-derived SCs can restore function to the deficient urethral sphincter commonly associated with UI. The cells derived from these sources are heterogeneous and senescent. It is difficult to characterize these mixtures and expand the sub-populations of cells that enhance efficacy. Human pluripotent SCs (hPSCs), such as human embryonic SCs (hESCs) and induced pluripotent stem cells (iPSCs), are an attractive source of SCs because of their ability to self-renew and to differentiate into all three germ layers. We investigated whether smooth muscle cell precursors (pSMCs) derived from hPSCs can restore urethral function. SUI animal models were generated by urethrolysis and ovariectomy in immuno-deficient rats. hPSCs were differentiated into pSMCs with a chemically defined protocol. pSMCs were derived from hESCs, episomal reprogrammed iPSCs (Epi-iPSC), and viral reprogrammed iPSCs. Rats were divided into treatment groups: control (intact rat), sham saline (surgery+saline injection), bladder SMC (surgery+human bladder SMC injection) and treatment (surgery+pSMC injection). pSMCs were injected peri-urethrally (2×10^6 cells/rat) 3 weeks after surgery. Urethral leak point pressure (LPP) to test urethral function was measured 5 weeks post injection. The SUI model demonstrated significantly lower LPP compared to intact controls 8 weeks after surgery ($P<0.05$). LPP of the iPSC-pSMC treatment groups were significantly higher compared to sham saline ($p<0.04$). In vivo pSMC integration was observed for at least 7 weeks with imaging and histologic staining for human SMCs. In addition to cell integration, histologic comparison of rat urethra between intact rat, sham (surgery+saline), and treatment (surgery+pSMC) groups show differences in elastin fiber pattern and content. Elastin gene expression is decreased in the shams versus controls ($p<0.03$), while treatment restored elastin expression to that of controls. Our data indicate that pSMCs derived from hPSCs can restore the urethra both histologically and functionally. These findings open the possibility of using autologous iPSCs for urologic conditions where smooth muscle cells are needed.

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T2088

FROM HEART TO MIND; CARDIAC AND NEURAL DIFFERENTIATION OF SINGLE EPISOME REPROGRAMMED FIBROBLASTS

Saini, Astha[1], Greenstein, Ashtyn[1], Sterneckert, Jared[2], Reinhardt, Peter[3], Reinhardt, Lydia[2] and **Cohen, Rick I.**[1], [1]Rutgers University, Stem Cell Research Center, Piscataway, NJ, U.S., [2]Technische Universität Dresden, Dresden, Germany, [3]AbbVie Deutschland GmbH & Co. KG, Ludwigshafen, Germany

Four Factor pluripotency reprogramming of somatic cells and subsequent multi-lineage directed differentiation can be most easily accomplished by robust genetically modifying/manipulating methods. More recently non-genetically modifying methods for induction have gained popularity as they lend themselves to translational and eventually clinically related research paradigms. Further, genetic-directed differentiation of somatic cells into other specialized cell types has been somewhat successful, however often limited in the amounts of material that can be produced from starting materials. In this study we developed an optimized single Episomal vector bearing multiple genes interspersed using using foot and mouth disease type “2A” motifs; Oct4-Myo-D-transactivating domain fusion; Sox2; KLF4; L-Myc; RFP Blasticidin-S-Deaminase fusion. This single entity plasmid electroporated into fibroblasts and cultured with optimized media and accompanying small molecule mixtures leads to reproducible reprogramming. Simplifications of xeno-free neuronal and cardiac differentiation methods relies on a 2 step procedure; for neuronal IPSCs first transition into highly expandable floor plate type neuro-epithelial cells (FPNE) using a cocktail of small molecule inhibitors; and secondly FPNE are differentiated into multiple neuronal cell types, specifically dopaminergic neurons in the presence of a cocktail of growth factors and small molecules. For cardiac differentiation, a robust upregulation of the TCF pathway using GSK-3-β inhibitor followed by a second step using Wnt inhibition leads to efficient appear-

ance of beating clusters within a 10-14 day period. It is our goal in this study to streamline these multiple differentiation pathways using non-animal components in order to ready methods for translational and clinical type research.

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T2090

DIFFERENTIATION, LINEAGE COMMITMENT AND GENE EXPRESSION ANALYSIS OF HUMAN CORTICAL NEURAL STEM CELLS DERIVED FROM PLURIPOTENT STEM CELLS

Ehsaei, Zahra[1], Fedele, Stefania[1], Collo, Ginetta[1,2] and Taylor, Verdon[1], [1]University of Basel, Basel, Switzerland, [2]University of Brescia, Brescia, Italy

The cerebral cortex is the most complex structure in the mammalian brain and contains approximately 10^9 neurons and 10^{10} glial cells (astrocytes and oligodendrocytes). The cortex is organized into and isocortex of six layers (layer I-VI) of pyramidal and interneurons. During development, neurons of the cerebral cortex are generated sequentially from stem cells and progenitors residing in the ventricular zone and subventricular zone of the neural tube and migrate to their perspective layer of the isocortex in an inside-out order. The mechanisms controlling the sequential formation of distinct neuronal subtypes through regulating neural stem cell fate remain unclear. We have investigated the heterogeneity of human cerebral cortex neural stem cells/progenitors derived from induced pluripotent stem cells (hiPSCs) and used a systems biology approach to examine changes in their gene expression profile with developmental stage and time. We performed clonal analysis of single neural stem cells and individual neural rosettes to study the precise lineage relationship between stem cells and neuron subpopulations. Our detailed transcriptome analysis has uncovered gene networks that likely control cell-type specific differentiation and cell fate choices in the human neocortex.

T2092

AN INVESTIAGTION OF G PROTEIN COUPLED ESTROGEN RECEPTOR IN HYPERTENSION USING INDUCED PLURIPOTENT STEM CELLS

Fredette, Natalie Catherine[1], Santostefano, Katherine[1], Prossnitz, Eric[2], Johnson, Julie[1] and Terada, Naohiro[3], [1]University of Florida, Gainesville, FL, U.S., [2]Univeristy of New Mexico, Albuquerque, NM, U.S., [3]University of Florida, Gainesville, FL, U.S.

The concomitant value of induced pluripotent stem cells (iPSCs) and gene editing techniques have presented novel opportunities for personalized medicine approaches, such as assessing functional SNP effects in multiple cell types. Our lab focuses on developing iPSC repositories with genome-wide SNP information as well as highly reproducible differentiation protocols for this purpose. Here, we present a novel application and proof of concept study of gene edited iPSCs from our iPSC repository derived from a pharmacogenomics cohort of hypertensive individuals. A SNP located in the coding region of the G protein-coupled estrogen receptor (GPER) (rs11544331) was recently shown to correlate with blood pressure (BP) in young women. Previous studies in animals indicate that this receptor has differing effects between endothelial cell (EC) and vascular smooth muscle cell (vSMC) functionality, thus complicating the overall effect of this SNP in blood pressure regulation and hypertension risk or protection. Using CRISPR technology on iPSCs derived from the confirmed heterozygous P16L female carriers in the library, we have targeted the GPER allele in an effort to study the GPER SNP variant in isolation or in a double allele knockout line. We propose to employ a dichotomous differentiation protocol to simultaneously produce endothelial and smooth muscle cells from isogenic GPER gene-edited iPSCs to assess mechanistic and physiologic readouts. These studies will decipher how this human GPER genetic variant would impact vascular responses leading to differential BP controls.

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T2094

HUMAN IPS/ES CELL SELF-RENEWAL UNDER GROWTH FACTOR- AND ECM-FREE CONDITION

Hasegawa, Kouichi[1,2], Yoshida, Noriko[1], Asari, Nami[1], Ikeda, Tatsuhiko[1], Manabe, Yuriko[1], Shahsavarani, Hosein[1], Nayer, Bhavana[2], Yasuda, Shin-ya[1] and Vartak-Sharma, Neha[1,2], [1]iCeMS, Kyoto University, Kyoto, Japan, [2]InStem, NCBS, Bangalore, India

Chemically defined conditions for large-scale production and quality-control of human pluripotent stem cells (hPSCs, including ES and iPS cells) is required for their application in transplantation therapies and drug screening. However, little is known about the molecular mechanisms of hPSC self-renewal, which impairs the identification of targets for applying chemical compounds to develop a chemically-defined culture system. Two growth factors bFGF and TGFβ are believed to be necessary extrinsic signaling molecule for hPSC self-renewal. Indeed most of the available culture systems include these growth factors. However, the molecular function of bFGF and TGFβ in the regulation of hPSC self-renewal are still poorly understood, and there are no compounds that can substi-

tute for these factors. In a hypothesis driven small chemical library screening approach, we have identified novel signaling cascades and chemical compounds that regulate hPSC self-renewal and differentiation. Utilizing these compounds, we have developed a growth factor-free hPSC defined culture medium in which only 2 proteins, Insulin and Transferrin, are required. All tested hPSC lines can be maintained. The pluripotent state of hPSC in the medium is slightly different from that in bFGF and TGFβ-dependent medium, and the efficiency of iPSC derivation in the medium is identical to that in the growth factor-including medium. We have also developed a novel sphere culture system with 2 polymers instead of using recombinant protein matrixes. These medium and culture system will not only dramatically reduce the cost of hPSC research and applications but also become a platform to study hPSC self-renewal and reprogramming.

T2096

PINPOINTING OF KEY DETERMINANTS OF OCT4 RESPONSIBLE FOR INDUCTION OF PLURIPOTENCY

Jerabek, Stepan[1], Ng, Calista[2], Esch, Daniel[1], Kim, Kee-Pyo[1], Cojocaru, Vlad[1], Velychko, Sergiy[1], Malik, Vikas[3], Yang, Xiaoxiao[3], Schöler, Hans[1] and Jauch, Ralf[3], [1]Max Planck Institute for Molecular Biomedicine, Muenster, Germany, [2]Institute of Medical Biology, Singapore, Singapore, [3]Guangzhou Institutes of Biomedicine and Health, Guangzhou, China

Epigenetic reprogramming of somatic cells to a pluripotent state holds great potential for regenerative medicine. The most widely used combination of transcription factors for this process consists of Oct4, Sox2, Klf4, and c-Myc. However, the biochemical characterization of the key determinants underlying the unique function of these four proteins remains largely incomplete. In our project, we focused on the master regulator Oct4, which cannot be substituted by any other member of the POU protein family. We sought to identify a minimal set of protein interfaces that are responsible for the ability of Oct4 to induce pluripotency. In this study, we narrowed down the attributes which render Oct4 a reprogramming factor to two protein interfaces: one affecting the selection of DNA-binding sites and the other influencing the ability to interact with cofactors. We had previously solved the crystal structure of another POU family member—Oct6—bound as homodimer to DNA. Recently, we observed that Oct6 and Oct4 exhibit a different propensity to form Oct-Oct homodimers versus Sox-Oct heterodimers. We then rationally created mutations that led to swapping of the binding preference between the two POU proteins. However, the switch of motif preference was not sufficient for converting Oct6 into a reprogramming factor, suggesting that Oct4-specific interactors are necessary for the induction of pluripotency. We recently found that the Oct4 linker region plays an integral role in protein-protein interactions; a few residues involved in Oct4-Sox2 cooperation had previously been described. Therefore, we designed a series of Oct6 mutants and determined the optimal combination for induction of pluripotency. In summary, by converting Oct6 into a reprogramming factor, we identified the key Oct4 determinants that drive the generation of induced pluripotent stem cells.

T2098

DEVELOPMENT OF A ROBUST HUMAN iPSC CULTURE-PROPAGATION STRATEGY THROUGH BOTULINUM HEMAGGLUTININ-MEDIATED SELECTIVE REMOVAL OF DEVIATED CELLS

Kim, Mee-Hae and Kino-Oka, Masahiro, Osaka University, Suita, Japan

Human induced pluripotent stem cells (hiPSCs) hold great promise for clinical and industrial applications because, like embryonic stem cells, they can self-renew and differentiate into multiple cell types; however, their use poses certain challenges, such the deviation of cells from the undifferentiated state during subculture. The undifferentiated state of hiPSCs depends on their cell–cell/substrate adhesion, and thus understanding the regulation of this adhesion is critical. In this study, we developed a robust and stable hiPSC culture-propagation strategy that involves botulinum hemagglutinin (HA)-mediated selective removal of cells that deviate from the undifferentiated state. We found that exposure to HA, a barrier-disrupting agent, selectively removed cells that deviated from the undifferentiated state in hiPSC colonies. After 24-h HA treatment, colonies containing deviated cells in central regions lost their adhesions with neighbors and shrank, and most tended to detach from the substrate. Following routine medium change, the vacated space inside colonies became filled by dividing neighboring cells, which, like undifferentiated cells, formed tight compact colonies. Immunofluorescence staining showed that all cells in the colonies were OCT3/4-positive. Subsequently, HA effects on hiPSC colonies displaying deviation were similar regardless of hiPSC lines, serum-free, and feeder-free culture conditions. We also tested whether enriched preparation of undifferentiated hiPSCs could be obtained performing several passages of the cells through HA-mediated removal method. After passage 5, the percentage of colonies displaying deviation was significantly greater in non-HA treated cells than that HA-treated cells. HA-treated cells maintained their undifferentiated state. These results indicate that HA-mediated selective removal method may be a useful tool for obtaining enriched preparations of undifferentiated hiPSCs. Given the progress in hiPSC expansion in monolayer cultures in closed-bioreactor manufacturing systems, we anticipate

being able to increase the scale of our process by several additional orders of magnitude.

T2100

THE ESTABLISHMENT OF THREE-DIMENSIONAL DISEASE SKIN MODEL FOR PERSONALIZED DRUG RESEARCH & DEVELOPMENT USING PATIENT- SPECIFIC INDUCED PLURIPOTENT STEM CELLS

Liu, Liping[1], Li, Shu[1], Gao, Yimeng[2], Xu, Hui[1], Hui, Lijian[2], Zheng, Yunwen[1,3] and LI, Yumei[1], [1]Jiangsu University Hospital, Zhenjiang, China, [2]Shanghai Institute for Biological Sciences, Shanghai, China, [3]University of Tsukuba, Ibaraki, Japan

Complex genetic cutaneous conditions are associated with genetic susceptibility which will lead to various treatment response in different individuals. It's necessary to establish a screening system for the specific patients with these diseases to identify drug effect. We hypothesized that a three-dimensional skin model based on patient specific iPSCs (induced pluripotent stem cells) could solve this issue. Here we collected white lesions of patients with vitiligo and normal skin of healthy individuals and established the vitiligo patient specific iPSC lines from dermal fibroblasts with Yamanaka's factors. Our preliminary data show that the iPSCs exhibit the essential characteristics of embryonic stem cells, and are similar to those of the healthy individuals derived in morphology, alkaline phosphatase activity, gene expression, surface markers, and epigenetic status of OCT4 expression. Additionally, both of the healthy and patients' iPSCs have normal karyotypes. In conclusion, we have generated iPSC lines of vitiligo patients. With iPSCs derived cutaneous cells, such as keratinocytes and melanocytes, we have established a three-dimensional disease skin model for vitiligo. Since iPSCs carry individual genetic messages, the drug screening could be performed with this personalized system to determine the most appropriate medical treatments not only for the vitiligo patients but also for other skin diseases such as psoriasis in perspective future.

T2102

OPTIMIZATION OF HUMAN CD34+ CELLS FOR REPROGRAMMING USING TAILORED MEDIA AND ALTERNATE TIMELINES

Mendoza, Alejandra V.[1,2], MacArthur, Chad C.[3], Vemuri, Mohan C.[1], Kuligowski, Sandra[1] and Lakshmipathy, Uma[1], [1]Thermo Fisher Scientific, Carlsbad, CA, U.S., [2]California State University, San Marcos, San Marcos, CA, U.S., [3]Life Technologies, Carlsbad, CA, U.S.

The focus on human fibroblasts as the somatic cell source for induced pluripotent stem cells (iPSC) is transitioning towards more easily reprogrammable cell types and/or methodologies. Somatic cells derived from blood are easier to obtain, and are more suited for a clinical grade workflow. In addition, these blood cells allow for minimal upfront manipulation of cells prior to reprogramming, thus reducing the potential for somatic mutations, while reducing the associated time and cost. The aim of this study was to optimize the media, cytokines, and timelines for reprogramming blood-derived cells with Sendai virus, while maintaining high reprogramming efficiency. Using CD34+ cells, two different media known to maintain CD34+ cells were used in combination with two known combinations of cytokine cocktails. Flow cytometric analysis indicated the novel media with cytokine cocktail 1 (CT1: SCF, GM-CSF, IL-3), showed a relatively higher percentage of CD34+ cells with 2-3 days of culture. The cells reprogrammed with tailored media and CT1 also resulted in a 3-fold increase in reprogrammed alkaline phosphatase-positive iPSC colonies. Additionally, this combination facilitated reduction of the reprogramming timeline from the standard three week timeline to a fourteen day protocol. Microscopic evaluation of the resulting colonies confirmed that the iPSCs were mature with ESC-like morphology and ready for downstream clonal selection and expansion. The combination of tailored media with the optimal cytokine cocktail, coupled with an improved protocol can enable reprogramming of other blood-derived cells, such as PBMCs, and small volumes of blood. Streamlining the process of iPSC generation by minimizing manipulation and timelines reduces the costs and effort involved with large-scale iPSC generation.

Funding Source: Funding provided by California Institute for Regenerative Medicine and ThermoFisher Scientific.

T2104

UNDERSTANDING SPINAL AND BULBAR MUSCULAR ATROPHY (SBMA) USING MOTOR NEURONS GENERATED FROM PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

Narayanan, Gunaseelan[1], Sheila, Marianne[2,3], Chai, Josiah[4] and Stanton, Lawrence[2], [1]Genome Institute of Singapore, Singapore, [2]Genome Institute of Singapore, Singapore, Singapore, [3]Genome Institute of Singapore, buona vista, Singapore, [4]National Neuroscience Institute, Singapore, Singapore

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is a progressive X-linked neurodegenerative disorder affecting males. Patients suffer from loss of lower motor neurons and typically display limb and bulbar muscle loss, dysphagia, dysarthria and gynaecomastia. SBMA is caused by expansion of the CAG repeat region in the first exon of the Androgen Receptor (AR) gene. Previous studies have suggested that dysregulation

of AR transcriptional activity contributes to the SBMA disease mechanism. To further investigate the dysregulation of AR transcriptional activity in SBMA, we aim to perform AR chromatin immunoprecipitation (AR ChIP) sequencing on motor neurons derived from patient-specific induced pluripotent stem cells. Using this approach, the differences in AR binding sites and the genes which are differentially regulated by AR between the healthy and SBMA motor neurons would be identified. These genes would be further studied to understand how dysregulation in AR transcriptional activity causes specific SBMA phenotypes. Furthermore, the healthy and SBMA motor neurons will be assessed for cell survival and morphological features such as neurite outgrowth to understand the selective loss of motor neurons in SBMA.

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T2106

INDUCTION OF PLASTICITY PHENOMENA IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CORTICAL NEURONS

Odawara, Aoi[1], Matsuda, Naoki[1], Arant, Ryan[2], Shi, Yichen[3] and Suzuki, Ikuro[4], [1]Tohoku Institute of Technology, sendai, Japan, [2]Alpha med scientific, Osaka, Japan, [3]Axol Bioscience Ltd, Cambridge, U.K., [4]Tohoku Institute of Technology, sendai, miyagi, Japan

Long-term potentiation (LTP) and long-term potentiation depression (LTD) in neuronal networks has been analyzed using in vitro and in vivo techniques in simple animals to understand learning, memory, and development in brain function. Human induced pluripotent stem cell (hiPSC)-derived neurons may be effectively used for understanding the plasticity mechanism in human neuronal networks, thereby elucidating disease mechanisms and drug discoveries. In this study, we attempted the induction of LTP and LTD phenomena in a cultured hiPSC-derived cerebral cortical neuronal network using multi-electrode array (MEA) systems. High-frequency stimulation (HFS) produced a potentiated and depressed transmission in a neuronal circuit for 1 h in the evoked responses by test stimulus. The cross-correlation of responses revealed that spike patterns with specific timing were generated during LTP induction and disappeared during LTD induction and that the hiPSC-derived cortical neuronal network has the potential to repeatedly express the spike pattern with a precise timing change within 0.5 ms. We also detected the phenomenon for late-phase LTP (L-LTP) like plasticity and the effects for synchronized burst firing (SBF) in spontaneous firings by HFS. In conclusion, we detected the LTP and LTD phenomena in a hiPSC-derived neuronal network as the change of spike pattern. The studies of plasticity using hiPSC-derived neurons and a MEA system may be beneficial for clarifying the functions of human neuronal circuits and for applying to drug screening.

T2108

HUMAN IPSC MODELS OF OBESOGENIC GENE-ENVIROMENT INTERACTIONS IN THE DEVELOPING ENDOCRINE SYSTEM

Rajamani, Uthra[1], Gross, Andrew[1], O'Campo, Camille[1] and Sareen, Dhruv[2], [1]Cedars Sinai Medical Center, Los Angeles, CA, U.S., [2]Cedars-Sinai Medical Center, Los Angeles, CA, U.S.

Exposure to environmental pollutants account for congenital anomalies in births in the U.S. and globally. Environmental toxicants are also possible potent endocrine disrupting chemicals (EDCs), which may play a causal role in early onset obesity by disrupting homeostatic metabolic and endocrine controls. There is, however, a paucity of data on developmental abnormalities in human tissues specially endocrine cells due to EDCs, in gut and brain. In this study, the effect of chronic exposure to 3 putative EDCs, perfluorooctanoic acid (PFOA; 2.5μM), butyl hydroxyltoluene (BHT; 10nM) and tributyltin (TBT; 10nM) was evaluated on the developing foregut organoids (iFGOs) and hypothalamic neurons (iHTNs) derived from human induced pluripotent stem cells (iPSCs) of normal (CTR; BMI<25) and super obese (SO; BMI>50) subjects. The iFGOs and iHTNs contained specific and characteristic markers of respective cell lineages including secretory endocrine cells. iFGOs showed foregut progenitors (Sox2), G cells (gastrin), parietal cells (Ghrelin) and Faveolar cells (Mucin5AC). The iHTNs expressed hypothalamic markers such as Neuropeptide Y (NPY), POMC (Proopioid Melanocortin) and endocrine markers by immunohistochemistry and quantitative RT-PCR. EDC exposure, particularly PFOA and BHT on iFGOs and all 3 EDCs on iHTNs, increased NF-κB phosphorylation by at least 52% (activation), suggesting pro-inflammatory triggers. EDC treatment also elevated NF-κB subunits p50 and p55 processing in both iFGOs and iHTNs. The iHTNs showed lower mitochondrial respiration with TBT ($p<0.01$), BHT ($p<0.01$) and combination ($p<0.01$) treatments. Quantitative proteomics revealed that pro-inflammatory signatures such as decreased NF-κB p100 (32%) and elevated HMGB1 (30%) were observed even at baseline (unexposed to EDCs) in SO-derived iFGOs compared to CTR samples. This study highlights the utility of investigating pervasive EDCs in relevant developing human tissues using iPSCs while also revealing role of chronic inflammation in obesity, as indicated by similarities between SO and EDC exposed iPSC-derived endocrine tissues. Such a human-based stem cell model paves way for more reliable toxicity screening of obesogenic EDCs and for identification of therapeutic targets that can cause abnormalities in the developing endocrine system.

Funding Source: Institutuional Funds

T2110

MULTI-OMICS ANALYSIS FOR ELUCIDATING THE POTENTIAL ROLE OF INTRACELLULAR BIOENERGETIC AND GENE EXPRESSION NETWORK ON CONTROLLING THE FATE OF HEMATOPOIETIC PROGENITORS USING PATIENT-SPECIFIC IPS CELLS

Saiki, Norikazu[1], Oshima, Koichi[1,2], Hirayama, Akiyoshi[3], Soga, Tomoyoshi[3], Tomita, Masaru[3], Nakahata, Tatsutoshi[1] and Saito, Megumu K.[1], [1]Center for iPS cell Research and Application, Kyoto University, Kyoto, Japan, [2]Institute for Cancer Genetics, Columbia University Medical Center, New York, NY, U.S., [3]Institute for Advanced Biosciences, Keio University, Yamagata, Japan

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 ↔ 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. Hematopoietic differentiation from RD-iPSCs was profoundly impaired compared to AK2-supplemented RD-iPSCs. It indicates this iPSC models can recapitulate the in vivo phenotype of RD. To elucidate the effect of ATP imbalance caused by AK2 deficiency on metabolic profile and gene expression, we acquired multi-omics data sets. AK2(-) iPSCs have latent alteration of metabolic profiles, and besides, despite the global transition of profiles in all of the clones during differentiation, AK2(-) cells still showed profiles that were distinct from AK2(+). Interestingly, malate and tryptophan were specifically accumulated in differentiated AK2(-) cells, and these metabolites were not accumulated in the PSC state. For detailed description of metabolic flux distribution of energy metabolism, we performed ^{13}C tracer analysis and a metabolic flux analysis (MFA) by establishing a stoichiometric model. The MFA revealed the initial distribution of pyruvate into the TCA cycle appears to be excessively skewed into the oxidative direction in AK2(-) clones. Moreover, these results were consistent with the difference in the expression profiles of the TCA cycle genes. On the other hand, the TCA cycle genes in differentiated AK2(-) clones expressed as opposite profile to those in the PSC state. Our findings suggest AK2-medieated putative phosphotransfer may contribute to balance the energy metabolic network and regulate the amount of specific intermediates. Looking ahead, these can be the key for complementation of differentiation.

T2112

DNA DOUBLE-STRAND BREAKS DETECTION IN PLURIPOTENT STEM CELLS WITH REGARD TO CELL CYCLE STAGES

Simara, Pavel, Tesarova, Lenka, Rehakova, Daniela, Matula, Pavel, Stejskal, Stanislav and Koutna, Irena, Masaryk University, Faculty of Informatics, Brno, Czech Republic

Human induced pluripotent stem cells (hiPSCs) play roles in both disease modelling and regenerative medicine. It is of utmost importance that the genomic integrity of the cells remains unharmed and the DNA repair systems are fully functional. In our research we focus on the detection of DNA double-strand breaks (DSBs) by phosphorylated histone H2AX (known as γH2AX) and p53-binding protein 1 (53BP1) in fibroblasts, three distinct lines of hiPSCs, and one line of human embryonic stem cells (hESCs). We measured both spontaneously occurred DSBs and DSBs induced by γ-irradiation and its decrease in time. Foci number was detected by fluorescence microscopy and EdU (5-ethynyl-2′-deoxyuridine) was used to discriminate between cell cycle stages. Discrimination between the EdU negative (G1) and positive (S/G2) populations allows excluding the replication-related foci and increase the accuracy of measurement. This is crucial when comparing the number of DSBs in cell types with different cell cycle speed (ie. somatic cells and pluripotent cells). EdU discrimination is also valuable when the cell cycle is being modified during experiments in a way that changes proportion of cells in the S/G2 stage (ie. by irradiation or using cell cycle synchronising agents). In EdU negative (G1) group, we detected low number of replication non-related DSBs in fibroblasts, while this number increases significantly after reprogramming into hiPSCs to decrease again after long-term in vitro passaging. However, hiPSCs in high passages responded weakly to γ-irradiation treatment in comparison to hiPSCs in low passage number, suggesting their DSB-repair capacity may be compromised.

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T2114

FUNCTIONAL MATURATION AND DRUG RESPONSES OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CORTICAL NEURONAL NETWORKS IN LONG-TERM CULTURE

Odawara, Aoi[1], Matsuda, Naoki[1], Arant, Ryan[2], Shi, Yichen[3] and **Suzuki, Ikuro**[1], [1]Tohoku Institute of Technology, Sendai, Miyagi, Japan, [2]Alpha med scientific, Osaka, Japan, [3]Axol Bioscience Ltd, Cambridge, U.K.

The functional network of human induced pluripotent stem cell (hiPSC)-derived neurons is a potentially powerful in vitro model for evaluating disease mechanisms and drug responses. However, the culture time required for the full functional maturation of individual neurons and networks is uncertain. We investigated the development of spontaneous electrophysiological activity and pharmacological responses for over 1 year in culture using multi-electrode arrays (MEAs). The complete maturation of spontaneous firing, evoked responses, and modulation of activity by glutamatergic and GABAergic receptor antagonists/agonists required 20–30 weeks. At this stage, neural networks also demonstrated epileptiform synchronized burst firing (SBF) in response to pro-convulsants and SBF suppression using clinical anti-epilepsy drugs. Our results reveal the feasibility of long-term MEA measurements from hiPSC-derived neuronal networks in vitro for mechanistic analyses and drug screening. However, developmental changes in electrophysiological and pharmacological properties indicate the necessity for the international standardization of culture and evaluation procedures.

T2116

DAMID-SEQ: A SENSITIVE TOOL TO INVESTIGATE TRANSCRIPTION FACTOR BINDING DYNAMICS DURING INDUCTION OF PLURIPOTENCY

Tosti, Luca, Ashmore, James, Tan, Nicholas, Chantzoura, Eleni and Kaji, Keisuke, University of Edinburgh, Edinburgh, U.K.

The stem cell field has been dramatically altered by the discovery that somatic cells can be transformed into pluripotent cells (induced pluripotent stem cells, iPSCs) through the overexpression of four transcription factors, namely Oct4, Sox2, Klf4, and c-Myc (OSKM). However, our ability to elucidate the underlying molecular mechanisms of this process is hindered by its low efficiency and the consequent scarcity of reprogramming intermediate cells. In particular, the investigation of genome-wide transcription factor (TF) binding dynamics during reprogramming by ChIP-seq has been hampered by the limited amount of cells. To overcome this limitation we have implemented the DNA Adenine-Methyltrasferase IDentification followed by Next-Generation Sequencing (DamID-seq). The E. Coli protein Dam is a GATC sequence-specific Adenine-Methyltrasferase. When Dam is tethered to the protein of interest (POI), it methylates GATC sequences near the POI binding sites and creates GA^{me}TC. The genome extracted from Dam-POI expressing cells can be digested by the GA^{me}TC specific-restriction enzyme DpnI. Subsequently, the DNA fragments flanked by GA^{me}TC can be amplified by adapter ligation and PCR, preceding next-generation sequencing. Since DamID-seq does not require any immunoprecipitation step, this technology allowed us to lower the number of cells required for the identification of TF binding sites. Using Dam-Oct4 fusion protein, here we demonstrate that DamID-seq can identify the genome-wide Oct4 binding sites using as few as 100 cells. We applied this technology to investigate changes of Oct4 binding sites in FACS-sorted reprogramming intermediate populations, paving the way for a better understanding of bottlenecks of reprogramming. This technology also provides us with a novel strategy to investigate TFs' targets in scarce cell types *in vivo*, including various stem cells.

T2118

DECONSTRACT SOMATIC CELL REPROGRAMMING HETEROGENEITY BY LIVE IMAGE AND RNA-SEQ IN SINGLE CELL LEVEL

Wang, Xiaoshan[1], Guo, Lin[2], Hutchins, Andrew[2], Wang, Bo[2], Liu, Jiadong[3], Gao, Mingwei[2,3], Chen, Jiekai[3] and Pei, Duanqing[1], [1]Guangzhou Institutes of Biomedicine and Health, Guangzhou, China, [2]Guangzhou Institutes of Biomedicine and Health, CAS, Guangzhou, China, [3]Guangzhou Institutes of Biomedicine & Health, Chinese Academy of Sciences, Guangzhou, China

Understand the mechanisms of somatic cells reprogramming is critical for its clinical application and stem cell biology. The biggest obstacle is the high heterogeneity during the process which is long time and low efficiency. Here, we utilized two high efficient (10%) reprogramming systems which can achieve iPSCs with germ line transmission ability in 7 days for OSK and 4 days for OvSvK, then, we used live cell station to follow the whole process of reprogramming in single cell resolution, and we performed single cell RNA-seq in five different stages of reprogramming, got 910 high quality single cells dataset totally. To compare the trajectory of reprogrammed cells and failed cells and combine the single cell transcritome, we found several new interesting features of reprogramming. First, there are several trajectory leading to successfully reprogrammed; Second, the daughter cells of reprogrammed cell have different cell fates; Third, cell cycle control system transition from somatic cell model to embryonic

stem cell model plays important role in reprogramming. Through this high resolution data, we got more detail information about somatic reprogramming mechanisms.

T2120

IDEAL XENO-FREE CULTURE CONDITIONS FOR HUMAN FIBROBLASTS THAT FACILITATE EFFICIENT REPROGRAMMING

Wetton, Nikki M.[1], MacArthur, Chad C.[2], Zarrabi, Aryan[1] and Lakshmipathy, Uma[1], [1]Thermo Fisher Scientific, Carlsbad, CA, U.S., [2]Life Technologies, Carlsbad, CA, U.S.

Patient derived human fibroblasts are the most widely used source for somatic reprogramming. Traditional methods require the use of fetal bovine serum containing media for the isolation and expansion of these cells. As resulting induced pluripotent stem cells find their way towards clinical applications, xeno-free workflow requirements have become essential.This study aims to identify ideal xeno-free media that best support fibroblast culture, health, and efficient gene transfer resulting in robust reprogramming. Human adult fibroblasts were cultured in five different xeno-free media systems, and their growth kinetics and cell health were measured in comparison to cells cultured in traditional FBS containing media. Whole well imaging and continuous monitoring was carried out using IncuCyte Zoom to track growth based on confluence. In addition, cells were transduced with a control GFP Sendai virus to assess the survival and percent GFP positive cells post transduction. Culture conditions that best supported robust fibroblast growth and efficient Sendai transduction were further used for the generation of iPSC from different adult and neonatal human fibroblasts in Essential 8 defined and feeder-free media. The identified xeno-free workflow offers flexibility and choices for patient sample processing to iPSC generation, critical for clinical-grade cells.

Funding Source: This research was funded by California Institute for Regenerative Medicine and Thermo Fisher Scientific.

T2122

DOWN-REGULATED CELL-MATRIX ADHESION PROMOTE MAINTAIN OF NAIVE-LIKE HUMAN PLURIPOTENT STEM CELLS

Yu, Leqian[1,2], Li, Junjun[1], Liu, Li[1], Fujimoto, Nanae[1,2], Nakajima, Minako[1,2], Chen, Yong[1,3] and Kotera, Hidetoshi[1,2], [1]Institutes for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto, Japan, [2]Department of Micro Engineering, Kyoto University, Kyoto, Japan, [3]Ecole Normale Supérieure, CNRS-ENS-UPMC UMR 8640, 24 Rue Lhomond, Paris, France

Recently, the naïve state of human pluripotent stem cells (hPSCs) has been achieved by gene modification (e.g. OCT4 and KLF4) and utilization of some complex culture formulations. However, the conventional method may not be suitable for the long-term maintenance of the naïve hPSCs. By using gelatin nanofibrous (GNF) substrate developed previously, we observed two types of hPSC colonies, which are morphologically different (flat and dome-like), similar to those of primed and naïve state cells respectively. In order to isolate the two types of colonies, we designed a single hPSCs culture platform by integrating polydimethylsiloxane (PDMS) multi-wells and GNF substrate. Single hPSCs were successfully loaded into PDMS wells and then cultured to form isolated clones. Our results showed that hPSCs in dome-like colonies have similar characteristics to naïve state cells, such as shorter doubling time and up-regulated expression of several naïve-related genes. Furthermore, mechanism study demonstrated that cell-matrix adhesion involved in interaction between colonies morphology and the naïve state core pluripotent factor (KLF4 and NANOG) expression through serum response factor (SRF) based auto-regulatory double loop. Thus, we suggest that the low cell-matrix adhesion substrates, such as GNF, is a good candidate for the naïve state PSCs culture.

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IPS CELLS: DIRECTED DIFFERENTIATION

T2128

AN HIPSC ORGANOID MODEL TO STUDY HEPATOCYTE LIPOPROTEIN METABOLISM

Abbey, Deepti, Hand, Nicholas and Rader, Daniel J, University of Pennsylvania, Philadelphia, PA, U.S.

ESC and iPSC-derived hepatocytes are attractive tools to study liver function, and to interrogate the impact of human genetic variation on hepatocyte biology. Significant

limitations to the usefulness of the widely used, conventional, two dimensional hepatocyte culture systems are that even the best protocols retain varying degrees of differentiation efficiency (~60-80% Alb+ cells) and that the terminally differentiated cells phenotypically resemble immature hepatocytes. The use of adult liver stem cells has shown promise relative to other starting cell types, however, the invasive techniques required to obtain this material limits the applicability of the approach. We have developed a novel 3D organoid culture as an efficient source of iPSC-derived hepatocytes (>90% Alb+ cells) that are more useful for the study of hepatocyte metabolic function (specifically those of cholesterol, bile acid, and lipoprotein). This protocol has been equally efficient with from iPSC from either fibroblast or buffy coat sources, obviating the need for adult liver stem cells. Using matrigel-induced culture, different stages of hepatocyte differentiation were achieved, recapitulating the in vivo chronological developmental stages. The hepatocytes within the differentiated organoids are polarized in nature and have a contiguous canalicular network. Importantly, these organoids also showed oleic acid-induced lipid (TG) accumulation, and secrete Albumin and ApoB100 making them suitable for studying lipid metabolism. Together this system, serves as a potential tool to interrogate the function of human variants identified by genome-wide association studies.

T2130

ANTIOXIDATIVE TREATMENT AS A TOOL FOR HUMAN IPSC-DERIVED NEURAL PROGENITOR FATE CONTROL: THE INFLUENCE ON GENETIC STABILITY

Buzanska, Leonora[1], Augustyniak, Justyna[1], Zychowicz, Marzena[1], Lenart, Jacek[1], Dzus, Malgorzata[1] and Stepien, Piotr[2], [1]Mossakowski Medical Research Centre, Warsaw, Poland, [2]Faculty of Biology, University of Warsaw, Warsaw, Poland

Understanding of how human iPSC and their neural progenitors maintain genetic stability are highly relevant for their safe possible therapeutic application. One of the possible mechanisms is the oxidative DNA damage generated by free radicals. Neural differentiation is linked to the metabolic switch from the glycolytic to the oxidative metabolism. While pluripotent stem cells due to glycolytic metabolism possess oxidative damage sensor system directing cells on repair or apoptosis pathways, neural progenitors are more susceptible to DNA damage depending on the ROS levels. The main goal of this study was to find out whether selected small molecules with antioxidant properties can enhance the genetic stability of human iPSC-derived neural progenitors (hiPS-NSC) and can influence their process of differentiation. The hydrogen peroxide was used to induce DNA damage and the three selected antioxidant small molecules were tested for their protective effect in hiPSC-NSC at different stages of neural development. Antioxidative properties of tested compounds were evaluated by: Alamar Blue viability assay, Trypan blue proliferation assay, Mitotracker red cmxros (mitochondrial membranes potenctial assay) and DCFHDA (ROS level assay). Positive antioxidative effect was observed after long term exposition (5 days) while 24 h treatment was not effective. Tested compounds showed protective effect by increasing viability and proliferation of hiPS-NSC in all tested doses. In our current experiments such effect is being linked to the phenotypic markers and mitochondrial biogenesis in hiPS-NSC at different stages of differentiation. For that purpose the expression of specific developmental (e.g. MAP2, TUBBIII) and metabolic switch related (e.g. MT-CO1, SDH-A, NRF-1, TFAM, PPARGC1A) genes is investigated at different level of expression (RNA and protein). Our preliminary data suggest that antioxidative treatment may serve as a tool for human iPSC-derived neural progenitor fate control.

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T2132

DETERMINING THE ORIGIN AND DEGREE OF FUNCTIONAL VARIABILITY IN PATIENT DERIVED IPSCs DIFFERENTIATED FOR DISEASE MODELING

Eade, Kevin[1,2], Lo Sardo, Valentina[2], Gantner, Marin[1,2], Hazen, Jennifer[2], Prins, Mitchell[1,2], Johnson, Alec[1], Westenskow, Peter[1,2], Baldwin, Kristin[2] and Friedlander, Martin[1,2], [1]The Lowy Medical Research Institute, La Jolla, CA, U.S., [2]The Scripps Research Institute, La Jolla, CA, U.S.

Disease modeling using patient derived iPSCs provides a promising tool to study otherwise inaccessible human disorders. While this approach allows for the investigation of diseases directly in human cells, the procedure involves multiple levels of manipulation with the potential to introduce variability. If not properly accounted for this variability decreases the power of experimental assays aimed to illuminate actual differences between affected and control cells, resulting in missed revelations or costly red herrings. In this study we derive retinal pigment epithelial (RPE) cells from iPSC lines designed to control for cell origin, reprogramming factor load, and differentiation protocols to determine the contribution of each step of reprogramming and differentiation to the variability observed in differentiation potential of iPSCs, and physiologically-relevant assays of mature RPE. From this we are able to predetermine experimental parameters including the number of iPSC clones per patient that will yield reliable results in a larger disease screen measuring cellular morphometry, phagocytic capacity, metabolic fitness,

and stress response. We also determined key time points to perform different functional assays so to minimize variability caused by the varying differentiation potential of each clonal line. Based on this study, we suggest that similar types of experiments are performed prior to beginning any disease modeling experiment to avoid misinterpretation of functional/anatomical analyses.

Funding Source: Lowy Medical Research Institute

T2134

NEUROMUSCULAR JUNCTION DEFECTS IN IPSC-DERIVED MUSCLE AND NEURONAL CO-CULTURES FROM PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

Haston, Kelly[1], Aron, Rebecca[1], Huebsch, Nathaniel[2], Conklin, Bruce[3,4] and Finkbeiner, Steven[1,5], [1]Gladstone Institutes, San Francisco, CA, U.S., [2]Gladstone Institutes of Cardiovascular Disease-Conklin, San Francisco, CA, U.S., [3]University of California, San Francisco, Gladstone, San Francisco, CA, U.S., [4]University of California, San Francisco, San Francisco, CA, U.S., [5]Taube/Koret Center for Neurodegenerative Disease and the Hellman Family Foundation Program in Alzheimer's Disease Research, San Francsico, CA, U.S.

The majority of pluripotent stem cell differentiation protocols produce cultures that contain one cell type, such as a motor neuron. However, *in vivo,* neurons are strongly influenced by other closely apposed cell types. An especially important cell type in amyotrophic lateral sclerosis is the skeletal muscle cell. Loss of the neuromuscular junction it forms with the motor neuron occurs during aging and is one of the earliest and most sensitive deficits in ALS and models of ALS. To address this concern, we developed a fully human i-motor neuron / i-skeletal muscle co-culture that formed functional neuromuscular junctions. We then applied this protocol to iPSC lines from patients with ALS due to mutations in TDP43, SOD1, and C9ORF72 as well as sporadic ALS. Characterization of the resulting cultures includes immunofluorescence for markers of i-MN, i-SkM, markers of the neuromuscular junction such as the acetylcholine receptor, and electron microscopy to confirm the presence of ultrastructural features of skeletal muscle, including sarcomeres and z-discs. Functional analysis includes sensitivity to curare, a well-established competitive inhibitor of the acetylcholine receptor and videography and calcium imaging of contracting cultures. Our preliminary data shows that within these co-cultures, there is spontaneous motion and calcium flux consistent with spontaneous action potential firing in neurons and rhythmic, contraction-linked calcium transients in skeletal muscle, and this behavior can be inhibited by curare treatment. We further observed that co-cultures differentiated from iPSCs of ALS patients with C9ORF72 mutations had significantly more fragmented mitochondria and less acetyl choline receptor clustering on iSkM than similar cultures differentiated from iPSCs of healthy volunteers. Although preliminary, these results raise the intriguing possibility that the protocol to differentiate iPSCs into i-MN / i-SkM co-cultures with functional neuromuscular junctions may reveal deficits in both i-MNs and i-SkM made from ALS patients and are therefore amenable to ALS disease modeling.

Funding Source: Supported by the Packard Center, the ALS association and the NIH Common Fund and NINDS

T2136

GENERATION AND DIFFERENTIATION OF MODY8 DISEASE-SPECIFIC IPSCs INTO PANCREATIC CELLS

Kahraman, Sevim[1], Teo, Adrian Kee Keong[1,2], Jackson, Nicholas[1], Martinez, Rachael[1], Raeder*, Helge[3] and Kulkarni*, Rohit[1], [1]Section of Islet Cell and Regenerative Biology, Joslin Diabetes Center, Harvard Stem Cell Institute, and Department of Medicine, Harvard Medical School, Boston, MA, U.S., [2]Institute of Molecular and Cell Biology, Singapore, Singapore, [3]Haukeland University Hospital, and the KG Jebsen Center for Diabetes Research, and University of Bergen, Bergen, Norway

MODY8 (maturity onset diabetes of the young, type 8) is an autosomal dominant monogenic form of diabetes. This disease is associated with frameshift mutations in the carboxyl ester lipase (*CEL*) gene which encodes one of the lipases and is expressed mainly in pancreatic acinar cells. Heterozygous mutations in the *CEL* gene result in childhood-onset exocrine pancreas dysfunction and diabetes during adulthood. However, it is unclear how CEL mutations cause development of diabetes in MODY8 patients, and hence we sought to establish an iPSC-based model of this disease to study disease mechanisms. In this study, we used episomal reprogramming to generate induced pluripotent stem cells (iPSCs) from skin fibroblasts of mutation carriers with or without diabetes at the time of biopsy. Fibroblasts were nucleofected with plasmids encoding four Yamanaka factors in combination with LIN28 and repression of p53. The efficiency of reprogramming was lower in all fibroblasts expressing the mutation, especially in those with diabetes, compared to healthy fibroblasts. Multiple iPSC clones derived from each subject (n=3-8), were morphologically indistinguishable from human ESCs, and expressed pluripotency markers. We confirmed presence of the single-base deletion (1686delT) in exon 11 of the CEL gene in the karyotypically normal MODY8-iPSCs. Healthy iPSCs were used to evaluate several differentiation methods to generate definitive endoderm (DE) followed by pancreatic progenitors (PP). Directed differentiation of iPSCs into DE showed rapid decrease

in expression of pluripotency markers and increase in expression of DE markers. The most efficient protocol for DE generation resulted in 77.4% CXCR4+, and 85.4% SOX17+ cell population in two days. Subsequent differentiation of DE cells into gut tube and then posterior foregut cells showed an increase in expression of PDX1, PTF1A, SOX9, and HNF6. Finally, PP cells were generated in vitro from posterior foregut cells (76.3% PDX1+). In conclusion, we report generation of MODY8 iPSCs and in vitro differentiation protocols for generating pancreatic progenitors, which provides an invaluable resource to model and phenotype endocrine versus exocrine defects in this form of monogenic diabetes.

T2138

CD24HIGH CHONDROCYTES DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS (HIPSC) ARE RESISTANT TO NFKB MEDIATED INFLAMMATORY RESPONSE

Lee, Ji Eun and Bhutani, Nidhi, Stanford University, Stanford, CA, U.S.

Diseases associated with human cartilage like Osteoarthritis have manifested age, mechanical stresses and inflammation as the leading causal and risk factors. Although inflammaging i.e. upregulation of inflammatory processes upon aging is well documented in multiple tissues, a detailed understanding of the responsible tissue-specific molecular mechanisms is lacking. Regeneration of human cartilage is inherently inefficient; therefore an abundant autologous source like human induced pluripotent stem cells (hiPSC) is especially attractive for engineering cartilage. An additional advantage is the generation of developmentally 'younger' chondrocytes akin to the neonatal chondrocytes that exhibit a superior potential for cartilage regeneration than adult chondrocytes. Towards this goal, we recently optimized a growth factor based protocol for differentiating hiPSC into articular-like chondrocytes (hiChondrocytes) within two weeks with an overall efficiency greater than 90%. Interestingly, we observed that the hiPSC-derived chondrocytes mimic neonatal chondrocytes functionally as well in an increased expression of the cell-surface marker, CD24. A low level of CD24 expression is present in hiPSCs and there was a gradual increased during chondrogenic differentiation. Molecular characterization identified an early $Sox9^{low}CD24^{low}$ pre-chondrogenic population that transitioned into a fully differentiated $Sox9^{high}CD24^{high}$hiChondrocytes population during hiPSC differentiation. Furthermore, we have observed that $Sox9^{high}CD24^{high}$ chondrocytes are resistant to cytokine-induced inflammation as compared to $Sox9^{high}CD24^{low}$ human adult chondrocytes. An acute loss of CD24 leads to an increase in NFκb activation and inflammatory gene expression as well as an increased response to IL1ß in chondrocytes. These findings provide evidence for a novel role for CD24 in cartilage function whereby it can modulate NFκb activity and the response to inflammatory cues. In this report, we have therefore identified CD24 as a novel regulator of cartilage function and response to age-associated inflammaging. $CD24^{high}$ hiPSC-derived chondrocytes therefore are a very attractive candidate for regenerative cell therapy in cartilage diseases with an inflammatory component including Osteoarthritis.

T2140

CHARACTERIZATION OF SKELETAL MYOGENIC CELLS DERIVED FROM HUMAN IPS CELLS

Narita, Asako[1], Masuda, Satoru[1], Wakamatsu, Toshifumi[1], Suzuki, Masatoshi[2], Fukada, So-ichiro[3], Uezumi, Akiyoshi[4], Takeda, Shin'ichi[1] and Miyagoe-Suzuki, Yuko[1], [1]National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan, [2]University of Wisconsin Madison, Madison, WI, U.S., [3]Osaka University, Osaka, Japan, [4]Fujita Health University, Toyoake, Aichi, Japan

Duchenne muscular dystrophy (DMD) is a severe muscle wasting disorder caused by mutations in the dystrophin gene. Transplantation of iPS-derived myogenic stem/progenitor cells into degenerative muscle is an attractive therapeutic strategy for DMD, and in fact xenograft experiments showed that human iPS cell-derived muscle stem/progenitor cells could repair damaged myofibers and restore dystrophin protein at the sarcolemma. To obtain a large number of myogenic cells from human iPS cells for clinical application, we modified the EZ-sphere method by introducing continuous stirring of the culture. After a six-week culture of spheres, myogenic cells that form multinucleated myotubes *in vitro* were induced. To enrich myogenic cells from the culture, cell surface antigens were screened by FACS. Interestingly, myogenic cells are highly enriched in the CD56(+) CD82(+) fraction. In addition, cell surface antigens useful for negative selection were also identified. Sorting the CD56(+) CD82(+) fraction, combined with negative selection by a magnetic cell separation system, enabled us to further enrich myogenic cells. Sorted cells were then intramuscularly transplanted into cardiotoxin-injured tibialis anterior muscles of immune-deficient dystrophin-deficient *NSG-mdx^{4cv}* mice. In the poster, we will describe the gene expression profiles of myogenic cells derived from human iPS cells and their regenerative activities.

T2142

SINGLE CELL LONGITUDINAL CHANGES IN MITOCHONDRIA AND LYSOSOMES IN A HUMAN NEURON MODEL OF PARKINSON'S DISEASE AND THEIR PROGNOSTIC VALUE FOR DEGENERATION

Ravisankar, Abinaya[1], Lee, Alicia[1], Fatima, Tehniat[1], Skibinski, Gaia[1] and Finkbeiner, Steven[1,2], [1]Gladstone Institute of Neurological Disease, the Taube/Koret Center for Neurodegenerative Disease and the Hellman Family Foundation Program in Alzheimer's Disease Research, San Francisco, CA, U.S., [2]Departments of Neurology and Physiology, University of California, San Francisco, San Francisco, CA, U.S.

Parkinson's disease (PD) is the second most common neurodegenerative disease, affecting 7–10 million people worldwide. α-synuclein is a major component of Lewy bodies, which is one of the pathological hallmarks of this debilitating disease. Duplication and triplication mutations in *SNCA*, the gene that codes for α-synuclein, is known to cause PD. Although α-synuclein has been linked to the dysfunction of mitochondria and lysosomes, its contribution to PD remains unclear. Using human neurons differentiated from induced pluripotent stem cells (iPSCs) obtained from healthy individuals, we are investigating the effects of α-synuclein overexpression on organelles such as mitochondria and lysosomes. We use robotic microscopy (RM) to longitudinally track live neurons over their complete life times. As we follow individual neurons, we have the dynamic range and sensitivity to capture cellular events that lead to occurrence of disease-associated phenotypes such as neuron survival and neurite arborization. Our method also provides sufficient resolution to identify dose-dependent effects of α-synuclein on cellular phenotypes, including mitochondria and lysosome morphology, which can be quantitatively measured and linked to the fate of the neuron. Using powerful statistical survival models, we can determine if morphological changes in mitochondria and lysosomes have prognostic significance in α-synuclein-associated neurodegeneration. We can also quantify how important they are and if there is interplay between them during neurodegeneration. Characterizing the effects of α-synuclein expression on neuronal phenotypes is critical for understanding the mechanism of α-synuclein-associated neurodegeneration and for developing therapeutic strategies for PD.

Funding Source: Michael J Fox Foundation, NIH Common Fund and NINDS

T2144

DIFFERENTIATION OF WELL CHARACTERISED HUMAN IPSCS TO SENSORY AND CORTICAL NEURONS FOR USE IN DRUG DISCOVERY

Tams, Daniel[1], Holder, Julie[1], Courtney, Aidan[2], Bruce, Kevin[2], Steeg, Rachel[2], Clare, Nicholas[1] and Hindhaugh, Lauren[1], [1]Roslin Cell Sciences, Cambridge, U.K., [2]Roslin Cell Sciences, Roslin, Midlothian, U.K.

Induced pluripotent stem cell (iPSC) models of human disease are rapidly becoming an integral part of drug discovery, disease stratification and basic research. The consistent derivation and banking of iPSCs from healthy donors or patients with disease causing genome mutations is an important step to fully utilise the potential of this technology. Robust characterisation of iPSCs as a population and at the single cell level is integral to ensuring a defined starting material for subsequent research. In drug discovery more predictive preclinical cellular disease models are required to assess and rank the efficacy of compounds in a cost-effective manner. iPSC derived models including neuronal, cardiomyocyte, pancreatic and hepatocyte cell types display the genetics and cellular function of primary human cells. They therefore offer an improved mechanism to improve the efficiency and economics of drug development. This poster describes protocols of cortical and sensory neuronal derivation from a well-defined starting population of human iPSC supplied by the European Bank of Induced pluripotent Stem Cells (EBiSC). These stem cell derivatives are produced under defined media conditions, are well characterised and can be used in high throughput assays demonstrating the application of this technology to multiple areas of drug discovery and development. Furthermore, applying these protocols to disease specific hiPSC lines enhances the assay relevance for drug screening and ultimately reduces drug attrition in later stage clinical trials.

T2146

SINGLE-CELL FUNCTIONAL AND TRANSCRIPTIONAL PROFILING OF ALS PATIENT AND ISOGENIC IPS CELL-DERIVED MOTOR NEURONS

Wiskow, Ole[1,2], Lee, Seung-Kyu[3], Ghosh, Sulagna[1,2], Sandoe, Jackson[4], Huang, Xuan[3], Woolf, Clifford[3] and Eggan, Kevin Carl[1,2], [1]The Broad Institute of Harvard and MIT, Cambridge, MA, U.S., [2]Harvard University, Cambridge, MA, U.S., [3]Boston Children's Hospital, Boston, MA, U.S., [4]Whitehead Institute, MIT, Cambridge, MA, U.S.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of the motor nervous system caused by the progressive loss of lower and upper motor neurons (MN). Affected motor neurons in rodent ALS models and human

post-mortem show increased oxidative stress, reduced mitochondrial function, altered subcellular transport, and activation of the ER stress and unfolded protein response pathways. Furthermore, clinical neurophysiological studies revealed that ALS patients exhibit a hyperexcitability phenotype in their neuro-motor circuit. In order to elucidate the correlation between these different molecular and functional hallmarks of ALS, we decided to analyze both the gene expression and the electrophysiological profile of single, ALS-affected motor neurons. Using this new experimental approach, we generated two different patient-specific iPS cell lines carrying the ALS associated A4V mutation in superoxide dismutase 1 ($SOD1^{A4V/+}$). Importantly, we then corrected the mutated allele to specifically analyze the effects of this mutation in our model, generating genetically corrected but otherwise isogenic $SOD1^{+/+}$ iPS cell lines. From these cell lines we generated reporter cell lines that express GFP under the control of the promoter of the motor neuron marker HB9 to allow for the specific isolation of single motor neurons. After differentiation, isolation and a three-week maturation period in the presence of glia cells we assessed and compared the electrophysiological characteristics of single motor neurons carrying either the $SOD1^{A4V/+}$ or the corrected $SOD1^{+/+}$ gene using patch clamp. Similar to the hyperexcitability phenotype in ALS patients, we could show that at the single-cell level $SOD1^{A4V/+}$ motor neurons were hyperexcitable compared to the isogenic control cells. Subsequently, the same motor neurons were retrieved and their gene expression analyzed using single-cell RNAseq. Interestingly, the single-cell RNAseq analyses revealed differential expression of genes involved in mitochondrial function, cytoskeletal and ion channel genes. Going forward we hope that the results of this study will provide new insights in ALS disease cause and progression and help us identify new therapeutic targets.

Funding Source: This work was supported by the ALS Association (ALSA).

IPS CELLS: EPIGENETICS

T2150

LARGE SCALE ANALYSIS OF LOSS OF IMPRINTING IN HUMAN PLURIPOTENT STEM CELLS

Bar, Shiran, Schechter, Maya and Benvenisty, Nissim, The Hebrew University, Jerusalem, Israel

Genomic imprinting is an epigenetic process resulting in a group of genes that are expressed only from one parental allele, while the other allele is silenced. Aberrations in imprinted genes are found in several developmental disorders and loss-of-imprinting (LOI) may be associated with tumor formation. In addition to genetic aberrations, pluripotent stem cells (PSCs) may acquire epigenetic abnormalities, such as loss of imprinting. Determining the extent in which LOI occurs in PSCs has important consequences on the use of these cells in regenerative medicine and disease modeling. In this study we performed a global analysis of loss of imprinting on 271 PSC samples from numerous sources, using RNA sequencing data. This allowed for statistically significant inquiries and for broad conclusions by comparing biallelic expression of various imprinted genes in different PSC types. Our results demonstrate that loss of imprinting is fairly common in PSCs and on average more than one imprinted gene is biallelically expressed in a given cell line. Interestingly, reprogrammed PSCs gain significantly higher imprinting aberrations than ESCs, and some aberrations could originate from a sub-population of the parental cell line that is used to generate the reprogrammed PSC. The biallelic expression persists during differentiation to both neural lineage and beta cells. The imprinted genes could be classified according to their LOI prevalence. Surprisingly, we establish that imprinted genes harboring a germline paternal iDMR are more vulnerable to imprinting aberrations than those with maternal iDMR, suggesting for possible differences in the maintenance of the parental iDMRs in PSCs. The relatively high frequency of LOI observed in our analysis, along with the known involvement of imprinting in cancer and disease, substantiate the need to closely examine PSC lines for imprinting aberrations.

T2152

MULTIPLE ANALYSIS FOR THE DNA METHYLATION CHANGED IN PARKINSON'S DISEASE SPECIFIC IPSC-DERIVED DOPAMINERGIC NEURONS

Kuzumaki, Naoko[1,2], Suda, Yukari[1], Igarashi, Katsuhide[3], Narita, Michiko[1], Takeshima, Hideyuki[4], Ushijima, Toshikazu[3,4], Hattori, Nobutaka[5], Suematsu, Makoto[6], Okano, Hideyuki[2,3] and Narita, Minoru[1,3], [1]Department of Pharmacology, Hoshi University, Tokyo, Japan, [2]Department of Physiology, Keio University, Tokyo, Japan, [3]L-StaR, Hoshi University, Tokyo, Japan, [4]Division of Epigenomics, National Cancer Center Res. Institute, Tokyo, Japan, [5]Department of Neurol., Juntendo University Graduate School Medicine, Tokyo, Japan, [6]Department of Biochemistry, Keio University School of Medicine, Tokyo, Japan

It is widely accepted that iPS cells (iPSCs) are pluripotent cells which give rise to all cells in the organism. In the present study, we evaluated the dopaminergic cell vulnerability under Parkinson's disease (PD) associated with dynamic changes in DNA methylation using disease specific human iPSCs. All of the clones were differentiated into dopaminergic neurons expressed tyrosine hydroxylase. Using CE-MS-system, we found the change in

the expression of several metabolites in glycolysis and glutathione metabolism pathways in PD-iPSCs derived dopaminergic neurons compared to control. Interestingly, the expression of 2-hydroxyglutaric acid and S-adenosylmethionone (SAM), which can lead to DNA methylation, was increased in PD-iPSC-derived dopaminergic neurons. Subsequently, we profiled DNA methylation in PD-iPSC-derived dopaminergic neurons using the Illumina Infinium HumanMethylation 450 BeadChips. We found the increased CpG-methylation at HTRA4 and MEG3 genes in PD-iPSC-derived dopaminergic neurons. These findings suggest that, although further studies are still needed, metabolic abnormality and epigenetic changes in PD-iPSC-derived dopaminergic neurons may, at least in part, contribute to neuronal dysfunction in PD.

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CHROMATIN IN STEM CELLS

T2154

CHD7 SPECIFIES STEM CELL IDENTITY AND NEUROGENIC POTENTIAL IN HUMAN NEURAL STEM CELLS

Chai, Muh Chyi[1], Sanosaka, Tsukasa[1], Zhou, Zhi[2,3], Koya, Ikuko[1], Satoe, Banno[1], Okuno, Hironobu[1], Okano, Hideyuki[1] and Kohyama, Jun[1], [1]Keio University, Tokyo, Japan, [2]Keio University, Tokyo, Tokyo, Japan, [3]Japan Society for the Promotion of Science, Tokyo, Japan

Precise control of tissue-specific gene expression is required to govern and orchestrate cellular identities and functions. Cis-regulatory machinery is a central player regulating the gene expression, yet the mechanistic principles underlying controlling cellular divergence contributed by somatic stem cells remain poorly understand. Using human neural progenitors (hNPCs) as an in vitro model, we demonstrated essential mechanisms to maintain stemness and neurogenic function in hNPCs regulated by Chromodomain Helicase DNA-binding protein 7 (CHD7), whose mutation is frequently observed in human CHARGE syndrome. Subsequently, non-neural highly related to mesodermal properties are upregulated in NPCs after CHD7 knockdown. We also demonstrated genome-wide profiling of CHD7-targets in hNPCs to systemically uncover regulatory mechanism governed by CHD7. CHD7 is highly associated with active enhancer chromatin state, characterized by H3K27Ac association. The CHD7-occupied regions is highly linked to spatiotemporal expression of central nervous system (CNS), unraveling the role of CHD7 in coordinating CNS-specific gene regulatory program. We further identified the downstream effectors of CHD7, which reportedly function to shape cellular identities by enhancing CNS-specifc program and repressing non-CNS-specific programs. These results indicate CHD7 as an information hub of epigenomic landscape in NPCs, allowing precise control of spatiotemporal dynamics of NPCs during human brain development.

T2156

DISSECTING REGULATORY MECHANISMS CONTROLLING SOX2 EXPRESSION

Kim, Seokho, Wang, Yuan, Gate, Rachel, Emmerson, Elaine, Knox, Sarah, Ye, Chun Jimmie and Shen, Yin, University of California, San Francisco, San Francisco, CA, U.S.

The transcriptional factor Sox2 plays essential roles in maintenance of multipotent stem cells populations in many cell types and embryonic developmental stages. The importance of Sox2 to organ development is shown in murine studies where ablation or overexpression of Sox2 leads to defects in formation of the nervous system, eye, and lung. More recent studies indicate Sox2 is differentially expressed in cells of the same lineage, and that this level corresponds to cellular outcome (e.g., differentiation, proliferation). Despite these findings, how Sox2 levels correlate with cell fate and the mechanisms that control Sox2 dosage are not well understood. Deciphering the fine-tuning of Sox2 has profound implications for the development of novel therapeutics targeting developmental defects, cancer as well as for the generation of induced pluripotent stem (iPS) cells whose differentiation is regulated, at least in part, by SOX2 activity. To provide mechanistic insights into the molecular basis for fine-tuning of Sox2 expression during development, we performed circularized chromosome conformation capture using high-throughput sequencing (4C-seq) and assay for transposase-accessible chromatin using sequencing (ATAC-seq) to identify regulatory elements interacting with SOX2 gene in human and mouse stem cells and developmental tissue samples**.** Our study reveals temporal and spatial-specific regulatory control of Sox2 expression during development.

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GERMLINE CELLS

T2160

Sirt1 REGULATES THE DNA-METHYLATION OF IMPRINTED AND GERMLINE LINEAGE GENES IN MURINE PLURIPOTENT STEM-CELLS BY ANTAGONIZING DNMT3L

KIM, Yonghwan, LIM, Jisun N, Heo, Jinbeom, Lee, Seungun and SHin, Dong-Myung, University of Ulsan College of Medicine, Seoul, Korea, South

Proper DNA-methylation is required for normal development. Although embryonic stem-cells (ESCs) highly express all types of DNA methyltransferases (Dnmts), these cells should maintain a low level of DNA-methylation at the promoters for several pluripotent and homeodomain-containing developmental master transcription factors to ensure their pluripotency and differentiation capacity. Here, we show that NAD-dependent deacetylase Sirt1 prevents in murine ESCs the DNA-methylation selectively on imprinted and germline genes by antagonizing Dnmt3L. Transcriptome and methylome analyses demonstrate that Sirt1$^{-/-}$ ESCs remarkably repressed the expression of some imprinted and germline genes, concomitantly increasing the DNA-methylation of their regulatory elements. Accordingly, Sirt1$^{-/-}$ ESCs highly expressed Dnmt3L, and its knockdown partially rescued abnormal DNA-methylation of affected Sirt1 target genes. The Sirt1 protein suppressed both the transcription and protein stability of Dnmt3L. In the embryoid body-based in vitro differentiation assay, Sirt1 deficiency delayed germline differentiation processes and spermatogenesis, which were significantly or partially rescued by reintroducing Sirt1 cDNA or Dnmt3Lknockdown, respectively. Thus, we suggest that Sirt1 protects DNA-methylation in germline genes in ESCs, thereby guaranteeing proper differentiation.

TOTIPOTENT/EARLY EMBRYO CELLS

T2164

ROLE OF PROSTAGLANDIN I2 ANALOGUE IN EARLY DEVELOPMENT AND SUBSEQUENT EARLY EMBRYOGENESIS IN PIGS

Kim, Ji-Su[1,2], Yoon, Seung-Bin[1,2], Choi, Seon-A[2], Jeong, Pil-Soo[2], Yang, Hae-Jun[2], Kim, Joo-Young[2], Park, Young-Ho[2], Song, Bong-Seok[2], Sim, Bo-Woong[2] and Kim, Sun-Uk[2], [1]University of Science and Technology, Daejeon, Korea, [2]Korea Research Institute of Bioscience and Biotechnology (KRIBB), Cheongju-si, Korea

Despite the several evidences concerning the presence of prostaglandin I2 (PGI2) in mammalian oviducts, its role in early embryonic development was largely unknown. Thus, the current study was carried out to examine the effect of PGI2 on in vitro developmental competence of porcine early embryos and underlying mechanism(s) by supplementing iloprost, a PGI2 analogue, into in vitro culture (IVC) medium. Especially, trophectoderm cell numbers were greatly increased, and cell survival was considerably improved in the blastocysts of 1 μM iloprost treatment group. Interestingly, Western blotting analysis showed that phosphorylation of Akt was markedly increased by treatment with 1 μM iloprost, suggesting the activation of phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K) signaling pathway. In addition, blastocyst formation rate, cell numbers and cellular survival were greatly reduced by Wortmannin, a potent PI3K inhibitor, which were significantly ameliorated by 1 μM iloprost treatment. Consistent with results from the IVF embryos, 1 μM iloprost improve the developmental competence in both parthenogenetically-activated and somatic-cell-nuclear-transferred embryos. Taken together, thμese results demonstrated that iloprost efficiently enhances the early embryonic development via Akt activation in pigs. Therefore, the current study strongly suggests that iloprost can be defined as a useful IVC supplement for massive production of porcine early embryos with high developmental competence.

T2166

DPPA3, A MATERNALLY DERIVED EPIGENETIC REPROGRAMMING FACTOR, IS ESSENTIAL FOR ENDOCYTOSIS IN EARLY MOUSE EMBRYOS

Shin, Seung-Wook and Dean, Jurrien, NIH, NIDDK, Bethesda, MD, U.S.

After mammalian fertilization, maternal control of gene expression is coordinately transferred to the newly established embryonic program in totipotent 1-cell zygotes. This maternal-to-zygotic transition (MZT) is accompanied by

degradation of maternal proteins by the ubiquitin-proteasome system (UPS) and activation of the embryonic genome. However, mechanisms underlying this changeover remain largely unknown. Here, we identify maternal proteins regulated by ubiquitin and investigate the effect of UPS cleavage on their localization and function following fertilization. In initial studies, we focused on Dppa3 (Developmental pluripotency associated 3), a maternal-effect protein also known as PGC7 or Stella. Dppa3 has been implicated in protecting embryonic DNA from TET3-mediated demethylation and maternal genetic ablation of Dppa3 results in cleavage-stage embryonic lethality. The Dppa3 protein is highly expressed during the MZT, but its abundance dramatically decreases after the 4-cell stage of embryogenesis. We document that maternal Dppa3 is partially cleaved in 2-cell embryos and determine the cleavage sites by Edman degradation after proteasome digestion of recombinant Dppa3 protein. Using mutant cRNA microinjected into 1-cell zygotes, we demonstrate that the normal export of Dppa3 from pronuclei to cytoplasm is prevented by point mutations that preclude cleavage and result in Dppa3 persistence in the nuclei after 2-cell embryos. Furthermore, we find that Dppa3 interacts with early and recycling endosome markers, Rab5 and Rab11 respectively, and endosomes and lysosome are abnormally aggregated in maternal Dppa3-deficient oocytes and 1-cell zygotes. Finally, we demonstrate that the arrest of early embryonic development at the 4-cell stage is rescued by microinjection of cRNA encoding the N-terminus of Dppa3 into the 1-cell zygote. Abnormal endosomes are rapidly recovered in the cytoplasm of rescued 1-cell zygotes. These results suggest that maternal Dppa3 is partially cleaved by proteasomes after fertilization and that the N-terminus of Dppa3 is essential for endocytosis during cleavage-stage mouse embryogenesis. Currently, we plan to determine epigenetic effects by comparing global DNA methylation patterns of transgenic embryos expressing control and mutant maternal Dppa3.

EMBRYONIC STEM CELL DIFFERENTIATION

T2170

LONG-TERM SELF-RENEWING HUMAN EPICARDIAL CELLS GENERATED FROM PLURIPOTENT STEM CELLS UNDER DEFINED XENO-FREE CONDITIONS

Bao, Xiaoping[1], Lian, Xiaojun[2] and Palecek, Sean[1], [1]University of Wisconsin-Madison, Madison, WI, U.S., [2]the Pennsylvania State University, University Park, PA, U.S.

The epicardium contributes both multi-lineage descendants and paracrine factors to the heart during cardiogenesis and cardiac repair, underscoring its potential for cardiac regenerative medicine. Despite significant advances in animal models, little is known about the cellular and molecular mechanisms that regulate human epicardial development and regeneration. Here we first generate a WT1-2A-eGFP knockin human pluripotent stem cell (hPSC) line and show that temporal modulation of canonical Wnt signaling is sufficient for epicardial induction from hPSCs under chemically-defined, albumin-free, animal component-free conditions. Inhibition of TGFβ signaling permitted long-term expansion of hPSC-derived epicardial cells, resulting in a more than 25 population doublings of WT1+ cells in homogenous monolayers. The hPSC-derived epicardial cells were similar to primary epicardial cells both in vitro and in vivo via morphological and functional assays, including RNA-seq. These findings improve our understanding of self-renewal mechanisms of the epicardium and have implications for stimulating epicardial regeneration via cellular or small molecule therapies.

Funding Source: This work was supported by NIH grant EB007534 (S.P.P.), and a fellowship from the University of Wisconsin Stem Cell and Regenerative Medicine Center (X.B.)

T2172

TRANSCRIPTOME AND DIFFERENTIATION IN VITRO OF HUMAN EMBRYONIC STEM CELL DERIVED INTERNEURON PROGENITORS

Chen, Christopher Y[1], Plocik, Alex[2], Anderson, Nickesha C.[1], Moakley, Daniel Finnell[1], Boyi, Trinithas[1], Dundes, Carolyn[1], Lassiter, Chelsea[1], Graveley, Brenton[2] and Grabel, Laura[1], [1]Wesleyan University, Middletown, CT, U.S., [2]University of Connecticut Health Center, Farmington, CT, U.S.

The medial ganglionic eminence (MGE) is a transient embryonic structure in the ventral telencephalon that is a major source of cortical GABAergic inhibitory interneuron progenitors. The homeobox domain-containing transcription factor NKX2.1 is highly expressed in the MGE and pre-optic area of the ventral subpallium and is essential for specifying cortical interneuron fate. Using a combination of growth factor agonists and antagonists to specify ventral telencephalic fates, we previously optimized a protocol for the efficient generation of NKX2.1-positive MGE-like neural progenitors from human ESCs. Using high throughput mRNA sequencing and quantitative PCR (qRT-PCR) analysis of the NKX 2 .1-positive progenitor population, we demonstrate that these cells display a transcriptome profile similar to the MGE and ventral telencephalon of both mouse and human MGE. NKX2.1 gene expression was more than 8-fold higher in the FACS enriched GFP-positive neural progenitors and RNA levels of MGE and subpallial markers MBIP, DLX1, and DBX1 were also elevated relative to the NKX2.1:GFP-negative population. Hierarchical clustering of the top 100 genes with

the largest calculated fold change demonstrated genes specific to the subpallium restricted to the NKX2.1:GFP-positive cell fraction, whereas the NKX2.1:GFP-negative population is associated with a more heterogeneous fate. Using astrocyte co-culture, we show that the MGE-like cell population differentiates into GABAergic interneurons, including SST and PV subtypes. These findings suggest that the MGE-like cell population can serve as an excellent model to study cortical interneuron differentiation and pathology, and can be used for the implementation of cell-based therapies to treat a variety of interneuron-related neurological disorders.

Funding Source: Connecticut Regenerative Medicine Research Fund

T2174

THYROID CONVERSION OF MOUSE ESC-DERIVED ANTERIOR FOREGUT THROUGH TRANSIENT OVEREXPRESSION OF NKX2-1

Dame, Keri[1], Cincotta, Steven[1], Sanghrajka, Reeti[1], Skvir, Nicholas[1], Wilson, Talitha[1], Zhang, Liye[1], Monti, Stefano[1], Kotton, Darrell[2] and Ikonomou, Laertis[2], [1]Boston University, Boston, MA, U.S., [2]Boston University School of Medicine, Boston, MA, U.S.

Thyroid lineages are derived from mouse embryonic stem cells (mESCs) through brief BMP4/TGF-β signaling inhibition at the definitive endoderm stage leading to anterior foregut endoderm, followed by FGF2/BMP4 treatment. These cells are characterized by expression of Nkx2-1, a homeodomain transcription factor expressed in the developing lung, thyroid, and forebrain. Currently, little is known about the specification process and the yield of progenitors derived is low. To investigate if transient Nkx2-1 expression can increase the efficiency of Nkx2-1$^+$ thyroid progenitor specification, we utilized a mESC line double knock-in GFP-T/hCD4-Foxa2 with a doxycycline inducible (Tet-On) Nkx2-1 transgene. Activation of the Nkx2-1 transgene for 24 hours at the anterior foregut endoderm (AFE) stage induces and maintains high levels of endogenous Nkx2-1 (up to 80% Nkx2-1$^+$ cells) as well as thyroid-specific markers including Pax8 (up to 60% Nkx2-1$^+$,Pax8$^+$ cells), Tg, Foxe1, Hhex, Nis, and Tshr at later stages (day 22+) in our protocol. These cells can be cultured in three-dimensional culture, where they mature and organize into follicle-like structures. Critical determinants of this thyroid lineage specification have been revealed by variations in developmental stage timing, signaling pathways, and sorting of subpopulations. Specifically, these experiments highlight a very narrow developmental time window of cellular competence to respond to exogenous Nkx2-1. They also show essential aspects including the derivation of anterior foregut endoderm populations with varied competence (marked by and sorted on Foxa2 expression) and the necessity of dual FGF2/BMP4 signaling activation. To provide further insights into the mechanisms of this thyroid specification from AFE, we are analyzing RNA-Seq data sets acquired from relevant stages to identify potential targets of Nkx2-1 and changes in global gene expression. The results demonstrate that Nkx2-1 can act as a stage-specific inductive signal during directed differentiation of mESCs to thyroid follicular cells. This method has provided novel insights into the thyroid specification process and exemplifies the potential of a more efficient system for deriving and studying thyroid cells, which can be used for in vitro modeling of development and disease.

T2176

NOD MOUSE HAPLOID GENETIC SCREEN AS A TYPE 1 DIABETES MODEL

Freeman, Brent, The Scripps Research Institute, La Jolla, CA, U.S.

Genetic screens have been shown to be a valuable tool in identifying cellular and molecular mechanisms underlying complex human diseases. One of the pitfalls of a genetic screen, however, is the ability for the two chromosomes of a diploid cell to compensate for any mutations. A cell with only a single chromosome would ensure that any introduced mutation would automatically affect the entire gene and therefore provide a much more reliable genetic screen. One way to overcome this limitation is to restrict the cell to a single set of chromosomes by deriving a line of haploid embryonic stem cells (hapESCs). These hapESCs can then be differentiated into any desired cell type. We are utilizing the non-obese diabetic (NOD) mouse model to study the interaction between immune cells and pancreatic beta-cells in type-1 diabetes (T1D). The hapESCs from NOD mice are differentiated into pancreatic beta-cells, in order to perform the genetic screen. To derive hapESCs, unfertilized oocytes were harvested from NOD mice and activated using $SrCl_2$. After three to four days, the resulting blastocysts were plated in vitro to derive hapESCs. Haploidity will be verified through staining with Hoechst 33432 and flow cytometry analysis; After flow cytometric enrichment, promising hapESC lines will then be subjected to spectral karyotyping (SKY) analysis to determine the overall chromosomal composition. The hapESCs will then be differentiation into pancreatic beta-cells. During this in vitro differentiation, the hapESCs will be exposed to a mutagen in order to induce random mutations, and subjected to a screen. The underlying mutations will then be identified using exome sequencing. Using our NOD hapESC screen will enable us to better understand mechanisms underlying T1D and uncover potential factors that can be explored for therapeutic purposes.

T2178

DEVELOPMENTAL & PHARMACOLOGICAL PROFILING OF HUMAN EMBRYONIC STEM CELL-DERIVED MEDIUM SPINY NEURONS

Hunt, Cameron, Monash University, Melbourne, Australia

Neuronal circuits of the basal ganglia play a vital role in regulating movement, reward & motivation. Signalling imbalances in response to degeneration or malfunction of neural subtypes in the basal ganglia are the centre of several neuropathologies including Parkinson's and Huntington's disease, HD. Although the molecular basis of HD is well understood with associated motor & non-motor issues resultant from compromised medium spiny neuron (MSN) populations in the dorsal and ventral striatum, respectively, pivotal mechanistic underpinnings which may contribute to the susceptibility of MSNs in HD remain unclear. The purpose of this study was to develop in vitro tools to investigate the development & pharmacology of bona fide human MSNs. The expression of dopamine-and-cAMP regulated phosphoprotein (DARPP-32) in the striatal neurons is widely used to identify MSNs. To this end, CRISPR-mediated gene editing technology was used to engineer human embryonic stem cell (hESC) reporter lines targeted to the DARPP-32 locus. Upon differentiation, these hESC lines have allowed identification, isolation and functional interrogation of in vitro-derived (IVD) DARPP-32+ neurons. Using an adherent monolayer differentiation strategy, reliant on dual-SMAD inhibition, hESCs developed into ventral forebrain progenitors expressing characteristic markers of striatal origin including MASH1, DLX2 & ISL1. Terminal differentiation of progenitors yielded both DARPP-32+GAD65/67+ & DARPP-32+VGLUT2+ neurons indicative of striatal MSNs with dorsal & ventral profile. The generation of multiple subclasses of MSNs was confirmed pharmacologically with IVD-MSNs exhibiting classical responses when challenged with D1 and D2-like dopamine receptor ligands.

T2180

GENERATION OF MESENCHYMAL PROGENTOR CELLS FROM HUMAN EMBRYONIC STEM CELLS DERIVED BY SOMATIC CELL NUCLEAR TRANSFER

Jun, Sung-Min[1], Lee, Jeoung Eun[1], Chung, Young Gie[2] and Lee, Dong Ryul[1], [1]CHA University, Gyeonggi-do, Korea, [2]CHA Health Systems, Los Angeles, CA, U.S.

Adult tissue-derived mesenchymal stromal cells (MSCs) have been considered to contribute to the recovery of tissue in damaged area, so they are expected to have great potential for regenerative medicine and clinical trials. However, the inability of mass production of functional cells from a single donor and donor-dependent variability in quality give limits for their clinical application. Therefore, the derivation of MSCs from human embryonic stem cells (hESC) is an alternative to adult MSCs regarding as scalability and consistent quality. In this study, we developed a simple and efficient protocol for differentiation of hESC into mesenchymal progenitor cells (MPCs), and then two hESC lines (H9 and CHA-hES 15) and 3 SCNT-hESC lines (CHA-hES NT4, NT5 and NT8) were differentiated into MPCs. We investigated MPCs derived from hESC and SCNT-hESC in terms of morphology, surface marker expression, proliferative capability, colony forming assay, and differentiation capacity into osteogenic, adipogenic and chondrogenic lineages, in order to evaluate ESC derived MPCs as a cell source for potential regenerative applications. With our protocol which is EB formation with TGF-β inhibitor and then outgrowth in DMEM (low glucose) supplemented with 10% FBS, we have got homogenous cell population by passaging without any specific selection steps after 5 passages. Based on our results, both hESC derived MPCs and SCNT-hESC derived MPCs were positive for markers associated with mesenchymal stem cells (CD29, CD44, CD90, CD105), but negative for embryonic stem cell markers (TRA1-60, SSEA4) and hematopoietic cell markers (CD34, CD45). There was any differences among hESC derived MPCs and SCNT-hESC derived MPCs in terms of MPC characteristics and proliferative ability, besides all of them showed better proliferative ability and shorter doubling time than BM-MSCs. Therefore, hESC and SCNT-hESC can be differentiated to MPCs by simple cultivation without any addition of specific cytokine or selection step, and these cells can be used as an alternative source of MSC for regenerative medicine.

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T2182

RECAPITULATING CRANIOFACIAL DEVELOPMENT IN PLURIPOTENT STEM CELL-DERIVED ORGANOIDS

Lee, Jiyoon and Koehler, Karl R., Indiana University School of Medicine, Indianapolis, IN, U.S.

Craniofacial development is fundamental to survival and social communication. However, millions of individuals suffer from improperly developed or surgically reconstructed facial features due to genetic mutations, removal of cancerous tumors, or severe burns. To better define facial development and identify novel cell therapies for facial defects, we will benefit from in vitro systems that model the mechanism by which the craniofacial complex arises in the embryo. The human face and mouth forms from an intermingling of ectoderm, endoderm, mesoderm, and cranial neural crest cells (CNCCs). Specifically,

we are interested in mechanisms dictating CNCC self-organization into facial features, such as the facial dermis and cartilage. Previously, we established a 3D culture system using pluripotent stem cells (PSCs), which gave rise to inner ear organoids as well as a diverse group of mesenchymal tissues, such as cartilage, skin, and muscle. Here, we show that two critical components of the craniofacial complex, the surface ectoderm and CNCCs, can be co-induced from human PSCs (hPSCs) in 3D culture. We used small molecules and recombinant proteins to control BMP, TGF-β, and FGF signaling in differentiating hPSC aggregates. After two weeks of guided differentiation, organoids emerged containing an inner layer of KRT5$^+$ surface epithelium and an outer layer of AP2$^+$ PDGFRa$^+$ CNCC-like cells, reminiscent of the cranial ectomesenchyme. Remarkably, these tissues self-organize into stratified epidermis and dermis, as well as cartilaginous masses, mimicking embryonic cranial development. Thus, our hPSC-based in vitro system offers an opportunity for in-depth investigation of the mechanisms underlying craniofacial development, modeling neurocristopathies, and identifying novel regenerative therapies.

T2184

OVEREXPRESSION OF GATA-2 ON HUMAN PLURIPOTENT STEM CELLS PROMOTES THE GENERATION AND EXPANSSION OF EARLY HEMATOPOIESIS

Ma, Feng[1], Zhou, Ya[1,2] and Cheng, Bo[1], [1]Institute of Blood Transfusion, Chinese Academy of Medical Sciences, Chengdu, China, [2]State Key Laboratory of Experimental Hematology, Tianjin, China

GATA-2 is essential for normal hematopoiesis which expresses highly in hematopoietic stem/progenitor cells (HSPCs), early erythroid cells, mast cells and eosinophils. The GATA-2 null mice died by E10-11 with deficit in definitive HSPCs, while overexpression of GATA-2 in cord blood CD34+ cells increased their property of quiescence, reflecting a diverging effect of GATA-2 on early and late hematopoiesis. Uncovering the mechanisim of GATA-2 controlling early development of human hematopoiesis from pluripotent stem cell (hPSCs) may be of great importance in conducting the generation of HSCs. We have established an efficient method to gain multipotential hematopoietic progenitors from hPSCs by coculture with mAGM-3 cells that favoring definitive hematopoiesis. Temporally inducible overexpression of GATA-2 in the hPSCs was established by a PiggyBac (PB) vector and its functional role in the early development of hematopoiesis analyzed by Doxycyclin (DOX) induction. In developing EB (which represents the initial hematopoiesis), overexpression of GATA-2 revealed that the proportion of CD34+cells increased 4.8 fold as compared without DOX. We then analyzed the proportion of hematopoietic cells in the PB-GATA-2-hESCs cocultured with mAGM-3 cells at day14 by adding DOX at a time-coursed pattern (day 0, 1, 2, 3, 4, 6, 8 and 10 respectively). At any timepoints, the proportion and absolute number of CD34$^+$CD43$^+$ cells were all increased. Along with CD34$^+$CD43$^+$ cell development, the proportion of CD34$^+$CD45$^+$ cells that could give rise to all hematopoietic lineage cells also increased consequently. Further assay by hematopoietic colony-forming potential of day-6 coculture-derived cells revealed that GATA-2 overexpression cells generated 43 fold increase in myeloid colonies, but not erythroid colonies. We are now examining the morphology, functional properties and transcriptional profile of CD34$^+$CD43$^+$ and CD34$^+$CD45$^+$ cells in GATA-2 overexpression hPSCs. Our GATA-2 overexpression model might lead to a possible way to gain mature hematopoietic cells, esp. HSCs, on a large scale. The pinpointed developing switch by overexpression of GATA-2 on early hemagenic endothelial cells (Flk+CD34+) would also lead us to further explore the molecular mechanism controlling the initiation of hematopoiesis

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T2186

GENERATION OF A 3D STEM CELL NICHE FOR THE REGENERATION OF SPIRAL GANGLION NEURONS USING PEPTIDE AMPHIPHILE NANOFIBER GELS

Morrissey, Zachery[1], Stupp, Samuel I.[2], Kessler, John[3] and Matsuoka, Akihiro[1], [1]Northwestern University, Chicago, IL, U.S., [2]Northwestern Univ, Evanston, IL, U.S., [3]Northwestern University, Chicago, IL, U.S.

The most common method to ameliorate sensorineural hearing loss (SNHL) is the use of a cochlear implant (CI). CIs electrically stimulate spiral ganglion neurons (SGNs) of the cochlea, which relay action potentials centrally to the cochlear nucleus (CN). However, for patients who have few functional SGNs, a CI is rendered mostly ineffective, as there are too few target neurons to relay the CI-encoded stimuli. This study aims to utilize human pluripotent stem cells (hPSCs) to develop otic neural progenitors (ONPs) that (1) are derived from as few non-human materials as possible to improve clinical relevance, (2) can be transplanted into an X-severe combined immunodeficient (X-SCID) rat model with significant viability, (3) differentiate into functional SGN-like cells, and (4) specifically innervate the CN. The larger aims of this study are to translate this research to clinical treatment in which CI technology is coupled with patient-derived induced pluripotent stem cells (iPSCs) that have been reprogrammed to ONPs. Promising results have been demonstrated from the first three lines of inquiry and our lab is currently pursuing research related to the fourth. To develop SGN-like

cells, human embryonic stem cell (hESC) lines H7 or H9 are differentiated using calibrated concentrations of exogenous ligands to guide differentiation along the SGN developmental lineage. These SGN-like cells are functionally responsive in 2D cultures. However, to create a better stem cell niche within the cochlea, 3D culture methods are needed. To address this, 2D cultures are developed into 3D spheroidal aggregates as cells reach the ONP stage and later cultured with BDNF-secreting iPSCs to provide neurotrophic support after the aggregates are transplanted. Aggregates are then inserted into biocompatible peptide amphiphile (PA) gels that promote neural differentiation and act as mechanical constructs that facilitate directional axonal outgrowth. These SGN-like cells have shown improved survival within the PA gels in vitro, as well as in vivo in X-severe combined immunodeficient (X-SCID) rats. Our current projects include optimizing 3D aggregation, identifying candidate molecular SGN axonal guidance mechanisms, and optimizing transplantation procedures and confirming the functionality of the SGN-like cells.

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T2188

SOX21 REGULATES NEUROECTODERMAL DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS

Ogawa, Kazuya, Kim, Suel-Kee, Jaishankar, Amritha, Hoeppner, Daniel, Seo, Seungmae, Wang, Yanhong, Shin, Joo-Heon, Stein-O'Brien, Genevieve, Colantuoni, Carlo, Chenoweth, Joshua and McKay, Ronald D., The Lieber Institute for Brain Development, Baltimore, MD, U.S.

We have developed a monolayer system for growing hPSCs and characterized the emergence of distinct anterior and posterior states. Using RNA-seq datasets and a computational tool, CoGAPS, we identified two patterns that captured gene expression changes in Noggin and SB-431542 (NSB) induced neuroectodermal differentiation. SOX21 was one of the most highly ranked genes in these patterns and did not contribute to any other condition patterns. Immunocytochemistry and gene inspection suggest that SOX21 is involved in the transition from neural specification and induction to commitment. We optimized gene editing technologies in human pluripotent stem cells to establish SOX21-KO cells using the CRISPR-Cas9 system to analyze the function of SOX21. We used two different gRNAs and obtained three homozygous KO (null) clones, which have two independent frame-shift mutations in the HMG domain. These SOX21-KO clones in NSB conditions showed clear elevation of NANOG and SOX2 on the edge of the epithelial sheet and down-regulated OTX2 expression on the core zone. When mesoendodermal differentiation conditions were applied on Day2, SOX21-KO clones showed elevated expression of BRACHYURY and GATA4 defining different regions of the core zone. These data suggest that SOX21 supports neuroectodermal differentiation in two-ways (1) transition from the pluripotent state (2) preventing the differentiation into anterior mesoendoderm.

T2190

IN VITRO GENERATION OF INSULIN-PRODUCING CELLS WITH INSULIN SECRETION KINETICS AND MITOCHONDRIAL RESPIRATION SIMILAR TO ADULT HUMAN ISLETS

Rezania, Alireza[1], Arora, Payal[1], Rieck, Sebastian[1], Ng, Bruce[1], Asadi, Ali[2], Quiskamp, Nina[2] and Kieffer, Tim[3], [1]Janssen, Raritan, NJ, U.S., [2]University of British Columbia, Vancouver, BC, Canada, [3]University of British Columbia, Vancouver, BC, Canada

We recently reported on the differentiation of pluripotent human stem cells into single hormonal insulin+ NKX6.1+ MAFA+ cells (stage 7 cells) that demonstrated glucose responsiveness in a static glucose challenge assay. Further evaluation using a more sensitive dynamic perifusion assay revealed sluggish insulin release kinetics in response to glucose challenge and marginal increase in insulin release after exposure to a GLP-1 mimetic peptide. Moreover, the single hormonal insulin+ cells did not show the typical calcium flux seen with adult human beta cells indicating clear functional deficiencies as compared to mature beta cells. Using a combination of RNA Seq analysis of the cells, empirical testing of various media compositions coupled with targeted small molecule screening, we generated insulin+ cells from human embryonic stem cells (hESCs) with insulin secretion kinetics and mitochondrial respiration similar to adult human islets. Newly generated insulin+ cells acquired some of the features attributed to mature beta cells, such as expression of UCN3, MAFA, GLP-1r, and GLUT1. Notably, these insulin+ cells survived within macroencapsulation devices following transplant in the subcutaneous space of immune compromised mice and yielded significantly faster production of circulating human C-peptide compared to previous reports, with significant glucose responsiveness as early as four weeks post-implant. These results show the feasibility of generating insulin-producing cells from hESCs in vitro with characteristics of mature human beta cells.

T2192

QUANTIFICATION AND CHARACTERIZATION OF GENE EXPRESSION VARIABILITY IN MOUSE EMBRYONIC STEM CELLS

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

Traditional measurements of gene expression at the RNA level typically use tens of thousands to millions of cells. These measurements therefore represent the mean expression level in a population of cells. New techniques developed in recent years allow measuring gene expression at the single cell level, thus providing information on the distribution of expression in the population. Previous studies used these methods to explore and define new subpopulations of cells and quantify variability around the mean. However, commonly used variability measures are heavily dependent on the mean. This becomes even more prominent when comparing between different conditions. Here, we developed a computational approach to correct for this bias. We show that our approach agrees with some known results in the embryonic stem cells field and demonstrate our method for comparison between knockout and wild type systems. Finally, we characterize genes which are differentially variable between the different conditions.

T2194

MAPPING HUMAN PLURIPOTENT-TO-CARDIOMYOCYTE DIFFERENTIATION: METHYLOMES, TRANSCRIPTOMES, AND EXON DNA METHYLATION "MEMORIES."

Tompkins, Joshua, City of Hope/Beckman Research Institute, Duarte, CA, U.S.

The differentiation of human cardiomyocyte from pluripotent cells provides an essential model for understanding cell fate mechanisms and offers substantial promise in cardiac regenerative medicine. For studies on in vitro human cardiomyogenesis we've utilized a human embryonic stem cell suspension bank, produced according to a good manufacturing practice, to generate cardiomyocytes using a fully defined and small molecule-based differentiation approach. Primitive and cardiac mesoderm purification was used to remove non-committing and multi-lineage cells and significantly aided our identification of key transcription factors, lncRNAs, and essential signaling pathways that define cardiomyogenesis. Multi-stage DNA methylomes ultimately reflected cardiomyocyte differentiation and led to the discovery of exon DNA methylation "memories" uniquely enriched among developmental transcription factors. Specifically, we have identified that transcription-linked methylation deposition can persist beyond gene repression providing a historical record of transient developmental gene activation. Current work is focused on understanding the function and predictive power of this new type of epigenetic "memory."

T2196

INHIBITION OF BONE MORPHOGENETIC PROTEIN PREVENTS HEPATIC DIFFERENTIATION IN AIRWAY PROGENITORS

Yao, Jiayi, Guihard, Pierre J, Blazquez Medela, Ana M, Boström, Kristina and Yao, Yucheng, University of California, Los Angeles, CA, U.S.

The crosstalk between endothelial cells and epithelial cells is critical for proper cell differentiation in organogenesis. Bone morphogenetic proteins function as the essential factors in regulating this crosstalk. Here, we report that the loss of a BMP inhibitor, Matrix Gla protein (MGP), disrupts the interaction of pulmonary endothelial cells and epithelial cells, resulting in abnormal hepatic differentiation in lung. We demonstrated that the dysregulated crosstalk of pulmonary endothelium and epithelium caused induction of HGF, which activated hepatic differentiation in airway progenitors. We showed that HGF induced Hnf4a, which competitively bound to Foxa2 from Nkx2.1, driving hepatic differentiation in MGP null lung. Limiting HGF in ECs reduced Hnf4a, abolished the binding of Hnf4a and Foxa2, and reduced the hepatic differentiation in MGP null lung. Together, our results suggest that the inhibition of BMP is essential in lung development by repressing hepatic differentiation.

T2198

CAPRIN1 IS A NOVEL REGULATOR OF STRESS GRANULE FORMATION AND DIFFERENTIATION IN MOUSE EMBRYONIC STEM CELLS

Azad, Gajendra Kumar, Pattabiraman, Sundar, Kaganovich, Daniel and Meshorer, Eran, The Hebrew University of Jerusalem, Jerusalem, Israel

Self-renewal and generation of specialized progeny through differentiation are hallmarks of pluripotent stem cells. Embryonic stem cells (ESCs) are the prototypical pluripotent stem cells that can form all different cell types. In recent years, a growing number of factors controlling pluripotency and differentiation of ESCs have been identified, most of which encode nuclear transcription factors. To reveal novel non-nuclear regulators of ESCs, we screened an endogenously-labeled fluorescent fusion-protein library in mouse ESCs generated in our lab. Searching for factors that are down-regulated during differentiation, we identified the cell cycle associated protein, CAPRIN1. In silico analysis revealed that the promoter region of Caprin1 is occupied by master regulators of ESCs: Oct4, Sox2, Nanog and Klf4, suggesting that Caprin1 might play an important function in ESCs. To study the role of CAPRIN1

in ESCs, we generated Caprin1-knockout (KO) ESCs using CRISPR/Cas9. Caprin1-KO cells showed altered gene expression in embryoid bodies (EBs) and failed to differentiate into NPCs (neuronal progenitor cells). Proteomic analyses in undifferentiated ESCs showed that CAPRIN1 associates with a variety of proteins including those playing pivotal roles in RNA metabolism. Interestingly, Caprin1 shows varied phenotypic changes in different cell cycle phases. It resides in small cytosolic granules during interphase, while these granules become completely diffuse during mitosis. Following stress, CAPRIN1 re-localizes into stress granules (SGs) and interacts with SG residents including G3BP, FUS, TIA1, etc. In contrast, CAPRIN1 does not interact with P-bodies, which are responsible for RNA turnover. Taken together, these results suggest a selective function of CAPRIN1 in ESCs and specifically in SGs, and identify a novel regulator of ESC early differentiation.

EMBRYONIC STEM CELL PLURIPOTENCY

T2202

DEEP SEQUENCING DEFINES THE TRANSCRIPTIONAL MAP OF SUSPENSION TE03 HESCS

Dvir, Shlomi[1,2], David-Eden, Hilda[2], Roytblat, Mark[2], Shariki, Kohava[2], Mandel-Gutfreund, Yael[1], Angel, Itzchak[2] and **Amit, Michal**[3], [1]Faculty of Biology, Technion, Haifa, Israel, [2]Accellta LTD, Haifa, Israel, [3]The Ephraim Katzir Department of Biotechnology, Braude College, Karmiel, Israel

Suspension models of human embryonic stem cells (hESCs) have the potential to revolutionize regenerative medicine by providing a valuable source of cells for clinical applications. However, no study to date has interrogated the transcriptome of suspension hESCs on a genome-wide scale. Here, we performed RNA-sequencing on suspension cultures of TE03 hESCs, embryonal carcinoma (2102Ep) and human foreskin fibroblasts (HFFs). To systematically identify genes that might be associated with the pluripotent state, we carried out differential expression analysis between TE03 and differentiated HFFs. First, we found 4,003 differentially expressed genes (DEGs) with a false discovery rate (FDR)-corrected P-value of 0.01 and 5-fold difference cutoff. Of these, 643 and 155 genes had more than a 100- and 300- fold increase in mRNA levels, respectively. As expected, well-established pluripotency markers, such as SOX2, OCT4, DPPA4 and LIN28A had the largest fold-change and smallest P-values. Second, we show that the vast majority of DEGs display a similar expression pattern across both TE03 and embryonal carcinoma, supporting the role of the identified DEGs in pluripotency, rather than as cell type-specific markers. Third, a comparison with previous reports shows that nearly all genes that were over-expressed in published studies of adherent hESCs are up-regulated in suspension TE03 cells. Gene set enrichment analysis further revealed that the top 3 most significantly enriched gene sets were tightly associated with stemness signatures. Finally, in concordance with the emerging link between pluripotency and long intergenic non-coding RNAs (lincRNAs), we found 245 lincRNAs with 56-fold increase, on average, in mRNA levels, the majority of which are up-regulated in TE03 hESCs. Taken together, our findings show that pluripotency-related signatures are strongly over represented among the list of DEGs. The detected DEGs include well-known as well as novel genes with strong fluctuations in expression levels, suggesting that these genes may shape the stem cell state.

T2204

CHROMATIN AND 3D ARCHITECTURE OF PRE-IMPLANTATION HESCS UNDERGO DISTINCT CHANGES DURING THE TRANSITION TO POST-IMPLANTATION HESCS

Battle, Stephanie Lauren[1], Doni Jayavelu, Naresh[1], Azad, Robert[1], Hesson, Jennifer[1], Ware, Carol B.[1] and Hawkins, David[2], [1]University of Washington, Seattle, WA, U.S., [2]Division of Medical Genetics, Department of Medicine, Department of Genome Sciences, Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Seattle, WA, U.S.

Mammalian embryogenesis results in substantial changes in morphology, gene expression and epigenomic profiles as it transitions from a totipotent to pluripotent cell mass. Soon after fertilization DNA methylation and histone modifications are dramatically remodeled during progression to the blastocyst stage. The early embryonic epigenome maintains an open chromatin structure as repressive heterochromatin is gained later during lineage commitment and differentiation. Shortly after fertilization, H3K4me1 and histone acetylation dramatically increase. This may reflect the activation of the zygotic genome, as these modifications are known markers of active enhancers and promoters.

Using hESCs that represent the pre-implantation blastocyst (naïve) and post-implantation epiblast (primed), we show that hESCs derived under naïve conditions have a chromatin landscape reflective of early embryogenesis. Naïve hESCs have double the enhancer repertoire of primed hESCs, 96% of which are hypersensitive in other human cell types. Half of the enhancers exist in a poised, H3K4me1 only state; however, these poised enhancers do not gain acetylation as they transition to the primed state. Instead, active and poised enhancers are decommissioned in a step-wise manner. We hypothesize that the expansion of H3K4me1 in the naïve epigenome is necessary for establishing an open chromatin structure, which is likely

a key aspect of defining pluripotency. Naïve hESCs also exhibit a dramatic reduction in H3K27me3 and H3K9me3, harboring a third of the bivalent promoters as primed hESCs. Super- or Stretch-enhancers are more abundant in the naïve state and reach sizes over 50kb in length. Naïve enhancers frequently extend through primed hESC CTCF binding sites. To compare the 3D architecture of naïve and primed cells, we have generated high-resolution global interaction maps using in situ DNase HiC. These are the highest resolution maps to date in human cells. Our high-resolution architecture maps allow us to detect differences in topological associated domains (TADs), and boundaries between naïve and primed cells. Taken together our data provide a detailed description of the structure of the naïve hESC genome and epigenome, and provide evidence for what changes must occur as embryonic cells prime themselves for differentiation.

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T2206

CLINICALLY COMPLIANT HUMAN PSC CULTURE CONDITIONS SUPPORT EFFICIENT CLONAL SURVIVAL AND RAPID SCALE-UP

Ericsson, Jesper, Sun, Yi, Xiao, Zhijie, Kallur, Therese and Hagbard, Louise, BioLamina AB, Sundbyberg (Stockholm), Sweden

The lack of defined, xeno-free, easy and robust methods for efficient expansion of human pluripotent stem cells (PSCs) has hindered both the advancement of basic research and human cell therapy, much due to high experimental variation and poor quality cells with phenotypic and genetic changes. Laminin-521 (LN-521) is a protein naturally expressed by human PSCs and is a critical factor of the pluripotent stem cell niche. Laminins influence adhesion, differentiation, migration, phenotypic stability, anoikis resistance and functionality of all cells associated to it. LN-521 is a human and recombinant protein and can easily be used as a cell culture substrate. When used for human PSCs they can be cultured for over 80 single cell passages without any abnormal genetic aberrations and with maintained expression of pluripotency markers. Human PSCs on LN-521 expand 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 ROCK inhibitor. Furthermore, LN-521 can be used as microcarrier coating for generating clinically relevant quantities of human PSCs thus offering a scalable and GMP-compatible bioprocessing platform. Moreover, true clonal culture, important for cell fate tracking, gene function analyses and editing, is possible by using LN-521 in combination with E-cadherin. Human embryonic stem cell (hESC) lines can even be derived from a single blastomere under chemically defined and xeno-free condition on LN-521, thereby circumventing the ethical issues associated with hESCs. The simplicity and reliability of the culture procedure, the rapid cell amplification and the genetic stability of the cells make LN-521 a suitable as reagent in clinical trials for human PSC-based therapy. In conclusion, we show that LN-521 is an optimal matrix for human PSC culture due to its biological relevance that allows derivation, clonal cultivation, stable long-term pluripotent cell growth and scalability. The robust method allows minimum culture maintenance and standardized protocols, which can easily be adapted to automation platforms, making LN-521 a suitable reagent choice for human cell therapy trials.

T2208

FUNCTIONAL SIGNIFICANCE OF OCT4 LINKER FOR MAINTAINING PLURIPOTENCY

Han, Dong, Wu, Guangming, Jerabek, Stepan, Adachi, Kenjiro and Schöler, Hans R., Max Planck Institute for Molecular Biomedicine, Muenster, Germany

In the classical reprogramming cocktail, Oct4 cannot be replaced by any POU family paralog factor without interfering with the maintenance of pluripotency and derivation of iPSCs. This raises the question which features render Oct4 unique and distinct from other POU family paralogs. Proteins of the POU family contain a bipartite DNA-binding domain, which consists of a POU-specific domain (POUS) and a POU-homeodomain (POUHD). Both are connected by a linker, hypervariable both in length and sequence, with no homology between the different POU factors. Our previous data revealed that, in striking contrast to Oct1, the linker of Oct4 is structured as an α-helix and exposed to the surface. Point mutations in this α-helix alter or even abolish the reprogramming activity of Oct4, but do not affect other fundamental protein properties. In the present study, we examined if the Oct4 linker is also important for maintaining pluripotency in ESCs and mouse development. We expressed Oct4 linker mutants in ZHBTc4 ESCs and tested whether the mutants could rescue ZHBTc4 cells when tetracycline was added to the culture medium. Overexpression-specific point mutants were able to maintain pluripotency just like the rescued ESCs, and many Oct4 downstream genes were not affected. However, the efficiency of rescued ESCs was much lower than that of wild-type Oct4, leading to impaired ESC proliferation, which resulted in severe lethality in mouse embryos in vivo. This meant that the specific mutation reduced the function of Oct4 in maintaining ESC pluripotency and proliferation. Furthermore, endogenous expression or overexpression of multi-point mutants could not rescue ZHBTc4 cells, while Oct4 downstream gene expression was not activated and the DNA-binding ability significantly decreased. Moreover, the transactivation activity of Oct4 could not maintain pluripotency in mouse embryos in vivo. In conclusion, our results all point to the hitherto unsuspected functional significance of the Oct4 linker for maintaining pluripotency.

T2210

REGULATION OF MOUSE EMBRYONIC STEM CELL HETEROGENEITY BY GLYCOLYSIS/ REACTIVE OXYGEN SPECIES AXIS

Khoa, LE TRAN PHUC[1,2], Tomoyuki, Tsukiyama[3], Ken, Matsumoto[3], Fujii, Setsuko[3], Satoru, Takahashi[4] and Masatsugu, Ema[3], [1]Human Biology Program, School of Integrative and Global Majors, University of Tsukuba, Tsukuba, Japan, [2]University of Tsukuba, Otsu-shi, Japan, [3]Research Center for Animal Life Science, Shiga University of Medical Science, Otsu, Japan, [4]Department of Anatomy and Embryology, University of Tsukuba, Tsukuba, Japan

Transcriptional factor heterogeneity has been appreciated as an inherent feature of embryonic stem (ES) cells as it intertwines with the balance between self-renewal capacity and lineage commitment trigged by differentiating stimuli. It is well-known that mouse ES cells vary in expression levels of many pluripotency makers (Nanog, Rex1, Tbx3, Klf4, etc.), fluctuating between substates of high and low expression. This discrepancy is associated with the ability of distinct subpopulations to either self-renew or commit toward differentiation. However, intrinsic factors enabling individual cells to either keep their current state or convert into another remain incompletely understood. To address this phenomenon, we first examined global gene expression of the purified Rex1-positive and Rex1-negative cells. Microarray analysis showed that highly expressing genes associated with glycolysis pathway were preferentially detected in Rex1-negative cells as compared to Rex1-postitive cells. Consistently, lactate production rate was higher in Rex1-negative cells than Rex1-positive cells, indicating that Rex1-negative cells possess an increased level of glycolysis relative to Rex1-postive cells. Interestingly, pharmacological or genetic inhibition of glycolysis pathway resulted in a significant enrichment of Rex1-positive cells without causing cell death. Importantly, we found that glycolysis inhibition caused an increase in the levels of reactive oxygen species (ROS) whose suppression by the antioxidant restored Rex1-negative state from the purified Rex1-negative fraction but not from the purified Rex1-positive one, suggesting a complex function of ROS in mediating the dynamic interconversion of each subpopulations. In fact, antioxidant-mediated ROS suppression alone remained the purified Rex1-positive cells in a Rex1-postive state over time compared to the control condition. In contrast, when cultured in the presence of antioxidant, the purified Rex1-negative cells could convert to Rex1-postive cells, but more slowly than in the control culture. Our findings suggest a novel function of in ROS in modulating the interconversion of distinct mouse ES cell substates with a stronger impact observed in Rex1-positive cells than in Rex1-negative cells.

T2212

NME6 AND NME7 SIMULTANEOUSLY REGULATES EIGHT KEY STEMNESS FACTORS FOR MOUSE EMBRYONIC STEM CELL RENEWAL AND THE ROLES IN EARLY DEVELOPMENT

Lin, Hsuan[1,2], Lin, Yu-Tsen[1,3], Kao, Yu-Ting[1], Wang, Chia-Hui[1], Wu, Cheng-Chung[1], Lu, Frank Leigh[2] and Lu, Jean[1], [1]Genomics Research Center, Academia Sinica, Taipei, Taiwan, [2]Department of Pediatrics, National Taiwan University Hospital and National Taiwan University Medical College, Taipei, Taiwan, [3]Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan

Objective. In contrast to the somatic cells, embryonic stem cells (ESCs) are characterized by its immortalization ability, pluripotency, and oncogenicity. Revealing the underlying mechanisms of ESC renewal is important. Methods. We performed a systematically functional screen in mouse ESCs with 4801 shRNAs that target 929 different kinases and phosphatases. Results and Conclusion. 132 candidate genes critical for both ESC expansion and stem cell marker expression were identified. Among them, the shRNAs of 27 genes induce significantly morphological changes. Then we chose non-metastatic cells 6 (Nme6, also named as Nm23-H6) and non-metastatic cell 7 (Nme7, also designated as Nm23-H7), members of nucleoside diphosphate kinase (NDPK) family, to study in-depth. Downregulation of Nme6 and Nme7 by two different shRNAs significantly hampered the expressions of multiple key factors for cell fate determination, which includes Oct4, Nanog, Klf4, c-Myc, telomerase, Dnmt3B, ERas and Nanog. Either knockdown of Nme6 or Nme7 reduces the formation of embryoid body and teratoma. The overexpression of either Nme6 or Nme7 rescues the expressions of multiple stem cell markers and the embryoid body formation in the absence of leukemia inhibiting factor (LIF). To further investigate the functional roles of Nme6 during early embryogenesis, we generated $Nme6^{-/-}$ knockout mice. We found the $Nme6^{-/-}$ knockout mice is embryonic lethal. All embryos died at embryonic day 6.5 to E7.5. To sum up, we reveal Nme6/Nme7 can modulate ESC renewal through 8 key stem cell regulators, and Nme6 is essential for the survival of early embryo.

T2214

ASSESSMENT OF REGULATORY NETWORKS IN HUMAN PLURIPOTENT STEM CELLS WITH HIGH-THROUGHPUT IMMUNOCYTOCHEMISTRY

Baryshnikova, MD, Natalya[1], Lenz, MSc, Insa[1], Keminer, Oliver[2], Brändl, MSc, Björn[1], Pless, Ole[3] and **Müller, Franz-Josef**[1], [1]Zentrum für Integrative Psychiatrie, Kiel, Germany, [2]Fraunhofer IME Screening Port, Hamburg, Hamburg, Germany, [3]Fraunhofer IME ScreeningPort, Hamburg, Germany

Self-renewal and multi-lineage differentiation capacity and human pluripotent stem cells is regulated by proteins interacting with each other and specific DNA sequences. While our ability to predict such networks based on our throughput nucleotide sequencing and hybridization data has increased enormously, relating these computational results back to the biological mechanism has been difficult. Is also become clear that differentiation conceptualized as the exit from the pluripotent self renewal state only occurs at distinct windows of opportunity in the G1 phase. As a result more than 90% of all cells in any given unsynchronized pluripotent stem cell culture are insensitive to differentiation signals and are unable to commit to a differentiation phase transition. These findings explain why it has been so difficult to mechanistically dissect differentiation on the single cell level. Here we present results from immunocytochemical measurements of central targets from proteins-interaction networks we and others have previously identified as associated with the regulation of the pluripotent state. Combining these with high throughput microscopy and automated image analysis allowed us to fine map the protein expression and in some cases activity of selected target proteins to the respective cell cycle phase. Correlative analysis of signal intensities mapped to distinct cell cycle phases revealed a dynamic view of pluripotent stem cells balancing phenotypic stability through self renewal with the ability to sense and integrate extrinsic differentiation signals. We also find that more future efforts will have to be directed towards better understanding technical variables in studies involving protein binding molecules in order to be able to confidently measure quantitative signals related to distinct proteins in single cells.

T2216

GENOME-SCALE DNA METHYLATION OSCILLATIONS IN PLURIPOTENT CELLS

Rulands, Steffen[1], Lee, Heather J[2], Clark, Stephen[2], Angermueller, Christoph[3], Smallwood, Sebastien[4], Krueger, Felix[2], Kelsey, Gavin[4], Stegle, Oliver[3], Simons, Benjamin David[1] and Reik, Wolf[2], [1]University of Cambridge, Cambridge, U.K., [2]The Babraham Institute, Babraham, U.K., [3]EMBL-EBI, Cambridge, U.K., [4]FMI, Basel, Switzerland

Pluripotent mouse embryonic stem cells (ESCs) display dynamic heterogeneity characterised by stochastic switching between transcriptional states. Recent work has also highlighted the dynamic nature of DNA methylation in these cells. Using single cell bisulfite sequencing we report coherent genome-scale oscillations in ESC DNA methylation that particularly affect CpG poor regions including distal enhancers. These oscillations are dependent on DNA methylation turnover by Dnmt3 and Tet enzymes; they arise during ESC priming and subside upon embryoid body (EB) differentiation. Signatures of DNA methylation oscillations are also found *in vivo* in epiblast cells that are exiting pluripotency. As a consequence of oscillations, differentiation primed ESCs are highly heterogeneous particularly in enhancer methylation. This methylation heterogeneity is linked at the single cell level with transcriptional heterogeneity particularly of pluripotency genes. Our observations suggest that regulated DNA methylation heterogeneity contributes to lineage priming in cells poised for differentiation.

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T2218

AN ISOGENIC EMBRYONIC STEM CELL PLATFORM FOR EVALUATING THE EQUIVALENCE OF EMBRYONIC STEM CELLS, NUCLEAR TRANSFERRED EMBRYONIC STEM CELLS, AND INDUCED PLURIPOTENT STEM CELLS

Stone, Lee[1,2], Chen, Chien-Hong[1], Yu, Chung-Ying[1], Chuang, Ching-yu[3], Shen, Chia-Nin[3] and Kuo, Hung-Chih[1,3], [1]Institute of Cellular & Organismic Biology, Academia Sinica, Taipei, Taiwan, [2]Translational Medicine Ph.D. Program, National Yang Ming University, Taipei, Taiwan, [3]Genomics Research Center, Academia Sinica, Taipei, Taiwan

Pluripotent stem cells (PSCs) can be derived from fertilized blastocysts (embryonic stem cells, ESCs), or be reprogrammed from somatic cells by somatic cell nuclear transfer (nuclear transferred ESCs, ntESCs) directly pluripotency reprograming by defined transcription factors (induced pluripotent stem cells, iPSCs). They are extremely important as the mechanisms underlying cellular differentiation and self-renewal can be studied. Also, patient-specific iPSCs and ntESCs are ideal for cell-based drug discovery and the study of disease pathogenicity. However, accumulating evidences indicated that the somatic cell reprogrammed PSC (SCPSCs) are prone to epigenetic and genetic abnormalities and these may hamper their clinical applications. Therefore, the strategy to establish an isogenic PSCs system will enable us to identify the potential defects in the SCPSCs through comparing ESC and their genetic identical SCPSCs. In this study, we have developed an efficient method to derive ESC (sbESC) from >50% of single 8-cell stage blastomeres. Employing the above strategy, we demonstrated that pairs of sbESCs and their isogenic sibling animal resulted from embryo transfer can be simultaneous generated with >20% matching rate. Accordingly, we are able to generate genetically identical ntESCs and iPSCs from the somatic cells of the genetic identical sibling animals of the sbESCs. Furthermore, combined genomic and epigenomic analysis of the isogenic pairs of sbESC, ntESC and iPSC revealed that there are varied degree of molecular defects in the SCPSCs in compare to ESCs.

T2220

COMPARISON OF TRANSCRIPTIONAL PROFILING OF LNCRNAS AND MRNAS IN FEEDER-DEPENDENT AND FEEDER-FREE CULTURE SYSTEMS OF HUMAN EMBRYONIC STEM CELLS

Xiao, Lu[1], Guo, Lei[1], Zhou, Xiaohua[1], Wei, Yanxing[1], Sun, Fei[1], Li, Qi[2], Long, Ping[2], Quan, Song[1], Ma, Yanlin[2] and Yu, Yanhong[1], [1]Nan Fang Hospital, Southern Medical University, Guangzhou, China, [2]Hainan provincial key Laboratory for Human Reproductive Medicine and Genetic Research, Affiliated Hospital of Hainan Medical University, Haikou, China

Human embryonic stem cells(hESCs), derived from the inner cell mass of human blastocysts, are considered to be the most pluripotent of all stem cells. Both feeder-dependent and feeder-free culture systems are applied to maintain self-renewal hESCs. However, hESCs of different culture systems showed different morphology, there might be different transcriptional and functional differences between these hESCs. To test this hypothesis, we first derived three hESCs lines under the culture system of human foreskin fibroblasts (HFF) combined with mTESR1, and the stem cell characteristics were identified. And then, we conducted a large-scale transcriptional profiling of lncRNAs and mRNAs in these three pairs of hESCs cultured under feeder-dependent and feeder-free culture systems using microarray technology. 23 LncRNAs and 15 mRNAs related to self-renewal, proliferation and differentiation were identified as significantly differentially expressed, which might result in differences in functions. These results will provide a large-scale of biological information that would eventually illuminate a key to get a better understanding of how different culture systems impact on stem cells, and figure out the best condition suitable for clinilcal therapy.

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EMBRYONIC STEM CELL CLINICAL APPLICATION

T2224

MUTATION DETECTION IN HUMAN PLURIPOTENT STEM CELL CULTURES

Baker, Duncan[1], Hirst, Adam[1], Codner, Gemma[2], Juarez, Miguel[3], Allison, Thomas[1], Wells, Sara[2], Andrews, Peter[1], Gokhale, Paul[1] and **Barbaric, Ivana**[1], [1]Centre for Stem Cell Biology, Sheffield, U.K., [2]MRC Harwell, Harwell, U.K., [3]School of Mathematics and Statistics, Sheffield, U.K.

Mutations in human pluripotent stem cells (hPSCs) gained during in vitro culture can confound experimental results and potentially jeopardize the outcome of clinical therapies. Particularly common genetic changes in hPSCs are whole or partial trisomies of chromosomes 1, 12, 17 and 20. Thus, hPSC cultures should be regularly screened for such mutations. Although a number of methodologies are used to assess hPSC genotypes, there has been no systematic evaluation of the sensitivity of the commonly used techniques in detecting low level mosaicism in hPSC cultures. We have preformed mixing experiments to mimic the naturally occurring mosaicism in hPSC cultures and have assessed the sensitivity of chromosome banding, qPCR, FISH and digital PCR in detecting variant cells in culture. Our analysis provides an important resource on the limits of mosaicism detection by the commonly employed methods, a necessary requirement for interpreting the genetic status of hPSC cultures.

T2226

GENERATION OF INACTIVATED HUMAN DERMAL FIBROBLASTS FOR USE AS FEEDER CELLS TO SUPPORT EXPANSION AND MAINTENANCE OF EUTCD-GRADE HUMAN PLURIPOTENT STEM CELLS

Man, Jennifer Sui-Sum, Nowell, Craig, Hunt, Charles and Stacey, Glyn, UK Stem Cell Bank, National Institute for Biological Standards and Control, Hertfordshire, U.K.

Human Embryonic Stem Cells (hESCs) are routinely expanded and maintained by co-culture with mitotically inactivated Human Dermal Fibroblasts (iHDFs), which provide secreted factors and matrix components essential for hESC growth. However, currently available iHDFs are often either not compliant with EU regulation or are only available through private commercial agreements. The UK Stem Cell Bank (UKSCB) aims to provide banks of iHDFs that are fully compliant with the EUTCD (European Tissues and Cells Directive) in order to reduce risk for patients and companies developing therapies. Importantly, iHDFs intended for use in the culture of EUTCD hESCs must be fully characterised, to confirm they are mitotically inactive and maintain their capacity to support the expansion of pluripotent hESCs. To establish a standard operating procedure (SOP) for in-house production of iHDFs for EUTCD-grade hESC lines, a qualification study has been performed comparing HDFs treated with Mitomycin C for varying durations. The degree of mitotic inactivation of iHDFs, their sterility, karyotype, phenotypic characteristics (morphology, expression profile), and ability to support expansion and maintenance of pluripotent hESCs have been assessed, in accordance with previously published and/or depositor's data have been assessed. Mitotic inactivation of HDFs after 3 and 4 hours of Mitomycin c treatment was assessed by analysing BrdU incorporation. Treatment for 4 hours resulted in the most effective mitotic inactivation with <5% cells displaying mitotic activity whilst maintaining >90% viability. A normal karyotype was detected in all treated groups and fibroblast identification was confirmed by flow cytometry analysis of a Vimentin$^+$:CD45$^-$:Oct4$^-$ cell population. Furthermore, all treated groups were able to support the maintenance of undifferentiated hESCs, which expressed markers associated with pluripotency and thus possessed a Oct4$^+$:Nanog$^+$:Tra-1-60$^+$:SSEA-1$^-$ hESC phenotype. Finally, hESCs maintained on all Mitomycin C treated groups retained the capacity for multi-lineage differentiation, as indicated by the induction of lineage associated genes. In light of the obtained results, a 4 hour inactivation protocol will be incorporated into the UKSCB SOP for culture of EUTCD grade hESCs.

CANCER CELLS

T2228

STUDIES OF THE SELF-RENEWAL AND THE NEURAL DIFFERENTIATION OF C6 GLIOMA CELLS

Chao, Chuan-Chuan[1], Kan, Daphne Wei-Chun[2], Lu, Kuo-Shyan[1] and Chien, Chung-Liang[1], [1]National Taiwan University College of Medicine, Taipei, Taiwan, [2]National Taiwan University Hospital, Taipei, Taiwan

The cancer stem cell (CSC), a subpopulation of cells in the tumors, has the properties of self-renewal, multipotency, and tumorigenicity. Selected C6 CSCs (C6-CSCs) were cultured *in vitro* as spheres using a serum-free medium containing basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) in our study. C6-CSCs could differentiate to neural cells after drug induction and showed remarkable similarities to neural stem cells (NSCs). Involvement of neural differentiation and tumor formation, microRNAs (miRNAs) play a regulatory role in CSCs by imperfect base-pairing binding to complementary sequences in the 3'-UTR of target mRNA transcripts.

However, the mechanism of miRNA-regulated self-renewal ability and multipotency in glioma cells is poorly understood. In this study, we presented the influences of drug challenge on rat C6 glioma cells in the aspects of cellular morphological changes and differentiation. Characterizations of neural cell markers including bIII-tubulin (for neurons), GFAP (for astrocytes) and CNPase (for oligodendrocytes) in the drug-treated cells have been shown that neural differentiation might be induced. A potential therapeutic application of anti-tumor drugs on the differentiation of cancer cells could be suggested. In this glioma cell model, we demonstrated that a miRNA microarray served as a good platform for investigating which miRNA contributes in the processes of sphere formation and neural differentiation. Spheres were formed at first to enhance the potential for multipotency, and then neural differentiation was induced by stimulation of 3-isobutyl-1-methylxanthine (IBMX). Several miRNAs involved in sphere formation were identified by the miRNA microarray, and miRNA-30c was confirmed to act an important role in sphere formation. Furthermore, miRNA-30c interfered the expression of GFAP by affecting the JAK-STAT3 pathway. These results suggest that miRNA-30c has a regulatory role in self-renewal and neural differentiation.

T2230

SINGLE CELL FUNCTIONAL CHARACTERIZATION OF NORMAL AND METASTATIC BONE MARROW NICHES

Gulati, Gunsagar Singh[1,2], Upton, Rosalynd[2], Seo, Elly[2], Marecic, Owen[2], Manjunath, Anoop[2], Lopez, Michael[3], Seita, Jun[1], Sahoo, Debashis[4], Leyrat, Anne[5], Gonzales, Michael[5], Neff, Norma[2], Sim, Sopheak[2], Quake, Stephen[2], Longaker, Michael[2,6], Weissman, Irving L.[2] and Chan, Charles KF[7], [1]Stanford University School of Medicine, Stanford, CA, U.S., [2]Stanford University, Stanford, CA, U.S., [3]Stanford university, mountain view, CA, U.S., [4]University of California, San Diego, San Diego, CA, U.S., [5]Fluidigm Corporation, San Francisco, CA, U.S., [6]Stanford University Medical Center Director, Stanford, CA, U.S. [7]Stanford University, Redwood City, CA, U.S.

Little is known about the specific bone marrow stroma that constitute the niche microenvironment of the hematopoietic stem cell (HSC) and metastatic prostate cancer (MPCa) cell. We find that HSCs and MPCa cell lines such as PC3, readily home to sites of new bone marrow niche formation induced by orthotopic transplant of purified skeletal progenitors into the renal capsule. We therefore hypothesize that HSCs and MPCa cells are both supported by specific stromal cells derived from skeletal progenitors in the bone marrow and that factors produced by these niche cells are sufficient for normal and metastatic cell survival and maintenance. Here we have developed new approaches to (a) identify the factors in the bone marrow stroma that support HSC and MPCa cell survival and maintenance in the bone, and (b) determine whether antibodies against these identified targets, used individually and in combination, can effectively treat metastatic prostate cancer. Using novel computational platforms to mine a database compiled from transcriptomes of prospectively isolated bone marrow stromal cells, we identified many potential ligand-cognate receptor interactions between stromal cells, HSCs, and MPCa cells. To experimentally screen and validate these targets, we utilized our previously described HSC bone marrow niche assay, which allows us to genetically alter heterotypic bone stromal populations and monitor whether knocking out certain stromal factors affects prostate cancer metastases to bone. We identified several novel pathways, including VEGF-C and Osterix, which are important for establishing both HSC and MPCa niches. We also tested various combinations of cytokines produced by skeletal stroma for their ability to maintain self-renewal and quiescence of single HSC and MPCa cells using the Fluidigm Polaris platform. Finally, we identified CDCP1, a surface protein exclusively expressed on HSCs and MPCSCs that may have a potential role in mediating homing and engraftment to the bone marrow. We are now investigating the mechanism by which CDCP1 may facilitate this process and exploring its therapeutic potential against metastatic prostate cancer. Preliminary results reveal that combined CDCP1 opsonization and CD-47 blockade promotes significant phagocytosis of PC3 cells by mouse and human macrophages.

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T2232

ASXL1 MAINTAINS THE STEMNESS OF HUMAN COLORECTAL CANCER STEM CELLS.

Isobe, Taichi, Zarnegar, Mark A. and Clarke, Michael, Stanford University, Stanford, CA, U.S.

Impaired cellular differentiation causes uncontrolled proliferation of stem cells and can be a cause of oncogenesis. The epigenome regulates the expression of genes that are essential for cellular differentiation. In colorectal cancer, it remains to be determined what aberrant epigenetic modifications perturb cellular differentiation resulting in oncogenesis. To identify epigenetic modifiers with aberrant gene expression, gene mutation or copy number alteration in colon cancer, H3K4me3- and H3K27me3-related genes were investigated with the public TCGA database. Among the genes examined, additional sex comb like 1 (ASXL1) was found to be more highly expressed in cancer

tissues than in normal tissues, and ASXL1 expression positively correlated with the expression of stem-cell related genes and negatively correlated with the expression of differentiation markers. In hematopoietic cells, ASXL1 has been reported to recruit Polycomb Repressive Complex 2 (PRC2) to the promoter regions of specific genes, such as homeotic genes, to regulate their expression. In normal colon, undifferentiated cells more highly expressed ASXL1 than differentiated cells. Immunofluorescent histology revealed that a subset of colorectal cancer cells had ASXL1 protein in their nuclei and that these cells did not express differentiation markers. Knockdown of ASXL1 reduced the amount of H3K27me3 in colorectal cancer cells and diminished organoid formation in vitro. Patient-derived cancer cells infected with ASXL1-shRNA expressing lentiviruses were injected into immunodeficient mice using the limiting dilution method. ASXL*1*-knockdown impaired the tumorigenicity of cancer stem cells in vivo and increased the expression of differentiation markers. In conclusion, ASXL1 contributes to maintain the stemness of colorectal cancer stem cells.

T2234

ELUCIDATING TUMOR PROMOTING MECHANISMS OF TERT PROMOTER MUTATIONS USING GENETICALLY DEFINED STEM CELL MODELS

Lorbeer, Franziska Katharina, Chiba, Kunitoshi, Wagner, Tina and Hockemeyer, Dirk, University of California, Berkeley, Berkeley, CA, U.S.

In many human tissues, continuous cell division is required for homeostasis. Yet, excessive proliferation is also a hallmark of cancer. Therefore, cellular mechanisms evolved to control and balance the regenerative capacity of human tissues. In somatic cells each cell division leads to telomere shortening a process that ultimately limits their proliferative capacity. In contrast human stem cells, prevent this attrition and retain the ability to divide thought the constitutive expression of the enzyme telomerase, which elongates shortened telomeres. Aberrant expression of telomerase is observed in more than 90% of all human tumors and it constitutes an important mechanism for their immortalization. Recently, highly recurrent mutations in the promoter of the TERT gene, which encodes the catalytic subunit of telomerase, have been reported in specific tumor types, e.g. melanomas. Yet, the functional consequences of these mutations are not understood. I have introduced mutations at the endogenous *TERT* locus of human pluripotent stem cells through genome editing as recently described in Chiba et al. This cell system is used to study the effect of *TERT* promoter mutations in non-transformed cells under conditions that closely resemble early tumorigenesis. The edited hPSCs can be differentiated in the physiologically relevant cell type. The comparison of wild type and mutant cells in an otherwise isogenetic background will provide a means model the early stages of cancer.

T2236

ROLE OF ALPHA-6 INTEGRIN IN CANCER STEM CELL PLASTICITY

Soto, Ubaldo[1], Bennett, Michael[1] and Alonzo, Janine[1,2], [1]Loma Linda University, Loma Linda, CA, U.S., [2]IMC University of Applied Sciences Krems, Krems, Austria

Stem cells are characterized as having high plasticity since they are able to change their phenotype in response to extracellular signals. Interestingly, most human tumors contain a minor subpopulation of high malignant cells with stem cell characteristic and high plasticity as well. These cells are known as cancer stem cells (CSC) or tumor initiating cells because of their strong capability to originate and maintain tumors. The phenotype of normal and cancer stem cells is tightly controlled by α-6 Integrin dependent pathways. α-6 Integrin, a stem cell marker, is expressed in two splicing forms, variant A and B. The former has been identified to play a role in tumor growth while the latter has been found to be responsible for cell stemness. We hypothesize that α-6 Integrin plays an important role in CSC plasticity. To test our hypothesis, we cultured MB-231 breast cancer cell line, which contains a high percentage of CSC. We treated MB-231 cells with conditioned media from induced pluripotent stem cells (iPSC) assuming that soluble factors secreted by normal stem cells (iPSC) could induce changes in CSC phenotype. Breast CSC are characterized as CD44+/CD24-/low /CD49f high and thus were able to evaluate potential changes in cell phenotype by FACS analysis. The gene expression of α-6 Integrin was analyzed by RT-qPCR. Our results have shown that indeed iPSC conditioned media was able to modify the CSC phenotype to a potentially less tumorigenic type. These modifications in CSC phenotype were accompanied with changes in α-6 Integrin expression and in the proportion of splicing variants A and B. In conclusion, our results have shown that it is possible to induce changes in the CSC phenotype and that α-6 Integrin is a good molecular marker for tracking these modifications.

T2238

MICRORNA-151 UP-REGULATES PLURIPOTENCY GENE EXPRESSION AND PROMOTES GASTRIC CANCER PROGRESSION

Hsu, Kai-Wen, Huang, Tzu-Ting and **Yeh, Tien-Shun**, National Yang-Ming University, Taipei, Taiwan

Gastric carcinoma is the third leading cause of lethal cancer worldwide. Gastric cancer progression is promoted by Notch1 receptor intracellular domain (N1IC), the activated form of Notch1 receptor. It was demonstrated that Notch pathways cross-talk with microRNAs (miRNAs) in

the regulation of tumorigenesis. We identified microRNA-151 (miR-151) as a Notch1 receptor-related miRNA in gastric cancer cells. Results showed that activation of Notch1 pathway enhanced miR-151 expression in gastric cancer cells. To check whether miR-151 affects the maintenance of cancer stem-like phenotype of SC-M1 gastric cancer cells, tumorsphere formation assay was employed. Tumorsphere formation ability of SC-M1 cells was enhanced after miR-151 overexpression, whereas repressed after miR-151 knockdown. Additionally, levels of mRNAs of pluripotency genes such as Nanog and SOX-2 were elevated after miR-151 overexpression in SC-M1 cells by quantitative real-time PCR analysis. Activities of reporter genes containing Nanog and SOX-2 promoters were induced after overexpression of miR-151. The N1IC-mediated increment of tumorsphere formation ability in SC-M1 cells was dampened after knockdown of miR-151. After Notch1 receptor knockdown or DAPT treatment, the reduced ability of tumorsphere formation in SC-M1 cells was restored by overexpression of miR-151. Furthermore, gastric cancer progression of SC-M1 cells including viability, colony formation, migration, and invasion was augmented by Notch1 pathway through miR-151. Taken together, these results suggest that Notch1 pathway interplays with miR-151 in controlling gastric carcinogenesis.

TECHNOLOGIES FOR STEM CELL RESEARCH

T3002

ANALYSIS OF PROTEIN INTERACTION OF LONG-NONCODING RNA BY CHROMATIN ISOLATION BY RNA PURIFICATION (CHIRP)

Asbrock, Nick[1], Chu, Vi T.[2] and Saito, Kan[1], [1]EMD Millipore, Temecula, CA, U.S., [2]EMD Millipore Corporation, Temecula, CA, U.S.

Gene regulation plays a critical role in complex cellular processes such as development, differentiation, and cellular response to environmental changes. While the regulation of gene expression by transcription factors and epigenetic influences has been well studied over time, pervasive genomic transcription and the role of non-coding RNAs in this process is a rapidly evolving field that remains to be thoroughly explored. Chromatin Isolation by RNA Purification (ChIRP) is one of the methods, which allows analysis of DNA, RNA and protein in the RNA complex by using probe-based hybridization to target RNA molecules in chromatin. After the purification of the complex, proteins can be isolated and analyzed by western blotting or mass spectrometry (ChIRP-MS). To enable the exploration of these RNA interactions in chromatin regulation, we have optimized the methods and developed ChIRP reagents. Using these reagents ChIRP experiments can be performed with reliable recovery of chromatin using lncRNA or other chromatin associated RNA as targets. We have performed ChIRP experiments with various cell lysates and capture oligos for several lncRNA targets. Isolated proteins are analyzed by western blotting for known RNA binding proteins. We successfully identified several lncRNA binding protein partners by the optimized ChIRP method. In summary the methods optimized allow discovery of RNA-associated proteins in addition to DNA and RNA sequences.

T3004

CREATING A TUNABLE STEM CELL: MODULATION OF OXYGEN AND ATMOSPHERIC PRESSURE DURING CULTURE ALTERS SIGNALING PATHWAYS GOVERNING STEM CELL MAINTENANCE AND DIFFERENTIATION

Cassereau, Luke[1], Li, Yunmin[1], Downie, Bryan[1], Przybyla, Laralynne[2], Chow, Tianna[1], Adams, Bruce[1] and Lim, James[1], [1]Xcell Biosciences, San Francisco, CA, U.S., [2]University of California, San Francisco, San Francisco, CA, U.S.

Stem cells have vast potential for use in reparative medicine due to their extensive ability to proliferate and differentiate into multiple cell lineages. Harnessing this potential requires developing techniques to culture these cells under conditions allowing robust production and consistent function of target adult cells of interest while avoiding unwanted genetic and epigenetic changes. Whereas extensive research has focused on soluble factors to optimize stem cell culture, part of the difficulty lies in identifying which extracellular factors influence differentiation of stem cells. Factors such as hypoxia, atmospheric pressure, and the composition and organization of the extracellular matrix (ECM) are important drivers of stem cell differentiation and cell function. However, no studies to date have systematically analyzed the contribution of these factors toward the maintenance and differentiation of stem cells. To address this, we have developed a novel primary cell culture bioreactor and biomimetic natural extracellular matrix substrates to provide tunable control of the microenvironment ex vivo. We altered oxygen concentration (1-5%) and atmospheric pressure (0-5 PSI) while culturing human pluripotent stem cells under canonical stem cell maintenance and differentiation protocols. We then assessed the impact of these conditions on stem cell state through transcriptome pathway analysis to determine the degree of stem cell maintenance or germ layer commitment during differentiation. As expected, modulation of oxygen concentration had profound affects on HIF1 mediated signaling pathways, but also led to differential expression of transcription factors involved in stem cell lineage commitment. In contrast, the introduction of atmospheric pressure led to changes in signaling pathways governing glucose and mitochondrial metabolism when

compared to standard stem cell culturing conditions. In summary, modulation of oxygen and atmospheric pressure levels during cell culture had direct effects on stem cell maintenance and differentiation and warrants further investigation.

T3006

FLUORESCENT PROBE INDUCES PLURIPOTENCY-SPECIFIC CELL DEATH BY VISIBLE LIGHT.

Cho, Seung-Ju, Sogang University, Seoul, Korea, South

After differentiation, the precise detection of undifferentiated pluripotent stem cells (PSCs) and a subsequent procedure to eliminate residual PSCs from a mixed population are critical for teratoma-free stem cell therapy. However, conventional sorting with antibody based fluorescence-activated cell sorting may have technical limitations in terms of their sensitivity and applicability. Herein, we show that CDy1, a PSC specific fluorescent probe, produced reactive oxygen species (ROS) enough to induce cell death upon visible light exposure. Irradiation with visible light induces cell death specifically in CDy1 positively stained PSCs, not in endothelial cells (EC-ESCs) derived form ESCs. CDy1 negatively stained EC-ESCs remain functional even after light exposure. Finally, cell death of PSCs by visible light exposure leads to inhibition of teratoma formation. This suggests that elimination of undifferentiated PSCs with PSC specific fluorescence probe with photodynamic feature would be a valid approach for safe PSC based cell therapy.

T3008

VERSATILE DRUG-ELUTING NANOSTRUCTURED DESIGNS FOR EFFECTIVE REGENERATION OF SOFT TISSUES

Mukherjee, Shayanti[1,2], Venugopal, Jayarama Reddy[2], **Gargett, Caroline**[1,3] and Ramakrishna, Seeram[2], [1]Hudson Institute of Medical Research, Clayton, Australia, [2]National University of Singapore, Singapore, Singapore, [3]Monash University, Clayton, Australia

Regeneration of soft tissues remains a challenge owing to complexity of generating functional vascular networks and structural features comprising many different cell types. Despite impressive milestones in cellular therapy, recent clinical trials highlight impediments to retention of locally administered cells at the site of injury posed by dynamic cytokine rich microenvironment resulting very low efficiency. We hypothesized that biodegradable nanostructured materials would provide a multimodal platform for controlled release of repair factors while promoting in situ retention of therapeutic cells for improved tissue functioning. In this study we fabricated and analysed the nanotopographically controlled core-shell nanofibers using co-polymers of Poly Lactic acid (PLA) and collagen. Specifically, we fabricated biomimetic PLA-based nanofibers with collagen (1:1) with a core containing the chemokine SDF-1 using scalable electrospinning. SEM confirmed porous, uniform nanostructures <500nm diameter. We noted a significant difference in degradation ($P<0.05$); <40% with and <20% without collagen. Characterization of the mechanical properties of random nanofiber electrospun meshes revealed a stiffness of 18MPa. Patterning the nanofibers by changing the collection method reduced the stiffness by 20 folds compared to PLA alone ($P<0.05$). The co-polymeric nanofibers displayed sustained release of chemokine SDF-1 resulting in the significant ($P<0.05$) recruitment of 200% more CXCR-4^{+} MSCs compared with a CXCR-4 inhibitor in a transmigration assay. In-vitro analysis of MSCs revealed high biochemical activity ($P<0.05$) and ability to penetrate the fibers. In a porcine model of MI created by ligation of left coronary artery , the nanofiber mesh readily integrated on the epicardium of the infarcted region and showed a 15% improvement in fractional shortening without the formation of scar tissue over control ($P<0.05$). Currently, we are developing these nanofiber based systems for vaginal wall applications for the treatment of pelvic organ prolapse, a major hidden disease burden affecting 25% of all women using MSCs from the endometrium. These are the first studies to explore the application of nanobiomaterials for repair, restoration and regeneration vaginal wall for improving women's health.

Funding Source: National University of SingaporeNational Research Foundation, SingaporeHudson Institute of Medical Research

T3010

PHYSIOLOGICALLY RELEVANT OXYGEN DURING CELL HANDLING AS WELL AS INCUBATION ENHANCES THE GROWTH OF HUMAN MESENCHYMAL STROMAL CELL CULTURES

Henn, Alicia, Darou, Shannon and Yerden, Randy A., BioSpherix, Parish, NY, U.S.

Reproducible research on any physiologic or pathophysiologic state requires relevant in vitro conditions for cells. It is well-known that when cultured at room oxygen levels, clinically important Mesenchymal Stromal Cells (MSC), experience oxidative stress. However, there is a common opinion that if cultured at physiologic oxygen, brief exposures to room air during cell handling won't affect the cells. So we tested the hypothesis that even if incubated at physiologically relevant oxygen levels, human bone marrow MSC exposed to room air during routine cell handling would have equivalent growth characteristics as MSC maintained in unbroken physiologic condi-

tions. Human MSC cultures were cultured in T flasks in 5% O2/5% CO2. Three cultures were housed in an external incubator fitted with an oxygen-controlled subchamber and handled in HEPA-filtered room air conditions (Room-Air). The other three cultures were housed within a closed processing chamber (Hypoxia Hood) that provided constant conditions for handling as well as incubation. We also measured pericellular oxygen levels with an oxygen probe. Cellular growth was monitored using the CytoSMART live cell imaging system which provided constant monitoring without disturbing the cells. Cell culture media were pre-equilibrated to the matching cell handling conditions before use. Pericellular oxygen levels in room-air handled flasks took over an hour to equilibrate to the incubator levels. Cell viability was greater than 90% at all times in both groups, but our data did not support our hypothesis. Statistically higher cumulative cell yields and faster cell growth were seen when cells were handled under full-time oxygen and CO2 control (Hypoxia Hood) than in Room-Air cell handling conditions (two-tailed T test, unequal variances). The cells also stayed in active cell growth for more passages. We conclude that breaking physiologic conditions even for routine cell handling had a detrimental effect on MSC growth. This was not an effect that can be easily seen by the cell culturist as cytotoxic effect, but was a cytostatic effect. Maintaining full-time physiologic gas levels during cell handling as well as incubation is beneficial for physiologically relevant research on clinically important MSC.

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T3012

COMPREHENSIVE MODELING OF PLURIPOTENCY AND THE EARLY EMBRYONIC CELL FATE UNIVERSE IN HUMANS THROUGH ROBOTIC MULTIDIMENSIONAL INTERROGATION

Jensen, Jan[1,2], Bukys, Michael[1], Sears, Katie[2], Joshua, Buzzard[2], Wessely, Oliver[2] and Finney, Krystal[3],
[1]Cleveland Clinic, Cleveland, OH, U.S., [2]Cleveland Clinic Lerner Research Institute, Cleveland, OH, U.S., [3]Trailhead Biosystems, Cleveland, OH, U.S.

Robust control of fate is imperative for successful development and clinical therapy potency of cell based products. Developmental biology is typically providing the underlying knowledge tapestry for protocol development; yet, our understanding of early human development is lacking. Commonly modeled on murine biology, we rely on information mainly based on single-factor testing. Even as such data leads to testable hypotheses using human pluripotent stem cells, our empirical approaches remain centered on a reductionist basis in which the experimental dimensionality is limited. Because of the combinatorial signaling involved in cell fate control during development, this conventional approach fails to interrogate the combinatorial space. To address this, we have built a technology platform that allows a substantially increased dimensionality to be interrogated during cell culture. The experimental designs are based on Design-of-Experiment principles and incorporate all classical morphogen pathways (RA, BMP, Activin, WNT, SHH, FGF). Measurement of a larger set of effector responses using Open Array TaqMan technology (>100 lineage specific genes /condition) provides a response matrix informing on cell fate. A typical experiment provides > 10,000 data points which are mathematically fitted onto the experimental design, typically represented by >2000 vertices of a 12-dimensional hypercube. As a result, this approach provides for mathematical modeling of the primary effects, and their interactions, of multiple effectors (pathways) while such simultaneously and together instruct cell fate. Using pluripotent stem cells, we here demonstrate how such a systems developmental biology approach allows for the extraction of the underlying signaling logic of radial and future anterior/posterior positions of the human blastodisc, providing a comprehensive representation of embryonic patterning prior to, and through gastrulation. Relevant to regenerative medicine, inspection of the elucidated spatial signaling logic rapidly provides growth factor/morphogen requirements for maximal induction of specific downstream cell fates. Our work therefore impacts protocol building and optimization for cells derived from all germ layers.

T3014

IMPROVED CLINICAL SCALE MSC ISOLATION AND EXPANSION WITH MATRIX- AND XENO-FREE MEDIUM.

Godthardt, Kathrin[1], Schreiner, Claudia[1], Reis, Monica[2], Freese, Katharina[1], Schult, Silke[1], Rockel, Thomas D.[1], Bosio, Andreas[1] and **Knöbel, Sebastian**[1],
[1]Miltenyi Biotec GmbH, Bergisch Gladbach, Germany, [2]Newcastle University, Newcastle upon Tyne, U.K.

The number of clinical PhII/III trials using mesenchymal stem cells (MSCs) is growing and there is an increasing need for a xeno-free and GMP compliant production of clinical scale MSCs. We have developed the MSC-Brew GMP Medium which supports the isolation and expansion of MSCs from various tissues. As the use of fetal calf serum (FCS) is undesirable with regard to adverse events and lot-to-lot variations, our medium is xeno-free and can be used without additional coating of cell culture vessels. Here, we compared our MSC-Brew GMP Medium with FCS- and platelet lysate (PL)-containing formulations. MSCs were isolated from human adipose tissue, bone marrow and umbilical cord using different cultivation media. Proliferation behavior was monitored for up to 5 passages followed by karyotyping. In parallel, the clonogenic potential was assessed using CFU-F assays. Differentia-

tion potential was tested at passage 3, representing the passage usually used in autologous clinical treatments. The clonogenic potential and proliferation rate for MSC-Brew expanded cells was higher than in FCS-expanded cells and comparable or higher than for PL-containing media. Cells displayed a normal karyotype and differentiated to chondro-, osteo- and adipocytes. The immunomodulatory properties of MSCs are the main reason for their great clinical potential. However, interaction of MSCs with cells of the innate- and adaptive-immune system are not completely understood. Therefore, we characterized the expression of surface molecules as potential candidates for immune cell interaction using a library of >300 monoclonal antibodies. To this end, we compared MSCs expanded in MSC-Brew with or without TNF-alpha/INF-gamma or Poly-IC induced licensing. The same cells were compared with respect to their immuno-suppressive properties using a standardized in vitro T-cell suppression assay. In summary, we demonstrate that the MSC-Brew GMP Medium supports efficient isolation and expansion of bona fide MSCs at relevant scale suited for cell therapy. Furthermore, we present a comprehensive characterization of expressed surface molecules which will contribute to deciphering the MSCs' immunological mode of action, and development of quality control assays for MSC cell products.

T3016

COMPARISON OF PLURIPOTENT STEM CELL PROCESSES AND HEALTH WHEN CULTURED ON MATRIGEL, GELTREX, AND LAMININ-521 USING STEMCELLQC, A VIDEO BIOINFORMATICS TOOLKIT

Lin, Sabrina C.[1], Antrim, Lauren[2], Loza, Antonio[2] and Talbot, Prue[3], [1]University of California, Riverside, Silverado, CA, U.S., [2]University of California, Riverside, Riverside, CA, U.S., [3]University of California Riverside, Riverside, CA, U.S.

StemCellQC is a powerful high content profiling toolkit that was developed to monitor the morphology, behavior and health of pluripotent stem cells in culture. Our objective in this study was to quantitatively compare the morphology, quality, and dynamic processes of human embryonic stem cells (hESC) grown on three different substrates using StemCellQC. H9 hESCs were cultured on Matrigel, Geltrex or laminin-521 for 48 hours in a BioStation CT high content incubator capable of live cell imaging. Time-lapse images were collected every hour and analyzed with StemCellQC. Each experiment was repeated three times and more than 10 videos were analyzed in each control and treatment group in each experiment. Attachment on laminin-521 was faster and more efficient than on Matrigel. Cells grew at about the same rate on the three substrates. Colonies were more motile on laminin-521, which facilitated migration and merging of small colonies and single cells. The rapid consolidation of colonies on laminin-521 improved colony survival, and there was less cell death on laminin-521 than on the other two substrates. The number of cells available after 48 hours of culture was highest on laminin-521 due to better attachment, motility, and less cell death. StemCellQC analysis was also done on normal versus abnormally shaped colonies, and on pluripotent versus differentiating colonies. Colony motility decreased significantly in differentiating colonies when compared to pluripotent colonies, and abnormal crescent shaped colonies were successfully distinguished from normal circular colonies. These data show that small changes in culture conditions can produce large changes in culture outcomes such as the yield of viable cells. This is the first application of StemCellQC, which was able to quantifiably distinguish between the performance of human pluripotent stem cells on three commonly used substrates. This application demonstrates the usefulness of StemCellQC in evaluating culture conditions for hESC and developing and improving cell cultures. In addition to the current application, StemCellQC could be used in drug testing, toxicological studies, translational laboratories, and in clinical labs where quality control of cultures is critical.

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T3018

INTRACELLULAR DETECTION OF HYPOXIA IN LIVE CELLS

Mandavilli, Bhaskar S., Chen, Aimei and Kang, Hee Chol, Cellular Analysis, Eugene, OR, U.S.

Hypoxia is an important phenomenon in many physiological processes and involved in many human diseases including cancer. Stem cells are grown at lower oxygen tensions to keep them in an undifferentiated state and support proliferation. The ability to measure the extent of hypoxia using live cell imaging is very valuable as it will allow scientists to monitor hypoxia in stem cells. Here, we describe a live cell-based method to conveniently measure hypoxia using a fluorescent hypoxia Probe, The hypoxia probe is oxygen sensing fluorescent probe, is quenched with increasing oxygen concentrations, and has excitation and emission peaks of 483 and 616 nm respectively. The probe is sensitive to varying concentrations of oxygen and can detect as low as 5% O_2 concentrations in cells. Hypoxia imaging of cells with this probe is reversible that allows dynamic measurements of hypoxia in cells. Using this probe, we measured hypoxia in several cell lines including A549, HeLa and U-2OS. Hypoxia was also measured to monitor stem cells in culture. The hypoxia probe was also used to measure hypoxia in stem cell derived spheroids and tumor spheroids.

T3020

MANUFACTURING SOLUTIONS FOR ROBUST T CELL EXPANSION

Murrell, Julie, Luther, Samantha, Lawson, Tristan, Verma, Anjali, Kehoe, Daniel, Rigby, Susan, Punreddy, Sandhya, Schnitzler, Aletta Christina and Rook, Martha, MilliporeSigma, Bedford, MA, U.S.

The long-term view of regenerative medicine therapies predicts an increased need for expansion solutions that ease scalability, utilize animal origin-free materials and are compatible with limited downstream processing steps. As more cell therapies progress through clinical testing, current in vitro culture methods are proving cumbersome to scale and lack robustness. Moreover, high quality animal origin-free reagents support the future implementation of scalable manufacturing solutions that will be required following clinical success. Here, we describe the implementation of single use bioreactors and high quality media for expansion of T cells. Bioreactor systems provide many advantages for expansion of cells compared to traditional methods, including the ability to monitor and control key process parameters. The presentation will review solutions for expansion of cells within the context of different upstream process development steps as well as scaling and processing with high yield, recovery, and viability as well as expected cell characteristics. Animal origin-free media formulations were also investigated and will be presented. Start to finish solutions for manufacturing, including high quality reagents, are key enabling technologies for success in commercializing cell therapies.

T3022

VEGF- AND AGIOPOIETIN1-SECRETING UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS BY ZINC FINGER NUCLEASES TARGETING AAVS1 LOCUS AMELIORATES CARDIAC DYSFUNCTION IN A RAT MYOCARDIAL INFARCTION MODEL

Park, Hye-Jeong[1], Bayasaikan, Delger[2] and Kwon, Tae-Hwan[1], [1]Kyungpook National University, Daegu, Korea, [2]Gachon University, Incheon, Korea

Cell-based therapeutic neovascularization is a promising method for treatment ischemic diseases. In this study, we report a safe and effective stem cell therapy system to secrete angiogenic factors in umbilical cord-derived mesenchymal stem cells (UC-MSCs) utilizing zinc finger nucleases targeting AAVS1 “safe harbor” locus for the treatment of acute myocardial infarction (AMI). We generated VEGF-or Angiopoietin1 (ANG1)-secreting MSCs to promote vascularization and restore heart functions in experimentally-created AMI rat models. AMI was induced by ligating the left descending coronary artery with subsequent injection of VEGF- or ANG1-MSCs into the peri-infarct zone. A significant increase in capillary density and reduction in infarct sizes were noted in the infracted hearts with coinjected group (VEGF/ANG1-MSCs) compared with VEGF- or ANG1-MSC-injected groups. Furthermore, the coinjected group showed significantly higher cardiac performance in echocardiography than VEGF- or ANG1-MSC groups at 4 weeks after myocardial infarction. Thus, these results demonstrated that the combination of VEGF- and ANG1-MSC treatment produced by site-specific integration of genes using AAVS1 targeting ZFNs can be valuable cell-based regenerative therapeutic strategy against AMI.

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T3024

NON-HUMAN PRIMATE MODEL OF SEVERE COMBINED IMMUNODEFICIENCY USING HIGHLY EFFICIENT GENOME EDITING

Sato, Kenya[1], Kumita, Wakako[1], Henry, Rachel[2], Sakuma, Tetsushi[3], Ito, Ryoji[1], Nozu, Ryoko[1], Inoue, Takashi[1], Okahara, Norio[1], Okahara, Junko[1], Weinstein, Edward[2], Yamamoto, Takashi[3], Okano, Hideyuki[4] and Sasaki, Erika[1,4], [1]Central Institute for Experimental Animals, Kawasaki, Japan, [2]Horizon Discovery, Saint Louis, MO, U.S., [3]Hiroshima University, Hiroshima, Japan, [4]Keio University, Tokyo, Japan

The common marmoset (Callithrix jacchus) is an experimental animal model with high utility, and the use of genetically modified marmoset is anticipated for research in various fields. DNA microinjection and/or the use of embryonic stem cells (ESCs) have been the methods of choice for generating genetic modified animals to date. However in many species, including the marmoset, targeted gene knock-out (KO)/ knock-in (KI) animals cannot be produced through traditional gene-targeting of ESCs as they lack the ability to contribute to the germline, although this is not the case in rodents. On the other hand, the recent development of innovative genome editing technologies such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeat /CRISPR-associated protein 9 (CRISPR/Cas9) can resolve this issue, and it is now possible to generate target-gene KO or KI animals without using ESCs. The interleukin 2 receptor subunit gamma (IL2RG) gene is one of the genes responsible for X-linked severe combined immunodeficiency (X-SCID). The immunodeficient mice are used widely in research. In this reason, the immunodeficient Non-human Primate models for use in biomedical science would constitute an excellent animal model. Here we report our attempt to generate an immunodeficient

marmoset by knocking out the IL2RG using two versions of ZFNs (HiFi-ZFN and eHiFi-ZFN) and TALENs. In our results, 7 of 21 neonates showed somatic mutagenesis in the IL2RG using ZFN and TALEN, moreover, 4 marmosets which dead case were observed lack of thymus. On the other hand, we have succeeded long term breeding in three IL2RG KO marmosets and analyzed immunological phenotypes. This study indicates that ZFN- and TALEN-mediated targeting of the marmoset IL2RG has the potential to produce an X-SCID marmoset model that is more similar to humans, and would contribute greatly to research in the very near future.

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T3026

SIMPLE, ROBUST 3-D MICROCARRIER CULTURE SUPPORTING IPS CELL GROWTH AND DIFFERENTIATION

Hendrickson, Sara, Leno, Gregory and **Sneed, Mechelle,** Primorigen Biosciences, Inc., Madison, WI, U.S.

Three-dimensional cell culture strategies have received considerable attention as a way to address the ongoing need for simpler, more reliable and cost effective processes for industrial scale production of stem cells and their progeny. Conventional two dimensional expansion methods often involve cumbersome manipulations that can negatively impact consistency of production while creating higher labor and materials costs. To address these shortfalls, commercial microcarriers have been introduced that provide a three dimensional surface for expanding stem cells in bioreactors or related vessels. Unfortunately, many such products are not optimal for such applications, with some even lacking key elements such as specific protocols for expanding pluripotent cells. These shortcomings are likely due to the difficulties in developing such microcarriers for stem cell production and differentiation, including technical barriers related to coating, sheer, and release, and failure to eliminate cumbersome preparation steps that add time and costs, and can introduce inconsistency into the production process. Primorigen has overcome these problems with a new approach for making 3-D surfaces viable for bioreactor-based production of stem cells. Internal studies have demonstrated that select types of carriers coated with full length recombinant proteins such as Vitronectin XF™ can generate reproducible yields of iPS cells in a single large vessel that are equivalent to the yields from 80-150 six well tissue culture plates, with data suggesting that yields equivalent to those of 250 plates and greater are possible with certain modifications under investigation by Primorigen. Primorigen will present data on the effectiveness of its new, simple and user friendly approach for pluripotent cell expansion and for scale up of iPSC differentiation to cells such as cardiomyocytes.

Funding Source: National Institutes of Health Institute for General Medical Sciences

T3028

GLOBAL REGULATORS OF ORGAN MATURATION

Uosaki, Hideki and Kwon, Chulan, Johns Hopkins University School of Medicine, Baltimore, MD, U.S.

Cellular maturation is a fundamental developmental process that occurs after terminal differentiation and completes at postnatal stages in many organs. We have recently revealed a transcriptional landscape of cardiac maturation with bioinformatics analysis of publically available microarray datasets. Here, we extended the analysis to brain, liver, and kidney with the goal to identify upstream regulators and pathways that commonly affect organ maturation. First, we performed genome-wide Principal Component Analysis using array datasets of all the organs combined. The analysis showed closely clustered patterns of the organs at early embryonic stages but they became segregated at later developmental stages. Second, we performed extensive analysis on each of the organs and identified first components that possess most variance information of original microarray datasets. Finally, using clustering information, we identified genes differentially regulated from stage to stage. The identified genes were further analyzed for upstream regulators using Ingenuity Pathway Analysis. Among more than 400 upstream regulators involved in the later developmental process, we found a small number of regulators were consistently activated or inactivated in all the organs. These regulators mostly changed their activity from neonate to adult, when the organs undergo major maturation. Our study provides insights into understanding global factors and mechanisms controlling organ maturation.

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T3030

EFFICIENT GENOME EDITING IN HUMAN MESENCHYMAL STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS BY OPTIMIZED VECTOR DESIGN AND SMALL MOLECULES

Zhang, Jian-Ping[1], Li, Xiao-Lan[1], Chen, Wan-Qiu[2], Arakaki, Cameron[2], Zhang, Lu[1], Wen, Wei[1], **Xu, Jing**[1], Yuan, Weiping[1], Cheng, Tao[1] and Zhang, Xiaobing[2], [1]Institute of Hematology and Blood Disease Hospital, Tianjin, China, [2]Loma Linda University, Loma Linda, CA, U.S.

CRISPR genome editing technology has been widely used in gene knockout (KO) and knockin (KI). However, genome engineering is less efficient in stem cells than in cells lines like 293T cells. Here we focus on vector design and small molecules to improve efficiency. Previous studies showed that truncated sgRNAs (17nt) substantially decrease off-target cleavage without affecting on-target disruption. Using a GFP reporter and lentiviral Cas9-sgRNA expression system, we found that both 17nt and the full-length 20nt sgRNAs induce ~95% KO in 293T cells, whereas the KO efficiencies are significantly lower in induced pluripotent stem cells (iPSCs) (50-70%) and mesenchymal stem cells (MSCs) (65-75%), which is associated with decreased Cas9 expression in stem cells. Furthermore, we observed some 20% reduction in KO efficiencies with the 17nt vs. 20nt sgRNAs in both iPSCs and MSCs. These data suggest on-target efficacy instead of off-target mutations is a major concern for genome editing in stem cells. We then sought to enhance homology-directed repair (HDR)-mediated precise KI, which is usually 0.1-1% for insertion or replacement of a large fragment in iPSCs. We first compared three donor templates and found that relative to PCR-derived donor template or conventional circular plasmid, a targeting vector that is in vivo cleavable due to the flanked two sgRNA recognition sequences, increases KI efficiency 2- to 3-fold. We also found that a 150-600bp homology in both arms leads to highest levels of targeting, whereas homology arm lengths out of this range significantly decrease KI efficiency. Even for inserts as long as 6kb, a 150-300bp homology is sufficient to achieve high-level targeting. In addition, cell cycle synchronization by nocodazole leads to a significant increase in gene targeting, whereas NHEJ inhibitors like SCR7, and HDR-enhancers effective in mouse ESCs like Brefeldin A and L755507, barely enhance KI efficiency of human iPSCs. Furthermore, after transient puromycin selection following transfection of Cas9-Puro, we observed KI efficiencies of 40% in MSCs and 8% in iPSCs. More recently, our pilot studies showed that the use of two factors increase KI efficiencies at the AAVS1 locus and PRDM14 stop codon to >10% in iPSCs. Our findings should have important applications in precision regenerative medicine.

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TISSUE ENGINEERING

T3034

RESCUING THE REGENERATIVE CAPACITY OF HUMAN MESENCYMAL STEM CELL POPULATIONS FROM ELDERLY DONORS

Block, Travis Jackson[1], Marinkovic, Milos[2], Marshall, Amanda[3], Dean, David D[1] and Chen, Xiao-Dong[1], [1]University of Texas Health Science Center at San Antonio, San Antonio, TX, U.S., [2]University of Texas Health Science Center at San Antonio, San Antonio, TX, U.S., [3]San Antonio Orthopaedic Specialists, San Antonio, TX, U.S.

Degenerative diseases are a major public health concern for the aging population. Mesenchymal stem cell (MSC)-based therapies have great potential for treating these diseases. However, the quantity and quality of MSCs declines with age and limits the potential of autologous stem cell therapies. Interestingly, we have observed that a sub-population of MSCs from elderly donors appear to possess a more "youthful" phenotype. The goal of the present study was to test a novel strategy for isolating and expanding this subpopulation, in order to obtain a large quantity of high quality autologous MSCs for use in treating age-related diseases. Sub-populations of elderly MSCs were isolated by fluorescence activated cell sorting based on size and expression of stage-specific embryonic antigen-4 (SSEA-4). These 4 sub-populations, as well as unsorted populations from young and elderly donors, were then cultured on tissue culture plastic and bone marrow-stromal cell derived extracellular matrix (ECM) provided by StemBioSys, Inc. Our experiments confirm that while the effect of age is not reflected in the expression of MSC surface markers (CD73, CD90, CD105), the overall quantity of MSCs from elderly donors is lower relative to young donors. In addition, overall quality is lower as they have decreased colony formation capacity and differentiation efficiency. In spite of this, we were able to obtain a sub-population of small size/SSEA-4 expressing MSCs from elderly donors that have a youthful phenotype that, after expansion on ECM produced by young bone marrow stromal cells, display restored (rejuvenated) capacity for proliferation and differentiation. These cells comprise roughly 5-10% of the total MSC population in elderly donors, and studies examining the effect of conditioned me-

dia from cultures of elderly MSCs on young cells suggest that the small size/SSEA-4 expressing cells are inhibited by secreted factors from the senescent and pre-senescent cells. By isolating this "youthful" population of cells and expanding them on a "young microenvironment" that promotes the maintenance of stemness, we are able to obtain a large quantity of high quality stem cells from elderly donors. The results of this study suggest that banking large quantities of high quality autologous MSCs for treating age-related diseases is feasible.

T3036

MICRO-CULTURE ARRAYS TO CONTROL THE GENERATION OF RETINAL ORGANOIDS

Decembrini, Sarah[1], Brandenberg, Nathalie[2], Hoehnel, Sylke[2], Arsenijevic, Yvan[1] and Lutolf, Matthias P.[2], [1]University of Lausanne, Jules-Gonin Eye Hospital, Lausanne, Switzerland, [2]Laboratory of Stem Cell and Bioengineering, Institute of Bioengineering, Lausanne, Switzerland

The feasibility to recapitulate mammalian retinogenesis in vitro by growing self-organizing retinal organoids from pluripotent stem cells was recently demonstrated by our and other laboratories. We observed that retinal organoids are not completely synchronized in culture, resulting in retinas with different sizes, shapes, and apical-basal orientation suggesting the need to improve the adopted cell culture conditions. We attempt here to standardize an approach, based on microwell arrays, that can be used to screen compounds to reproducibly generate retinal organoids meanwhile saving time, space and materials. Different biomaterials were tested as culture substrates of microwell arrays. The stiffness, quantity of hydrogels, well shape and size, volume of the reaction and medium composition as well as the effect of cell number were evaluated in respect to the efficiency to generate retinal organoids. Different qualities and stiffness of hydrogels were tested in order to establish the best culture substrate supporting retinal organoid generation. Agarose, noble agarose, polyethylene-glycol (PEG) and polydimethylsiloxane (PDMS) were assessed. The results highlight the higher propensity of 5% w/vol PEG, to generate retinal organoids in comparison to all the other tested materials. AggreWell™ (Stemcell Technologies) served in primisas template to construct the desired well topography. Flat- or V- bottom wells, round or square with a diameter of 400 or 800 µm were tested. The shape and size of the microwells tested were not able to support the generation of the retinal organoids. A standard photolithography was developed to generate an unlimited well size and geometry. The optimal microwell size and shape was identified in 1.5mm height (1.7 max), 1.7mm width, U-bottom wells. Such microwells allow the aggregate formation in less than 6 hours. Different medium conditions, supplements, matrigel concentrations and surviving factors were assessed to fine-tune the retinal organoid production. The results suggest the requirement of factors to induce retinal organoids different than those utilized with plastic wells. We conclude that the microwell plates support the survival and differentiation of retinal organoids and can be used to screen morphogens aimed to improve the development of retinal organoids.

T3038

INTERVERTEBRAL DISC REPAIR BY AUTOLOGOUS ADIPOSE-DERIVED STEM CELLS

Han, Inbo, CHA University, Seongnam-si, Korea

Intervertebral disc (IVD) degeneration has been considered as a major cause of low back pain. The surgical treatments may lead to accelerated degeneration of the adjacent segments. Therefore, there is a strong clinical demand for developing cell based therapies for regenerating the degenerated IVD. We designed a pilot study to assess feasibility and safety of autologous adipose-derived mesenchymal stem cells (AD-MSCs) in patients with discogenic back pain. Ten patients with chronic low back pain due to IVD degeneration received autologous AD-MSCs disc injections. Preoperative and postoperative evaluation included visual analogue scale (VAS), Oswestry disability index (ODI), short form (SF)-36, T2-weighted magnetic resonance imaging (MRI). IVD degeneration was grade according to Pfirrmann grade. Clinical evolution was followed for 1 year. All subjected presented a significant reduction in ODI and VAS and increase in SF-36. In addition, water content was slightly increased at 6 months. This study provides evidence of safety and feasibility of autologous AD-MSCs for IVD degeneration and intradiscal injection of AD-MSC may be a valid alternative treatment for chronic discogenic back pain.

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T3040

MINIATURIZED IPS-CELL-DERIVED CARDIAC MUSCLES FOR PHYSIOLOGICALLY RELEVANT DRUG RESPONSE ANALYSES

Huebsch, Nathaniel[1], Loskill, Peter[2], Deveshwar, Nikhil[2], Spencer, Ian[3], Judge, Luke[1], Mandegar, Mohammad[1], Fox, Cade[4], Mohammed, Tamer[1], Ma, Zhen[2], Mathur, Anurag[2], Sheehan, Alice[1], Truong, Annie[5], Saxton, Mike[1], Yoo, Jennie[1], Srivastava, Deepak[3], Desai, Tejal[4], So, Po-Lin[1], Healy, Kevin E.[6] and Conklin, Bruce[4], [1]Gladstone Institutes of Cardiovascular Disease-Conklin, San Francisco, CA, U.S., [2]University of California, Berkeley, Berkeley, CA, U.S., [3]Gladstone Institutes of Cardiovascular Disease, San Francisco, CA, U.S., [4]University of California, San Francisco, San Francisco, CA, U.S., [5]Gladstone Institutes, San Francisco, CA, U.S., [6]University of California, Berkeley, CA, U.S.

Tissue engineering approaches have the potential to increase the physiologic relevance of human iPS-derived cells, such as cardiomyocytes (iPS-CM). However, forming Engineered Heart Muscle (EHM) typically requires >1 million cells per tissue. Existing miniaturization strategies involve complex approaches not amenable to mass production, limiting the ability to use EHM for iPS-based disease modeling and drug screening. Micro-scale cardiospheres are easily produced, but do not facilitate assembly of elongated muscle or direct force measurements. Here we describe an approach that combines features of EHM and cardiospheres: Micro-Heart Muscle (μHM) arrays, in which elongated muscle fibers are formed in an easily fabricated template, with as few as 2,000 iPS-CM per individual tissue. Within μHM, iPS-CM exhibit uniaxial contractility and alignment, robust sarcomere assembly, and reduced variability and hypersensitivity in drug responsiveness, compared to monolayers with the same cellular composition. μHM mounted onto standard force measurement apparatus exhibited a robust Frank-Starling response to external stretch, and a reproducible, dose-dependent inotropic response to the b-adrenergic agonist isoproterenol. Based on the ease of fabrication, the potential for mass production and the small number of cells required to form μHM, this system provides a potentially powerful tool to study cardiomyocyte maturation, disease and cardiotoxicology in vitro.

Funding Source: National Institutes of Health

T3042

PRODUCTION OF HUMAN CARDIAC TISSUES USING PLURIPOTENT STEM CELLS AND PHOTOCROSSLINKABLE HYDROGEL BIOMATERIALS

Kerscher, Petra[1], Seeto, Wen J[1], Head, Sara E[1], Kaczmarek, Jennifer A[1], Turnbull, Irene[2], Hodge, Alexander J[1], Kim, Joonyul[1], Seliktar, Dror[3], Easley, Christopher J[1], Costa, Kevin D[2] and **Lipke, Elizabeth Ann**[1], [1]Auburn University, Auburn, AL, U.S., [2]Icahn School of Medicine at Mount Sinai, New York, NY, U.S., [3]Technion-Israel Institute of Technology, Haifa, Israel

Detecting cardiotoxicity of prospective pharmaceuticals is a long, expensive, and uncertain process, with about 28% of drugs being withdrawn due to unexpected side effects. Human pluripotent stem cell-derived cardiomyocytes (SC-CMs) have high potential to be used not only in regenerative medicine, but also in high-throughput drug testing and personalized medicine. In this study we present a straight-forward and highly reproducible cardiac tissue production process using a single-cell handling step to encapsulate and differentiate hiPSCs within two different photocrosslinkable hydrogels to form 3D developing human engineered cardiac tissues. To form cell-laden tissues, hiPSCs were either combined with liquid poly(ethylene glycol) (PEG)-fibrinogen or methacrylated gelatin (GelMA) polymer precursor and photocrosslinked to form microisland tissues. Encapsulated hiPSCs were then cultured in mTeSR-1 media for three days prior to initiation of cardiac differentiation (day 0). Tissues were characterized using phase contrast and confocal microscopy, flow cytometry, RT-qPCR, electrophysiology, SEM, and TEM. Cells remained viable, grew, and remodeled their hydrogel microenvironments to form dense and uniformly contracting cardiac tissues over time. Beginning on day 7 areas of spontaneous contraction increased in number and strength, resulting in uniformly contracting tissues by day 10, which continued to contract for several months in vitro. Tissues were composed of >70% CMs (cardiac troponin T positive, n=3) and temporally expressed cardiac markers and functional proteins of developing and maturing CMs. Able to be exogenously paced at up to 3.0 Hz, CMs responded appropriately to drug treatment, including isoproterenol and propranolol. Long-term cultured tissues developed features of mature CMs including gap junctions, intercalated discs, and even T-tubules. This reproducible tissue production platform was successfully extended to create highly reproducible and spherical microspheres using a microfluidic system. HiPSCs encapsulated within microspheres differentiated into spontaneously contracting cardiac spheroids which responded to pharmaceutical and electrical stimuli, and maintained their spontaneous contractile function after sheer through 18g and 23g injection needles.

T3044

A POLYETHYLENE GLYCOL POLYMER UTILIZING THE MATRIKINE TENASCIN C ENHANCES MULTIPOTENT STROMAL CELL SURVIVAL AND IMPROVES SKIN WOUND HEALING OUTCOMES IN MICE

Nuschke, Austin[1,2], Yates, Cecelia[1,2], Rodrigues, Melanie[3], Whaley, Diana[1], Taylor, Donald[1] and Wells, Alan[1,2], [1]University of Pittsburgh, Pittsburgh, PA, U.S., [2]McGowan Institute for Regenerative Medicine, Pittsburgh, PA, U.S., [3]Stanford University, Stanford, CA, U.S.

Mesenchymal stem cells (MSCs) remain of great interest in regenerative medicine due to their ability to home to sites of injury, differentiate into a variety of relevant lineages, and modulate inflammation and angiogenesis through paracrine activity. Many studies have found that despite the great promise of MSC therapy, cell survival upon implant is highly limited and greatly reduces the therapeutic utility of MSCs across a variety of regenerative contexts. Thus, strategies to improve MSC survival are of great interest in an effort to improve upon current clinical outcomes for cell therapy. The matrikine Tenascin C, a protein expressed often at the edges of a healing wound, contains unique EGF-like repeats that are able to bind EGFR at low affinity and signal without inducing receptor internalization, allowing for pseudo-constitutive activation of downstream pro-survival signaling. Previously, we have shown that this protein is able to protect MSCs from induced cell death in vitro. In this study, we exploited the EGFR binding activity of Tenascin C by utilizing the protein in a matrix-based polymer for skin wound healing, incorporating human bone marrow-derived MSCs into the polymer prior to application to mouse punch biopsy wound beds. We found that the functionalized polymer was able to promote MSC survival long term in vivo, yielding detectable MSCs after three weeks of treatment. This extended MSC survival in turn led to associated improvements in wound healing such as dermal maturation and collagen content. This was most marked in a model of hypertrophic scarring, in which the scar formation was limited. This approach also reduced the inflammatory response in the wound bed, while increasing the numbers of endothelial cells. Ultimately, this matrikine-based approach to improving MSC survival may be of great use across a variety of cell therapies utilizing matrices as delivery vehicles for cells.

Funding Source: NIGMS, University of Pittsburgh Center for Medical Innovation

T3046

DECELLULARIZED MURINE AND PORCINE KIDNEYS USED AS A THREE-DIMENSIONAL SUBSTRATE FOR IN VITRO CULTURE OF WHOLE ORGANS

Whiteley, Jennifer[1], Chow, Theresa[1,2], Kaths, Moritz[2,3], Selzner, Markus[2,3] and **Rogers, Ian**[1,2], [1]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada, [2]University of Toronto, Toronto, ON, Canada, [3]University Health Network-Toronto General Hospital, Toronto, ON, Canada

According to the Kidney Foundation of Canada 3450 Canadians are on a waiting list for a kidney transplant. The development of an alternative to donor organ transplant would significantly reduce the number of patients on dialysis and greatly improve patient quality of life. Stem cells are capable of differentiating into multiple cell types suggesting their potential for cell therapy. The next challenge is to produce whole organs for transplantation. This goal will be limited by the ability to produce all of the required cell types as well as our ability to produce a three-dimensional structure. Using decellularized organs may provide a solution to both problems. Decellularization involves the gentle removal of cells using a mild detergent solution without damaging the underlying extracellular matrix (ECM). Once the ECM is stripped of cells, it can provide both a mechanical and biological support for stem and progenitor cells. The method of decellularization, pioneered by Dr. Doris Taylor, demonstrates that cardiomyocytes and endothelial cells can be used to repopulate the acellular organ, re-establishing some of its original properties. Our lab is focused on the decellularization of murine and porcine kidneys. We propose that acellular kidneys can be used as substrates for three-dimensional tissue culture, providing an improved environment for stem cell differentiation and ultimately for rebuilding patient specific organs for transplantation. For both goals having an ECM that has retained its protein composition and structure is critical. It is also important to demonstrate the compatibility of human cells on xeno-ECM, as these are candidate substrates for experimental cell culture systems and the development of whole organs. Detergent concentration, solution volume, time and pressure were all parameters that had to be considered to achieve complete removal of cells without damaging the ECM. We used a positive pressure pump system that removed cellular debris, giving us optimal acellular substrates. The ability of the acellular ECM to support cell growth was tested using endothelial cells, stromal cells, and differentiated mouse ES cells. Our results indicate that the decellularized organ is suitable for three-dimensional cell culture and has great potential for the eventual production of whole organs.

T3048

DISTINCT TEMPORAL EXPRESSION OF INTESTINAL EPITHELIAL MARKERS OCCURS DURING GENERATION OF HUMAN AND MOUSE TISSUE-ENGINEERED SMALL INTESTINE

Schlieve, Christopher Ross, Schall, Kathy, Fowler, Kathryn, Hou, Xiaogang and Grikscheit, Tracy, Children's Hospital Los Angeles, Los Angeles, CA, U.S.

Tissue-engineered small intestine (TESI), derived from multicellular clusters of autologous intestinal epithelium and mesenchyme termed organoid units, could provide a potential source of patient-specific transplantable tissue for short bowel syndrome patients. Previous studies demonstrate the presence of all native cell lineages in mature TESI. However, the process of TESI formation remains unknown. Elucidating integral cellular mechanisms may enhance TESI formation and function. Human and mouse TESI (hTESI or mTESI) were graded based on histology: grade 0:no growth and scaffolding present, grade 1:round clusters of small epithelium, grade 2:flat epithelium, grade 3:simple invaginating epithelium, and grade 4:resembling native epithelium with crypt-like structures. Each grade was stained for differentiated epithelial and mesenchymal cell types; E-cadherin/villin (enterocytes), lysozyme (Paneth cells), mucin (goblet cells), chromogranin A (enteroendocrine cells), smooth muscle actin (muscle), Tuj-1 (neurons), GFAP and s100 (glia) and Ki67 (proliferating cells). Proliferating cells, nerves and muscle appeared in grade 1 TESI. However, goblet cells and enterocytes develop later in mTESI (grade 2) as compared to hTESI (grade 1). Enteroendocrine cells emerged in grade 3 for mTESI, but appeared earlier at grade 1 for hTESI. Paneth cells were identified in fully developed mTESI and hTESI at grade 4 and did not appear in early stages. Human TESI expressed mature epithelium and mesenchymal markers at earlier stages of development than mouse TESI. However, fully differentiated grade 4 mTESI and hTESI contained Paneth cells. Although Paneth cells are integral to the intestinal stem cell niche and support Lgr5+ stem cells that give rise to mature epithelium, our data suggest an alternative pathway is crucial for the maintenance and generation of TESI.

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T3050

USING HUMAN IPS CELLS TO CREATE AN IN VITRO MODEL OF PERFUSED TUMOR AND CARDIAC TISSUE

Weng, Kuo-Chan, Kurokawa, Yosuke, Shirure, Venktesh S and George, Steven C, Washington University in St Louis, St Louis, MO, U.S.

Cardiovascular disease and cancer remain major health threats in developed countries "Organ-on-a-chip" disease platforms provide potential avenues for preclinical drug discovery and personalized medicine that are cost effective. Here we developed an integrated microfluidic device that supports both tumor cells and iPS-derived cardiac tissue with a perfused living dynamic network of microvessels derived from the same iPS cells. The device design allows the vessel networks to form and supply nutrition to the tissues, and then assess simultaneously the anti-tumor and cardiotoxicity of drugs. A TGF-β-modulatory protocol was used for endothelial cell (iPS-EC) differentiation, and magnetic-activated cell sorting was used to select for CD31+ cells. By manipulating the WNT signaling pathway, we differentiate cardiomyocytes from human iPS cells with a GCaMP6 fluorescence reporter in the AAVS1 safe harbor locus to facilitate imaging intracellular calcium dynamics and to quantify the drug response. We first introduce iPS-derived endothelial cells and fibroblasts in a fibrin gel to form the vessel network in a central chamber (~ 0.05 cm^3). A contiguous and perfused vessel network develops in 5-7 days. We then introduce tumor cells and iPS-derived cardiomyocytes in a fibrin gel (10 mg/ml) in separate chambers (~ 0.03 cm^3) that are adjacent to the vascular network. The tumor and cardiac organ systems are supported by perfusion of a single medium (EGM-2) through the vascular network. In this fashion, candidate drugs can also be delivered through the vessel network. The tumor and cardiac tissues can be maintained for a minimum of seven days. Our perfused microfluidics device integrates three organ systems (tumor, cardiac, vasculature) on a single platform. The technology employs iPS-derived cardiomyocytes, endothelial cells, and stromal cells, and may be used as a high throughput drug-screening platform for personalized medicine applications.

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T3052

DIFFERENTIATION OF MOUSE PLURIPOTENT STEM CELLS IN THREE-DIMENSIONAL CULTURES UNDER DEFINED CONDITIONS

Zujur, Denise Carolina[1], Kanke, Kosuke[2], Chung, Ung-il[3] and Ohba, Shinsuke[3], [1]The University of Tokyo, Tokyo, Japan, [2]The University of Tokyo, Graduate School of Medicine, Tokyo, Japan, [3]The University of Tokyo, Graduate School of Engineering, Tokyo, Japan

Offering greater physiological relevance than conventional two-dimensional cultures, three-dimensional (3-D) systems have become a promising alternative for bridging the gap between in vitro culture and living tissues. This is especially important for embryonic stem cell research, since they are derived from a highly organized 3-D structure. In this regard, there is a clear need for developing suitable 3-D culture platforms for the maintenance and differentiation of pluripotent stem cells (PSCs), which may significantly accelerate the understanding and translation of PSCs. Here, we developed an in vitro fully-defined strategy in which mouse PSCs are maintained and differentiated into specific lineages in a 3-D manner. Mouse PSCs were successfully maintained within atelocollagen sponges, displaying expressions of pluripotency genes and proteins as well as teratoma formation. Under further differentiation treatments, they gave rise to endodermal, mesodermal, and ectodermal cells in the 3-D culture, as indicated by downregulation of pluripotency genes and specific upregulation of marker genes of each lineage. In addition, the PSC-derived mesoderm was subsequently differentiated into bone-forming osteoblasts in the 3-D culture using the small molecule-mediated strategy that we previously reported; expressions of mesoderm markers (T and Mixl1) were downregulated, while those of osteoblast precursor markers (Runx2 and Col1a1) were upregulated, followed by the sequential expression of mature osteoblast markers (Sp7, Ibsp, and Bglap). Furthermore, terminal differentiation of the PSC-derived osteoblasts into osteocytes was evidenced by the expression of Dmp1, Sost, and Rankl as well as by visual recognition in histological sections of the 3-D complexes. Thus, the present strategy, in which the maintenance and subsequent differentiation of PSCs were performed in 3-D cultures under defined conditions, generated 3-D bone-like tissues consisting of PSC-derived osteogenic cells and functional extracellular matrices. The strategy may also be applicable to the generation of ectoderm- or endoderm-derived tissues when combined with appropriate differentiation systems.

T3054

THREE-DIMENSIONAL BIOENGINEERED LUNG TISSUE FOR PERSONALIZED DISEASE MODELING

Wilkinson, Dan Charles[1], Alva-Ornelas, Jackelyn[2], Sucre, Jennifer[1], Durra, Abdo[2], Richardson, Wade[1], Jonas, Steven J.[1,2], Vijayaraj, Preethi[3], Paul, Manash K.[2], Dunn, Bruce[1] and Gomperts, Brigitte[2], [1]University of California, Los Angeles, CA, U.S., [2]David Geffen School of Medicine at University of California, Los Angeles, CA, U.S., [3]University of California, Los Angeles, CA, U.S.

Lung diseases are among the leading causes of morbidity and mortality worldwide and account for many billions of dollars of healthcare expenditure. As the population ages, the burden from chronic lung diseases is expected to increase, with higher morbidity and mortality rates attributable to them. The development of therapies for lung diseases has been hampered by a lack of human disease models as traditional in vitro cell culture and animal in vivo studies do not faithfully reproduce the human diseases. Here we present a method for the generation of self-assembled human lung tissue and its potential for disease modeling and drug discovery for diseases characterized by irreversible, progressive scarring such as Idiopathic Pulmonary Fibrosis (IPF). This bioengineered lung organoid contains multiple lung cell types assembled into the correct anatomical location thereby allowing cell-cell contact and recapitulation of the lung microenvironment. Tissue formation occurs due to the overlapping processes of cellular adhesion to multiple alveolar sac templates, bioreactor rotation, and cellular contraction. Though the main benefit of this method is that of modularity; different cell types and extracellular matrix components may be easily added or subtracted allowing for improved experimental control and hypothesis testing. Addition of TGFb-1 to the lung tissue resulted in phenotypic scarring typical of that seen in IPF. Our bottom up approach for synthesizing patient-specific lung tissue in a scalable system allows for the development of relevant human lung disease models with the potential for high throughput drug screening to identify targeted therapies.

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REGENERATION MECHANISMS

T3056

SUPPRESSION OF SWI/SNF COMPONENT ARID1A LEADS TO INCREASED BETA-CELL REGENERATION DURING DEVELOPMENT AND IN DIABETES.

Chuang, Jen-Chieh[1], Wu, Linwei[1], Wang, Sam[1], Nassour, Ibrahim md[1], Sun, Xuxu[2], Celen, Cemre[1], Repa, Joyce[1] and Zhu, Hao[3], [1]University of Texas Southwestern Medical Center, Dallas, TX, U.S., [2]University of Texas Southwestern, Dallas, TX, U.S., [3]Children's Research Institute, UT Southwestern Medical Center, Dallas, TX, U.S.

The epigenetic mechanisms that regulate pancreatic β-cell regeneration are mysterious. The SWI/SNF chromatin-remodeling complex is thought to enforce cellular differentiation and repress proliferation, so we reasoned that SWI/SNF might play a critical role in tissue homeostasis. Arid1a is a DNA-binding subunit of SWI/SNF that is highly expressed in quiescent β-cells and is then suppressed in mouse islets and β-cell lines undergoing proliferative expansion (after exposure to high-glucose, Igf2, or Il-1β). To determine if Arid1a suppression is functionally relevant, we inducibly deleted Arid1a in adult Ubc-CreERT; Arid1a$^{Fl/Fl}$ mice. At baseline, these whole-body Arid1a knockout (KO) mice possessed normal glucose clearance in glucose- and insulin-tolerance tests. After exposure to the β-cell ablating toxin streptozotocin, KO mice showed higher plasma insulin, exhibited increased glucose clearance, and were almost completely protected from type 1 diabetes. Arid1a KO mice had increased β-cell proliferation and β-cell mass after partial pancreatectomy. In a distinct tet-on mouse model, conditional Arid1a deletion after streptozotocin and specifically in β-cells was also able to block the onset of diabetes, demonstrating a β-cell autonomous effect and therapeutic relevance. Transcriptome analysis in isolated β-cells revealed increased EGFR signaling in the absence of Arid1a. Moreover, Arid1a knockdown in β-cell lines led to increased proliferation and hyper-responsiveness to exogenous EGF ligand. Pharmacological EGFR inhibition with Canertanib in cell lines and KO mice was able to rescue the proliferation and glucose phenotypes associated with Arid1a loss. These data collective show that Arid1a is a novel and potent epigenetic regulator of β-cell regeneration.

T3058

GENE EXPRESSION PROFILING WITHIN THE DERMAL STEM CELL LINEAGE

Hagner, Andrew, Alpaugh, Whitney, Workentine, Matthew, Raharjo, Eko and Biernaskie, Jeff, University of Calgary, Calgary, AB, Canada

Within each hair follicle (HF), specialized mesenchymal cells provide instructive signals to epithelial cells to maintain continuous tissue regeneration. We recently demonstrated the existence of a self-renewing dermal stem cell (DSC) that functions to populate both mesenchymal compartments within the HF: the inductive dermal papilla (DP) and connective tissue sheath (CTS), which wraps around the transient portion of the follicle. In order to identify: 1) mesenchyme-derived activators of HF regeneration, and 2) DSC-lineage determinants, we developed a novel FACS strategy to isolate DSCs or their differentiated progeny (DP and CTS) from early growth follicles, then performed RNA-seq to identify gene expression signatures for each population. Signature genes were validated using qPCR and immunohistochemistry. Ongoing work is examining the functional role of certain candidate genes within hfDSCs and HF regeneration, in part using a conditional knockout strategy and topical application of candidate agonists. Together, our findings impart novel insights on the molecular regulators of the hfDSC lineage and the potential activation signals of epithelial progenitors at the onset of HF regeneration.

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T3060

ROLE OF GLIAL SCAR IN HUMAN IPSC-DERIVED LT-NES BEHAVIOR AFTER TRANSPLANTATION IN STROKE-INJURED MICE

Laterza, Cecilia[1], Hara, Naomi[1], Ge, Ruimin[1], Tornero, Daniel[1], Milos, Pekny[2], Lindvall, Olle[1] and Kokaia, Zaal[1], [1]Lund Stem Cell Centre, Lund University, Lund, Sweden, [2]Center for Brain Repair and Rehabilitation, Göteborg, Sweden

The transplantation of human iPSC-derived neural cells in experimental ischemic stroke has been already demonstrated to be an efficacious and safe therapeutic strategy. In this project we aim to evaluate the influence of glial scar on the therapeutic potential of human iPSC-derived neuroepithelial-like stem (lt-NES) cells after their transplantation in storke-injured mice. Astrocytes are a key cell type playing active role in physiology and pathology of the CNS. After stoke, reactive astrocytes play both detrimental and beneficial effects: the capacity of reactive astrogliosis in limiting the tissue damage at the acute phase is balanced against restricted regenerative potential later

on. To pursuit our aim we used a transgenic mouse lacking both Vimentin and GFAP. $Vim^{-/-}GFAP^{-/-}$ mice show impaired astrocyte activation and glial scar formation upon CNS injury. We induced cortical lesion by occlusion of distal branch of middle cerebral artery both in $Vim^{-/-}GFAP^{-/-}$ and WT mice and transplanted $1x10^6$lt-NES cells close to the penumbra. Mice were sacrificed 2 months later. Our preliminary data confirmed the absence of glial scar formation in $Vim^{-/-}GFAP^{-/-}$ mice. We detected some GFAP-positive cell in $Vim^{-/-}GFAP^{-/-}$ mice, but they were exclusively coming from grafted cells. Moreover, we showed that the absence of glial scar did not affect the lesion size. Then we evaluated how glial scar affected the properties of grafted cells and we found that transplanted lt-NES cells showed a higher proliferation rate in $Vim^{-/-}GFAP^{-/-}$ mice compared to WT mice. Finally we investigated the inflammatory reaction both surrounding the penumbra and the injection site of lt-NES cells and we observed an increase in the number of activated inflammatory cells in the ipsilesional hemisphere of WT and not of $Vim^{-/-}GFAP^{-/-}$ mice. We are currently analyzing the integration and differentiation potential of transplanted lt-NES cells in $Vim^{-/-}GFAP^{-/-}$ mice. At the end of this project, our data will shed light on the interplay between astrocytes and lt-NES cells, contributing to the improvement of cell-based treatment strategies, both in promoting brain repair and in reducing neurological impairment after stroke.

T3062

INFLAMMATORY MEDIATORS REQUIRED FOR MUSCLE STEM CELL FUNCTION DURING REGENERATION

Ho, Andrew T.V.[1], **Palla, Adelaida R.**[1], Magnusson, Klas E.G.[2], Blake, Matthew R.[1], Holbrook, Colin A.[1] and Blau, Helen M.[1], [1]Baxter Laboratory for Stem Cell Biology, Institute for Stem Cell Biology and Regenerative Medicine, Stanford School of Medicine, Stanford, CA, U.S., [2]KTH Royal Institute of Technology, Stockholm, Sweden

The elderly suffer from progressive skeletal muscle wasting and regenerative failure which decreases mobility and quality of life. Crucial to muscle regeneration are adult muscle stem cells (MuSCs) that reside in niches in muscle tissues, poised to respond to damage and repair skeletal muscles throughout life. During aging, the proportion of functional MuSCs markedly declines, hindering muscle regeneration. To date, no therapeutic agents are in clinical use that target MuSCs to combat this regenerative decline. Using in silico screens for factors that enhance MuSC functions, we discovered that factors associated with early inflammation control the stem cell state, are capable of overcoming the aged MuSC signaling defects, and enhance regenerative potential. Some of these immunomodulaltors act as potent inducers of MuSC function essential to muscle regeneration while others promote MuSC commitment. In addition, we identified an intrinsic molecular defect in aged MuSCs, which led to impaired muscle regeneration. Most notably, intramuscular injection of small molecules, mimicking the inflammatory mediators observed in young healthy muscles in response to damage, suffices to induce a significant increase in endogenous stem cell numbers and myofiber sizes in aged muscles two weeks later. Our findings suggest a novel therapeutic target in exploiting inflammation signaling pathway to augment the regenerative capacity of muscles in the aged and other muscle disease states.

ETHICS AND PUBLIC POLICY; HISTORY OF STEM CELL RESEARCH; SOCIETY ISSUES; EDUCATION AND OUTREACH

T3068

THE LURE OF STEM CELL TOURISM: AN ANALYSIS OF YOUTUBE VIDEOS FROM PROVIDERS OFFERING UNPROVEN STEM CELL INTERVENTIONS

Master, Zubin[1], **Hawke, Bethany**[1], Chico, Alexandra[1], Zarzeczny, Amy[2] and Caulfield, Timothy[3], [1]Alden March Bioethics Institute, Albany Medical College, Albany, NY, U.S., [2]University of Regina, Regina, SK, Canada, [3]Health Law Institute, University of Alberta, Edmonton, AB, Canada

Stem cell tourism describes the market for unproven stem cell-based interventions (SCBIs). This increasingly global industry is expanding to include tightly regulated jurisdictions, most notably in the U.S. Providers primarily advertise on a direct-to-consumer basis over the internet, including through patient testimonial videos on clinic websites and YouTube. Patient narratives have been found to be more persuasive than text-based statistical information because they appeal to intuitive reasoning. Previous studies have examined text-based advertisements from providers, including website content and use of Twitter, but there is no comprehensive analysis of YouTube videos examining how they are used to attract patients and perhaps to fuel hope. Our study analyzes 159 YouTube videos from providers offering unproven SCBIs for 5 conditions. Using quantitative and qualitative methods, we evaluate both visual and auditory content. The videos studied averaged 5 minutes in length with 3,500 views and had up to 2,047 subscribers. The majority of providers were reportedly based in India (30%), followed by the U.S. (12%). Most videos (90%) were patient testimonials describing their therapeutic journey or answering questions posed by the provider. Almost all videos (91%) included patients describing treatment benefits; 58% of videos also included purported demonstrations of efficacy. Some patients discussed their hopes associated with the treatment

(26%), offered praise (54%) and recommended (29%) the SCBI or the provider. Our qualitative assessment suggests providers use patient testimonials as “validation or testament” that SCBIs work, and to dispel fears or uncertainty. We found almost no discussion of costs, ethical considerations or regulatory measures. Only 10% of videos mentioned risks, almost all of which were minimized. Videos may encourage targeted audiences to identify with and trust the individuals represented because of shared experiences with similar medical struggles. Given the risks and uncertainties associated with unproven SCBIs, there is a need to ensure availability of evidence-based information to support informed decision-making by patients. Our results indicate that narrative-based countermeasures may offset positive but perhaps unbalanced appeals facilitated by providers.

T3070

CONTRASTING VIEWS ON EMBRYO RESEARCH AND FUNDING: A SURVEY OF US PHYSICISTS AND BIOLOGISTS

Matthews, Kirstin RW, Rice University, Houston, TX, U.S. and Tsao, Sharon, Rice University Baker Institute for Public Policy, Houston, TX, U.S.

In the United States, the public and scientists are engaged in ongoing discussions related to embryo research, including the use of embryonic stem cells and the creation of embryos for research. While the public has been regularly surveyed on the topic, scientists are rarely assessed. In this project, we surveyed 3,989 randomly selected physicists and biologists in the United States across 78 universities and research institutes on social and political factors impacting their work as scientists, achieving a 57% response rate. Participants were asked to agree or disagree with two statements related to embryo research: “Scientists should be able to create human embryos for medical research purposes” and “The government should support research using cells derived from lab-created human embryos.” Our results showed that of the 1,966 scientists who responded, 52% supported human embryo research and 59% supported its federal funding. There was no difference for either statement based on discipline or rank of the university (‘elite’ versus ‘non-elite’). Of note, men were more likely to agree with either statement than women. And, as expected, those who disagreed were more likely to self-identify as being religious. Interestingly, a group of 92 respondents who answered “strongly disagree” or “somewhat disagree” to the first statement on human embryo research gave a contradictory response of “strongly agree” or “somewhat agree” to the statement on its federal funding. These scientists were more likely to be biologists and to work at ‘elite’ universities, though there was no difference between genders. They were also less likely to be religious. Overall, the survey points to a favorable environment for controversial studies involving the creation of embryos for research purposes and the funding of this research. This data also seems to demonstrate that scientists make complex value judgments, weighing personal beliefs about areas of moral ambiguity separately from how they regard broader decisions related to what the field should be doing. This is especially salient when biologists considered morally gray areas.

Funding Source: Research for the study was conducted with a major grant from the Templeton World Charity Foundation (Grant TWCF0033/AB14) Elaine Howard Ecklund, PI; Kirstin R.W. Matthews and Steven W. Lewis, co-PIs. Additional support was provided by Rice University

T3072

ATTITUDES OF SCIENTISTS AND THE PUBLIC TOWARD SCIENCE COMMUNICATION CONCERNING STEM CELL RESEARCH AND REGENERATIVE MEDICINE

Shineha, Ryuma[1], Yashiro, Yoshimi[2], Inoue, Yusuke[3], Ikka, Tsunakuni[4] and Kishimoto, Atsuo[3], [1]Seijo University, Tokyo, Japan, [2]Kyoto University, Kyoto, Japan, [3]The University of Tokyo, Tokyo, Japan, [4]National Center of Neurology and Psychiatry, Kodaira, Japan

In modern times, there is a growing need for scientists' active participation in science communication. However, scientists’ current attitudes toward science communication and their gap with the public are unclear. In order to consider the effective participation of scientists in science communication, a survey on scientists’ attitude is necessary. At the same time, we have to examine gap of attracted topics between scientists and the public. To this end, a questionnaire survey to researchers in stem cell science fields and the public was conducted in 20015. As the result over two thousand of the public responses, and over one thousand responses of stem cell scientists were obtained. From the comparative analysis of their answers, we found that there are gap of attracted topics between what scientists would like to tell and what the public want to know. Simultaneously, there are different attitudes toward media discourses on stem cell science. In addition, we examined hurdle for scientists to participate in communication activities, and pointed out several issues of science communication: infrastructure of communication activities, including evaluation system and science policy.

Funding Source: This research is funded by the Ministry of Education, Culture, Sports, Science and Technology, Japan (Modeling of risk communication).

LATE BREAKING ABSTRACT

T4002

THE REGULATORY ROLE OF NUCLEAR RECEPTOR LRH-1 IN HUMAN PROSTATE CANCER STEM CELL MAINTENANCE

Wang, Yuliang and Chan, Franky Leung, The Chinese University of Hong Kong, Shatin, Hong Kong

Emerging evidences suggested that cancer stem cells (CSCs) endowed with capacity of self-renewal and differentiation are involved in tumor initiation, progression, metastasis and therapy-resistance. Agents specifically targeting and eradicating the CSC population in tumors may provide an attractive therapeutic strategy for the advanced prostate cancer management. Nuclear receptor liver receptor homolog-1 (LRH-1, NR5A2*)* is a transcription factor playing a central role in stem cell biology and in human cancers, and thus may represent a potential target to develop CSC-specific treatment. In this study, we investigated the regulatory role of LRH-1 in prostate cancer stem cell (PCSC) maintenance. Our preliminary data demonstrated that LRH-1 was highly expressed in prostate cancer cells with stem-like cell properties; and LRH-1overexpressed prostate cancer cells exhibited increased expression of CSC specific markers and enhanced self-renewal capacity *in vitro,* while knockdown of LRH-1 could reduce the expression level of CSC markers, and suppress the sphere formation capacity *in vitro* and tumorigenicity *in vivo* in PCSCs. Moreover, we showed that LRH-1-induced PCSC maintenance might be mediated by its transcriptional control of expression of some core pluripotency-associated transcription factors (e.g. Oct4 and Nanog). Taken together, LRH-1 may play a tumor-promoting role in prostate cancer development via its maintenance of PCSC population, and targeting to LRH-1 is of promising therapeutic significance in advanced prostate cancer.

Funding Source: This study was supported by a grant from The Hong Kong Food and Health Bureau (Health and Medical Research Fund /HMRF, project number 02130066)

T4004

EPIDERMAL YAP DRIVES ROCK-DEPENDENT MECHANOSIGNALLING AND β-CATENIN ACTIVATION TO INDUCE KERATINOCYTE PROLIFERATION IN THE MOUSE SKIN IN VIVO

Akladios, Bassem[1], Mendoza-Reinoso, Veronica[1], Hardeman, Edna[1], Khosrotehrani, K Kiarash[2], Key, Brian[3], Samuel, Michael[4] and **Beverdam, Annemiek**[1,3], [1]The School of Medical Sciences, UNSW Australia, Sydney, Australia, [2]University of Queensland Centre for Clinical Res, Herston, QLD, Australia, [3]The School of Biomedical Sciences, The University of Queensland, Brisbane, Australia, [4]Centre for Cancer Biology, SA Pathology and the University of South Australia, Adelaide, Australia

One in three cancers diagnosed globally is a skin cancer (WHO). Yes-associated protein (YAP) is a pivotal regulator of stem cell proliferation and of organ size that is active in human cancer. Mechanical force is increasingly recognized to be a crucial player in the regulation of tissue homeostasis and in cancer development, with YAP rapidly taking the center stage as a key mechanoprotein. We have recently generated a viable and fertile transgenic mouse model that expresses a constitutively active form of YAP protein mutant YAP2-5SA-ΔC in basal keratinocytes. YAP2-5SA-ΔC mice display a dramatic expansion of basal epidermal stem/progenitor cell populations.

Wnt/β-catenin signalling also controls epidermal development and regeneration. Here we describe increased nuclear β-catenin activity in the hyperproliferating keratinocytes of YAP2-5SA-ΔC epidermis, and increased β-catenin transcriptional activity in the skin of live YAP2-5SA-ΔC/TOPFLASH mice. Loss of β-catenin in basal keratinocytes of YAP2-5SA-ΔC/K14-creERT/CtnnB1lox/lox mice resulted in reduced epidermal proliferation of basal keratinocytes and a striking rescue of the hyperplastic abnormalities. These data show that YAP requires β-catenin activity to drive keratinocyte proliferation. In addition, we found highly prominent cortical actin in the epidermal keratinocytes of YAP2-5SA-ΔC mice and increased ROCK activity. This was accompanied by increased expression of vimentin, increased collagen content, and increased expression of ECM molecules such as Tenascin-C, Serpins and metalloproteases, demonstrating extensive tissue remodelling in the dermis of YAP2-5SA-ΔC mice. Moreover, we noticed increased phosphorylation of FAK, AKT and GSK3β in the YAP2-5SA-ΔC epidermis, showing that β-catenin is activated in response to integrin-dependent signalling due to remodelling of the extracellular matrix.

Altogether, these data show that mechanoprotein YAP activates RhoA/ROCK-dependent mechanosignalling in mouse skin in vivo, eventually resulting in the activation of β-catenin and keratinocyte proliferation. Our studies have far-reaching implications for our understanding of

the aetiology of human (skin) cancer and of regenerative disease in tissues displaying increased YAP, β-catenin and ROCK activity.

Funding Source: National Health and Medical Research Council (NHMRC) Australia, UNSW Australia International Postgraduate Award, LASPAU International PhD scholarship.

T4006

A NEW TOOL FOR ANALYSIS OF HUMAN GENETIC DISEASES-3D HUMAN LIVER ORGANOIDS

Guan, Yuan, Xu, Dan, Garfin, Phillip M, Nishimura, Toshihiko, Michie, Sara, Enns, Greg, Sage, Julien and Peltz, Gary, Stanford University, Stanford, CA, U.S.

A major unsolved problem is defining the role of NOTCH and other signaling pathways in solid organ development in humans. We developed a new in vitro model system where induced pluripotent stem cells (iPSCs) differentiate into 3-dimensional human liver organoids (3D-hLOs) through stages that resemble human liver during its embryonic development. The organoids have a complex 3D- structure: there are sheets of hepatocytes, and cholangiocytes are organized into epithelia that surround the lumina of bile duct-like structures. During organoid formation, the interaction between hepatocytes and JAG1-expressing cholangiocytes is required for bile tube formation. We analyze Alagille Syndrome, a genetic disorder where NOTCH signaling pathway mutations impair bile duct formation, to demonstrate how 3D-hLOs could be used to study the pathogenesis of diseases affecting liver development.

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T4008

ANTIDEPRESSANT TREATMENT STIMULATES LIVE NEUROGENIC SIGNAL IN THE HUMAN HIPPOCAMPUS

Valenzuela, Michael[1], Li, Yao[2], Han, Xu (Wendy)[3], Suo, Chao[4], Elvasashagen, Torbjorn[5], Stosic, Marija[3], Schrantee, Anouk[6], de Ruiter, Michiel[6], Yu, Hui Jing[7], Haque, Muhammad[8], Loo, Colleen[9], Pujol, Jesus[10], Cardoner, Narcis[10], Pantelis, Christos[11], Yucel, Murat[12], Sachdev, Perminder[13], Velakoulis, Dennis[11], Contreras-Roderiguez, Oren[10], Urretavizcaya, Mikel[14], Menchon, Jose M.[14], Wagshul, Mark E.[7], Milanovic, Snezana[15], Ratai, Eva-Maria[15], Schagen, Sanne[16], Lampit, Amit[4], Boen, Erland[5], Malt, Ulrik Frederik[5], Liu, Zhangdong[3], Krupp, Lauren[17], Lucassen, Paul[18], Reneman, Liesbeth[6] and Maletic-Savatic, Mirjana[17], [1]School of Psychiatry, UNSW, Sydney NSW, Australia, [2]Shanghai Jiao Tong University, Shanghai, China, [3]Baylor College of Medicine, Houston, TX, U.S., [4]University of Sydney, Sydney, Australia, [5]Oslo University Hospital, Oslo, Norway, [6]University of Amsterdam, Amsterdam, Netherlands, [7]Stony Brook University, Stony Brook, NY, U.S., [8]Texas Children's Hospital, Houston, TX, U.S., [9]Wesley Hospital, Sydney, Australia, [10]Hospital del Mar, Barcelona, Spain, [11]University of Melbourne, Melbourne, Australia, [12]Monash University, Melbourne, Australia, [13]University of New South Wales, Sydney, Australia, [14]University of Barcelona, Barcelona, Spain, [15]Massachusetts General Hospital, Boston, MA, U.S., [16]Netherlands Cancer Institute, Amsterdam, Netherlands, [17]Stony Brook Medicine, Stoney Brook, NY, U.S., [18]SILS-CNS University of Amsterdam, Amsterdam, Netherlands

Adult hippocampal neurogenesis produces circuit-integrating neurons *de novo*, in animals implicated in cognition, mood and stress regulation. However, functional importance in humans has proven elusive in the absence of a live and non-invasive measure.Here, we first introduce an analytical advance (SVD-Bank) that allows fully automated, replicable and objective quantitation of the 1.28ppm spectroscopic signal from any 3T MR scanner, a signal that is highly enriched in neuroprecursor cells in vitro and in vivo.Using this new technology, neurogenic (hippocampal) and non-neurogenic (cortical) brain areas are clearly distinguishable in adults, and a linear decline in hippocampal signal documented across the human lifespan in a large multicentre study ($r=-0.19$, $p=0.004$, $N=164$). Age is further revealed to be an important moderator in the context of depression: 1.28ppm signal is depleted in late life depression (-55%, $p=0.038$) but not in unmedicated depression in youth (-15%, p=NS), whilst the association between antidepressant selective serotonin receptor

inhibitor medication and neurogenic signal is highly age dependent.

Strikingly, in medication-resistant depressed individuals, electroconvulsive treatment provokes a two-fold signal rise (p=0.02, N=20) within the first week of treatment. Initial 1.28ppm signal change is a promising therapeutic biomarker because it predicts structural plasticity several weeks later (r=0.7, p=0.003) as well as final clinical outcome, based on either categorical definition (odds ratio 10.6, p=0.037) or continuous depressive symptoms (r=-0.53, p=0.036). Overall, SVD-Bank analysis of the 1.28ppm signal provides compelling new evidence for the importance of hippocampal neurogenesis in human depression and treatment.

T4010

HUMAN SKELETAL MUSCLE-DERIVED STEM CELLS USE SPECIFIC INTEGRIN TO PROGRAM CELL DIFFERENTIATION ON EXTRACELLULAR MATRIX MICROENVIRONMENT VIA AUTOCRINE SECRETION OF GROWTH FACTORS

Hiyama, Taiki, Ozeki, Nobuaki, Hase, Naoko, Yamaguchi, Hideyuki, Kawai, Rie, Mogi, Makio and Nakata, Kazuhiko, University of Aichi-Gakuin, Nagoya, Japan

We established conditions for efficient conversion of α7 integrin-positive human skeletal muscle-derived stem cells ($\alpha7^+$hSMSCs) to osteogenic cell lineages (odontoblasts, osteoblasts, and chondrocytes). In the present study, the possibility of utilizing mimicking extracellular matrix (mECM) for odontoblast, osteoblast, and chondrocyte engineering using $\alpha7^+$hSMSCs was investigated. After culture for 5 days, the cell-cultured tissue culture polystyrene plates were washed twice with phosphate-buffered saline, and the cellular components were removed from the ECM by incubation with PBS containing 0.5% Triton X-100 and 20 mM NH_4OH for 5 min at 37°C, similar to a previously reported method for decellularization. Cells were cultured for 7 days on mimicking odontoblast ECM (mECM-OD), osteoblast ECM (mECM-OB), and chondrocyte ECM (mECM-CC) and the expression levels of the indicated markers were assessed by qPCR or Western blot analysis. The bone morphogenetic protein (BMP)-4, BMP-2, and transforming growth factor (TGF)-β3 protein levels in conditioned medium ($\alpha7^+$hSMSC + mECMs) were determined by corresponding ELISAs. The adhesion of differentiated and undifferentiated $\alpha7^+$hSMSCs to substrata of mECM-OD, mECM-OB, or mECM-CC were assayed in the presence of the indicated anti-integrin monoclonal antibodies. As the results, we developed a mECMs during each osteogenic culture, and decellularized these mECMs. Each osteogenic differentiation from $\alpha7^+$hSMSCs was effectively performed by culture on mECMs without addition of growth factors. Contact between stem cells and mECMs resulted in potent release of required growth factors, and led to matching differentiation. Since an anti-α7 integrin antibody, but not anti-aV or anti-aVβ3 integrin antibody, suppressed adhesion between stem cells and mECMs, and differentiation, α7 integrin is an important component for first contact with the ECM as a mechanical sensor protein. α7 integrin recognized specific profiles in mECMs, and led to suitable differentiation of osteogenic cells. These results demonstrate that human skeletal muscle stem cells determine their fate through recognition of specific ECM profiles by α7 integrin, leading to autocrine secretion of specific growth factors, and resulting in differentiation of osteogenic cells.

T4012

THE ROLE OF RGS2 AND RGS4 IN ADIPOGENIC AND OSTEOGENIC DIFFERENITATION OF HMSCS

Zhao, Yuanxiang and **Madrigal, Alma**, California State Polytechnic University at Pomona, Pomona, CA, U.S.

Human mesenchymal stem cells (hMSCs) are multipotent adult stem cells capable of giving rise to mature cell types including osteocytes, adipocytes and chondrocytes. These cells can be derived from bone marrow or adipose tissue and differentiated *in vitro* into specific mature cell types upon receiving external stimuli. Understanding the molecular mechanisms underlying hMSC differentiation into osteocytes and adipocytes is critical in the development of treatment strategies for bone- or adipose-related diseases and there are evidences supporting a cellular and systemic connection between the two. Through a microarray study comparing gene expression profiles between hMSCs undergoing adipogenesis and control cells, a number of genes whose expression was significantly up- or down-regulated during early adipogenesis were uncovered, including RGS2 and RGS4, members of the Regulators of G-protein Signaling factors family. By RT-PCR, time dependent gene expression patterns of both genes were examined at 12h, 24h, 36h, 48h, 72h, 96h, 5D, 6D, 7D and 16D post differentiation induction. RGS2 was significantly up-regulated in response to adipogenic or osteogenic induction as compared to untreated cells, with the strongest gain during the initial commitment phase (Day 1- 3). On the contrary, RGS4 expression was down-regulated throughout the entire adipogenesis process, but up-regulated by osteogenic induction that appeared to coincide with osteoblast maturation (after Day 3). Functional studies demonstrated that expression knock-down of RGS2 during the commitment phase by *siRGS2* significantly inhibited both adipogenesis and osteogenesis without affecting the total cell numbers, suggesting that RGS2 plays a positive role in both processes. Furthermore, such inhibitory effect during Adipogenesis was a result of fewer cells committing to adipogenic lineage. Similarly,

expression knock-down of RGS4 by *siRGS4* resulted in significant inhibition of adipogenesis, but such effect was a result of both reduced total cell numbers and reduced percentage of cells committing to adipogenic lineage. Our preliminary data also indicated that RGS4 played a positive role during osteogenesis. In summary, both RGS2 and RGS4 are implicated to be positive regulators during adipogenic and osteogenic differentiation of hMSCs.

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T4014

GENERATION OF FUNCTIONAL CARDIOMYOCYTES FROM THE SYNOVIOCYTES OF PATIENTS WITH RHEUMATOID ARTHRITIS VIA INDUCED PLURIPOTENT STEM CELLS

Jung, Seung Min[1], Lee, Jaecheol [2], [3]Kim, Youngkyun, [4]Yi, Hyoju, [5]Ju, Ji Hyeon, [1]Yonsei University College of Medicine, Seoul, Korea, South, [2]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, CA, U.S., [3]College of Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea, South, [4]College of Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea, South [5]Seoul St. Mary's Hospital, Seoul, Korea, South

Although cardiovascular disease is a leading cause of morbidity in rheumatoid arthritis (RA), the pathophysiologic research is still limited. This study was aimed to generate cardiomyocytes from induced pluripotent stem cells (iPSCs) of RA patients and characterize the differentiated cardiomyocytes. Using lentiviral vectors, fibroblast-like synoviocytes (FLSs) from patients with RA and osteoarthritis (OA), and dermal fibroblasts of a healthy control were successfully reprogrammed into RA-iPSCs, OA-iPSCs, and control-iPSCs, respectively. The pluripotency of iPSCs was confirmed by quantitative reverse transcriptase polymerase chain reaction and immunofluorescent staining. Established iPSCs were differentiated into cardiomyocytes by a small molecule-based monolayer differentiation protocol. Within 12 days of cardiac differentiation from patient-specific iPSCs and control iPSCs, sponatenously beating cardiomyocytes (iPSC-CMs) were observed. All iPSC-CMs exhibited reliable sarcomeric structure stained with antibodies against cardiac markers, as well as similar expression profiles of cardiac-specific genes. Intracellular calcium signaling was recorded to compare the calcium handling properties between cardiomyocytes differentiated from three different groups of iPSCs. RA-iPSC-CMs had a lower amplitude and shorter duration of calcium transients, compared to other control groups. Peak tangential stress and maximum contractile rate were also decreased in RA-iPSC-CMs, suggesting a reduced contractility. This study shows the successful generation of functional cardiomyocytes from pathogenic synovial cells in RA patients through iPSC reprogramming. Research using RA-iPSC-CMs might provide an opportunity to investigate the pathophysiology of cardiac involvement in RA.

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T4016

ASSESSMENT OF ELECTRICAL, TRANSDUCING AND MECHANICAL COMPONENTS IN SPONTANEOUSLY BEATING SYNCYTIA OF ICELLS/hiPSC-DERIVED CARDIOMYOCYTES

Zeng, Haoyu, Balasubramanian, Bharathi, Dech, Spencer, Lis, Edward, Imredy, John, Lagrutta, Armando and Sannajust, Frederick, Merck & Co., West Point, PA, U.S.

Human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CMs) syncytia have drawn wide attention as emerging, critical tools in an integrated cardiac safety evaluation of drug candidates. In this context, our objective is to systematically evaluate properties of syncytial beating in commercially available hiPSC-CMs, specifically CDI/iCells®. In this study, we used a non-invasive platform, ACEA-CardioECR, to monitor the response of iCell syncytia to several classes of pharmacological reference agents, to help interpret results with unknown compounds. When we challenged iCell syncytia with Ltype Ca^{2+} channel blockers, verapamil, nifedipine, and diltiazem, we observed the previously reported, yet atypical response of increased field potential (FP) rate and reduced impedance (IMP) amplitude (contractility-like). When we applied different pharmacological agents affecting heart rate (ivabradine, adenosine, isoproterenol, and forskolin), we noted effects corresponding to the action of each agent on its respective molecular pathway(s) (ivabradine's inhibition of I_f "funny" pacemaker current, forskolin's activation of adenylyl cyclase, isoproterenol's activation of adrenergic pathways, and adenosine's activation of adenosine-1 receptor and G_i-mediated action), but no increased in IMP amplitude was detected with forskolin or isoproterenol. We studied intracellular Ca^{2+} handing mechanisms on iCells with ryanodine, acting on the ryanodine receptor to deplete sarcoplasmic-endoplasmic reticulum Ca^{2+} stores; thapsigargin, acting on the SERCA pump; and SEA-0400, acting on the Na^+-Ca^{2+}–exchanger-1 (NCX1). We found that 3μM ryanodine only reduced FP rate but exerted no impact on IMP amplitude; 1 μM thapsigargin showed similar effects to ryanodine; and SEA-0400 only increased FP rate without impact on IMP amplitude. When we challenged iCell syncytia with the

myosin inhibitor: blebbistatin, in addition to an anticipated suppression of IMP amplitude, we observed increased FP rate in a concentration-dependent manner. In summary, our findings indicate that iCell spontaneously beating syncytia exhibit a mature phenotype in some pathways modulating FP rate and IMP amplitude reflecting contractility-like mechanics, but an immature phenotype in their intracellular Ca^{2+} handling.

T4018

E2F4 IS ESSENTIAL FOR MURINE PRE-IMPLANTATION DEVELOPMENT

Arand, Julia, Wossidlo, Mark and Sage, Julien, Stanford University, Stanford, CA, U.S.

The fusion of a sperm with an oocyte starts a complex program that results in a new organism. Human pre-implantation development is very inefficient with only 30-50% of early embryos surviving the first five days. In contrast, mouse pre-implantation development is more efficient, with multiple pups per litter. Normal developing human embryos are characterized by tight cell cycle parameters up to the 4-cell stage, where deviations correlate with arrest before the blastocyst stage. Based on this requirement for a strict cell cycle regulation, we hypothesized that members of the core cell cycle machinery may play critical roles in pre-implantation embryos. To test this idea, we analyzed the role of cell cycle factors on mouse pre-implantation development. In particular, we concentrated on retinoblastoma (Rb) pathway members, which show different expression patterns between mouse and human embryos and may explain the differences in efficiency between the two species. We found that loss of Rb function did not affect pre-implantation development in mice. In contrast, E2F4 is essential for the development beyond the 2-cell stage. E2F4 is a major repressor E2F in cells - it is usually involved in transcriptional repression. Interestingly, our data suggest that E2F4 plays an activating role in 2-cell embryos and is one of the first transcription factors needed for embryonic genome activation. Understanding essential cell cycle factors in pre-implantation development can translate into improved treatments in human reproductive medicine and into the better understanding/derivation of pluripotent stem cells for regenerative medicine.

T4020

GENERATION AND CHARACTERIZATION OF AN OLIGODENDROCYTE PROGENITOR CELL (OPC) LINE DERIVED FROM HUMAN EMBRYONIC STEM CELLS (HESC)

Du, Jing[1], Kelland, Eve[1], Ramirez, Maria[1], Cannon, Sophie[2] and Weiner, Leslie P.[1], [1]University of Southern California, Los Angeles, CA, U.S., [2]The University of Toledo, College of Medicine, Toledo, OH, U.S.

Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease characterized, in part, by demyelination and axonal loss in the central nervous system. In response to this injury oligodendrocyte progenitor cells (OPCs) are recruited to demyelinated areas and undergo differentiation and remyelination thus leading to improved neurological outcome. However for reasons that are not well understood this repair process appears to fail. In order to further the development of novel therapies that can promote protection and remyelination in MS it is important to study and understand the functions of human OPCs during MS disease pathogenesis. In this study, we have derived OPCs from the human embryonic stem cell H1 line and, using clonal expansion, created an OPC line. We characterized the physical properties and tested the functions of this line using immunocytochemistry, PCR, western blot, karyotyping, fluorescent protein labeling and in vitro cell differentiation and myelination. Our results showed that this OPC line, and a subset of these cells transduced with a fluorescent protein, all expressed the OPC markers Olig2, NG2 proteoglycan and PDGFR-alpha, as well as the immature oligodendrocyte marker O4. In addition, they expressed mRNA for the myelin proteins PLP, MBP, CNPase, MAG and OMGP. Following in vitro differentiation of these cultures there was increased expression of the oligodendrocyte markers O4, GalC, O1, Oligodendrocyte, CNPase and MBP that was accompanied with an oligodendrocyte-like branched morphology. In additional in vitro myelination experiments we demonstrate that when these OPCs were co-cultured with rat embryo spinal cord explants the OPCs differentiated to mature oligodendrocytes and interacted with spinal cord neural filaments. Immunocytochemistry and electron microscopy analysis demonstrated evidence of myelin formation. In summary we show that this OPC line is easy to maintain in culture and that the cells can be cryopreserved and cell banked. Furthermore, this OPC line is useful for the study of human OPC and oligodendrocyte development, pathology, drug screening, drug toxicity analysis, and high–content high-throughput assays to screen small molecules for neuroprotective and remyelinative actions.

T4022

DETAILED CHARACTERIZATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS MANUFACTURED FOR THERAPEUTIC APPLICATIONS

Ahmadian Baghbaderani, Behnam[1], Syama, Adhikarla[1], Sivapatham, Renuka[2], Pei, Ying[3], Mukherjee, Odity[4], Fellner, Thomas[1], Zeng, Xianmin[5] and Rao, Mahendra S.[6], [1]Lonza, Walkersville, MD, U.S., [2]Buck Institute for Research on Aging, Novato, CA, U.S., [3]XCell Science Inc, Novato, CA, U.S., [4]Centre for Brain development and Repair, Bangalore, India, [5]XCell Science, Novato, CA, U.S., [6]Q therapeutics, Salt Lake City, UT, U.S.

We have recently described manufacturing of human induced pluripotent stem cells (iPSC) master cell banks (MCB) generated by a clinically compliant process using cord blood as a starting material (Baghbaderani et al. 2015). In this manuscript, we describe the detailed characterization of the two iPSC clones generated using this process, including whole genome sequencing (WGS), microarray, and comparative genomic hybridization (aCGH) single nucleotide polymorphism (SNP) analysis. We compare their profiles with a proposed calibration material and with a reporter subclone and lines made by a similar process from different donors.We believe that iPSCs are likely to be used to make multiple clinical products. We further believe that the lines used as input material will be used at different sites and, given their immortal status, will be used for many years or even decades. Therefore, it will be important to develop assays to monitor the state of the cells and their drift in culture. We suggest that a detailed characterization of the initial status of the cells, a comparison with some calibration material and the development of reporter sublcones will help determine which set of tests will be most useful in monitoring the cells and establishing criteria for discarding a line.

T4024

USING EXTENDED LIVE CELL TIME-LAPSE IMAGING TECHNOLOGY FOR DISEASE PHENOTYPES AND SURVIVAL ANALYSIS OF HUMAN ES AND IPS-DERIVED NEURONS

Shin, Hye Young[1], Pfaff, Kathleen Lindahl[1], Davidow, Lance[1], Sun, Chicheng[1], Uozumi, Takayuki[2], Kiyota, Yasujiro[2] and Rubin, Lee L.[1], [1]Harvard University, Cambridge, MA, U.S., [2]Nikon Corporation, Tokyo, Japan

The ability to generate neurons from human patient stem cells has brought about new possibilities for how neurodegenerative diseases can be modeled and interrogated for drug discovery. We are developing live cell imaging assays using the Nikon BioStation CT to uncover morphological changes that accompany motor neuron (MN) differentiation, stress responses, and death. In developing this platform, we have tested several different embryonic stem cell lines that express MN lineage reporters and compared the survival of wild-type vs. Type I Spinal Muscular Atrophy patient-derived MNs. The BioStation CT is capable of performing long-term imaging (up to several weeks) and utilizes multifactorial imaging analysis algorithms paired with robust cell tracking technology. Key attributes of individual MNs including cell body size, neurite number and neurite length of individual neurons are tracked following neurotrophic withdrawal and recovery. These analyses uncovered an informative metric of neuron health by detecting early changes in neurites and the number of neurite-cell body junctions, or nodes. We find that more mature neurons, as defined by these criteria, exhibit better survival in stress conditions. We are initiating studies to examine other disease-relevant neuron populations by live cell imaging analysis, including dopaminergic neurons (affected in Parkinson's Disease) and cortical neurons (affected in schizophrenia, autism and Alzheimer's Disease). These methods may be useful in uncovering new classes of therapeutic molecules with potential for intervening early in the progression of these diseases.

Funding Source: Nikon, Harvard Stem Cell Institute NINDS P01NS066888

T4026

HUMAN IPSC-DERIVED NEURAL CREST CELLS PROMOTE SCARLESS WOUND HEALING ON MOUSE CORNEA.

Yoshida, Satoru, Yamazoe, Katsuya, Yasuda, Miyuki, Tsubota, Kazuo and Shimmura, Shigeto, Keio University School of Medicine, Tokyo, Japan

Neural crest cells (NCCs) have the ability to differentiate into various types of cells and contribute to various tissues including nervous system, connective tissues, pigment cells, cartilage and bone. We took notice of the multipotency of NCCs and considered the possibility that NCCs are involved in scarless fetal wound healing. To address the role of NCCs in scarless wound healing, we employed induced pluripotent stem cells (iPSCs)-derived NCCs and alkaline burned model of mouse cornea. The iPSC-derived NCCs were obtained by dual Smad inhibition method, and the cells were injected into injured mouse cornea. We found that iPSC-derived NCCs injection into injured mouse cornea significantly reduced the scar formation due to injury compared with fibroblast injection. Cytokine array analysis revealed that the expression patterns of cytokines in iPSC- derived NCCs correspond to that in fetal wound healing. In particular, low expression levels of inflammatory cytokines IL-6 and IL-8, and high expression levels of macrophage migration inhibitory factor (MIF)

and FGF4 were found. Immunohistochemical analysis showed that infiltration of macrophages into injured corneal stroma was repressed by iPSC- derived NCCs injection. Theses results suggest that scarless wound healing by iPSC- derived NCCs was caused by anti-inflammatory cytokines.

Funding Source: This work was supported by JSPS KAKENHI Grant Number 80574912 and Highway Program for Realization of Regenerative Medicine from AMED.

T4028

KRT8-BASED LINEAGE TRACING REVEALS LONG-LIVED EPITHELIAL CELLS IN MURINE FALLOPIAN TUBE AND TARGETS LGR5+ STEM CELLS IN OVARIAN SURFACE EPITHELIUM

Park, Eunsil and **Li, Zhe**, Brigham & Women's Hospital and Harvard Medical School, Boston, MA, U.S.

Epithelial ovarian cancer (EOC) is the most lethal malignancy of the female reproductive system, largely due to the fact that most EOCs are diagnosed at advanced stages. Thus, determination of the cellular origin of EOC is important for better diagnosis, treatment and prevention of this malignancy. Both ovarian surface epithelial (OSE) and fallopian tubal epithelial (FTE) cells have been proposed as cells of origin of EOCs. However, whether and how these epithelial systems are sustained by long-lived stem/progenitor cells and whether such cells are targets of transformation remain poorly understood. Recently, a population of LGR5+ cells in the murine ovarian surface epithelium (OSE) was identified. Importantly, these LGR5+ OSE cells have been implicated as stem cells to sustain the OSE lineage and as the cellular origin of EOC. In fallopian tube, although a small population of LGR5+ cells was also observed, it was reported that the adult fallopian tube epithelium (FTE) was not maintained by LGR5+ cells, thus raising a question as to whether the adult FTE contains other stem/progenitor cells to sustain this lineage. Keratin 8 (K8, encoded by *Krt8*) is a keratin marker expressed in FTE and some OSE cells. By co-immunofluorescent staining, we found that in OSE, K8+ cells largely overlapped with LGR5+ cells. Intrabursal injection of Cre-expressing adenovirus under the control of Krt8 promoter *(*Ad-K8-Cre*)* to conditional Cre-reporter mouse lines, Rosa26-LSL-YFP *(*R26Y*)* or Rosa26-LSL-tdTomato *(*R26tdT*)*, revealed that Ad-K8-Cre could target both OSE and FTE cells. By intrabursal injection of *Ad-K8-Cre* to mice carrying R26tdT and an Lgr5-GFP reporter, we found that most tdTomato+ OSE cells were also GFP+, suggesting that *Ad-K8-Cre* could target LGR5+ OSE stem cells genetically. Since Ad-K8-Cre only leads to transient Cre expression in K8+ cells, we used it to perform pulse/chase lineage-tracing studies. Upon long-term chases (up to 6 months), we found both FTE and OSE contained long-lived cells to sustain these epithelial lineages. This observation was confirmed by another lineage-tracing approach based on the K8-CreER transgenic mouse line (upon tamoxifen induction). Currently, we are targeting K8+ OSE and FTE cells by Ad-K8-Cre injection to model EOC development.

Funding Source: This research was supported by DOD W81XWH-14-1-0280.

T4030

QUANTIFICATION OF DOPAMINERGIC NEURON DIFFERENTIATION AND NEUROTOXICITY VIA A GENETIC REPORTER

Cui, Jun[1], Rothstein, Megan[1], Bennett, Theo[1], Zhang, Pengbo[2], Xia, Ninuo[1] and Reijo Pera, Renee A.[1], [1]Montana State University, Bozeman, MT, U.S., [2]Stanford University, Stanford, CA, U.S.

Human pluripotent stem cells provide a powerful human-genome based system for modeling human diseases in vitro and for potentially identifying novel treatments. Directed differentiation of pluripotent stem cells produces many specific cell types including dopaminergic neurons. Here, we generated a genetic reporter assay in pluripotent stem cells using newly-developed genome editing technologies in order to monitor differentiation efficiency and compare dopaminergic neuron survival under different conditions. We show that insertion of a luciferase reporter gene into the endogenous tyrosine hydroxylase (TH) locus enables rapid and easy quantification of dopaminergic neurons in cell culture throughout the entire differentiation process. Moreover, we demonstrate that the cellular assay is effective in assessing neuron response to different cytotoxic chemicals and is able to be scaled for high throughput applications. These results suggest that stem cell-derived terminal cell types can provide an alternative to traditional immortal cell lines or primary cells as a quantitative cellular model for toxin evaluation and drug discovery.

T4032

DEVELOPMENT OF AUTOMATED CELL CULTURE PROCESS FOR IPS CELLS

Saito, Hikaru[1], Kato, Midori[1], Kiyama, Masaharu[1], Sekiya, Sayaka[2], Yoshikawa, Manabu[2], Yoshida, Kenji[2], Kishino, Akiyoshi[2], Kimura, Toru[2], Takahashi, Jun[3] and Takeda, Shizu[1], [1]Research & Development Group, Hitachi Ltd., Hatoyama, Japan, [2]Regenerative & Cellular Medicine Office, Sumitomo Dainippon Pharma Co., Ltd., Kobe, Japan, [3]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

Induced pluripotent stem (iPS) cells have the ability to self-renew and differentiate into all types of cells. These cells are considered to be cell sources for use in regenera-

tive medicine, and techniques for treating a wide range of diseases are being developed. However, cost reduction of cell production and maintenance of sterility are the main issues to the spread of regenerative medicine. An automated closed cell culture system is considered to be a platform that can be used to address these two challenges. We have been developing an automated closed cell culture system that can maintain sterility. The purpose of this study was the development of mass-production processes using closed cell culture system for iPS cells. The quality of iPS cells cultured in closed system was compared with that of manually cultured cells (open system). The effect of closed culture and cell seeding through a tube on cell quality was also analyzed in this study. As the results, the expression levels of pluripotent markers SSEA-4 and TRA-1 in the closed system were equal to those in the open system. Next, iPS cells were differentiated to dopaminergic progenitors using closed system and verified the quality of cells. Floor plate marker CORIN expression rate was between 12.7-58.6%, which met the criteria. In addition, the effect of cell seeding through tube on the quality of cells was analyzed because cells suffer from shear stress when they pass through the tube. After being fed through the tube, the cells were cultivated for one week, then the growth rate and pluripotent markers were analyzed. As the results, cell seeding through the tube did not affect the cell growth rate and pluripotent marker gene expressions. The above results indicate that a closed cell culture system can be applied to the production of iPS cells. Thus, the basis for the mass-production processes of iPS cells was determined.

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T4034

FAR-INFRARED IRRADIATION INCREASES THE OSTEOGENIC DIFFERENTIATION OF HUMAN TONSIL-DERIVED MESENCHYMAL STEM CELLS

Kim, Ha Yeong[1], Yu, Yeonsil[1], Kim, Han Su[2] and Jo, Inho[1], [1]Ewha Womans University School of Medicine, Seoul, Korea, [2]Ewha Womans University School of Medicine, Department of Otorhinolaryngology-Head & Neck Surgery, Korea

Far-infrared (FIR) irradiation has a variety of biological effects including the improvement of vascular function and the inhibition of cancer cell proliferation. However, the role for FIR irradiation in osteogenesis and its underlying mechanism are yet to be fully understood. Using human tonsil-derived mesenchymal stem cells (TMSC), we investigated whether FIR plays a role in regulating the osteogenic differentiation. TMSC were acutely exposed to FIR irradiation (3 – 25 μm wavelength) at room temperature for 0, 15, 30, and 60 min, and further incubated in the culture chamber (at 37 °C under 5% CO_2) for up to 48 h. To investigate osteogenic potential, TMSC after acute exposure to FIR irradiation were cultured in osteogenic differentiation medium for up to 14 days. Expression levels of bone-specific markers including osteocalcin and alkaline phosphatase were investigated using Western blot analysis. Our results showed that FIR irradiation and further incubation in CO_2 chamber for up to 48 h increased protein expression of osteocalcin in a dose- and time-dependent manner; the maximal increase was found for 60-min FIR irradiation and further 24-h incubation. However, the level of alkaline phosphatase expression was not altered under this condition. Calcium chelating compounds (BAPTA-AM and EGTA) reversed the stimulatory effect of FIR irradiation on osteocalcin expression. After 14-day incubation with osteogeinc differentiation medium, significantly higher levels of osteocalcin expression and calcium deposition, as measured by Alizarin Red S staining, were found in FIR-irradiated TMSC compared with unirradiated control TMSC. This study suggests a possibility of a role for FIR irradiation in enhancing osteogenic differentiation of TMSC.

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T4036

GENERATING HUMAN IPSC-DERIVED CHOROIDAL ENDOTHELIUM TO STUDY MACULAR DEGENERATION.

Songstad, Allison Elaine, Worthington, Kristan S., Chirco, Kathleen R., Giacalone, Joseph C., Burnight, Erin R., Whitmore, S. Scott, Riker, Megan J., Stone, Edwin M., Mullins, Robert F. and Tucker, Budd A., University of Iowa Carver College of Medicine, Iowa City, IA, U.S.

Age-related macular degeneration (AMD), the most common form of irreversible blindness in developed countries, involves dysfunction of choroidal endothelial cells (CEC), retinal pigment epithelial cells (RPE), and photoreceptor cells (PRs). While human induced pluripotent stem cell (iPSC)-based strategies to replace PRs and RPE are a major scientific focus, success of these approaches may also require the replacement of lost CECs, which degenerate early in AMD pathogenesis. To that end, we designed a novel stepwise differentiation protocol to generate iPSC-CECs. Fibroblasts from a donor with normal ocular history were isolated from a skin biopsy and reprogrammed into iPSCs via transduction with a non-integrating Sendai virus driving expression of *OCT4, SOX2, KLF4*, and *c-MYC*. Pluripotency was assessed via rt-PCR, TaqMan Scorecard Assay, and immunocytochemistry (ICC). RNA-seq analysis of cultured monkey RF/6A CECs identified

seven secreted proteins known to be involved in vascular development. Using a Taguchi statistical screening strategy, media were developed using combinations of the seven proteins to determine each factor's effect on CEC differentiation. In a separate strategy, each protein was excluded one at a time from media containing all other proteins of interest to identify those that were necessary for CEC differentiation. Both methods showed that iPSCs cultured in media containing CTGF and TWEAKR differentiated into CECs at a higher percentage than those cultured with other media combinations. The resulting iPSC-CECs expressed the EC-specific markers CD31, CD34, FOXA2, ICAM1, TIE2, and vWF, and the CEC-restricted markers CA4 and TTR. CTGF was necessary to drive CEC differentiation, whereas TWEAKR enhanced CEC differentiation by inducing endogenous CTGF secretion. Conversely, CTNNB1, PDPN, RTN4, SHC1, and VEGFB did not play a role in CEC differentiation. The iPSC-CECs fed CTGF alone formed vascular tube networks morphologically identical to those formed in primary human CEC cultures. Now, this newly established differentiation method is being used to generate CECs from AMD patient-specific iPSCs. In addition to being useful for cell replacement therapy, patient-specific CECs, in combination with RPE and PRs, will be invaluable to the accurate interrogation of AMD pathophysiology.

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T4038

INVESTIGATING THE FUNCTION OF NOVEL NOTOPHTHALMUS VIRIDESCENS GENES USING DROSOPHILA MELANOGASTER AS ANIMAL MODEL

Mehta, Abijeet Singh[1], Madrigal, Agustin[2], Singh, Amit[1] and Tsonis, Panagiotis[1], [1]University of Dayton, Dayton, OH, U.S., [2]Miami University, Oxford, OH, U.S.

Notophthalmus viridescens (Red-spotted newt) possess amazing capabilities to regenerate their organs including tail, limb, heart, brain, spinal cord, lens and other tissues. We and our colleagues using a de novo assembly of the newt transcriptome combined with proteomic validation have identified a novel family of proteins expressed in adult tissues and during regeneration in newts. These proteins, some having new sequence motifs, have no counterparts in public databases. The presence of a putative signal peptide suggests that all these proteins could be secreted. To obtain a quick glimpse into possible function of some of these newt-specific genes (and given certain restrains with transgenic newts, such as time) we have attempted to express these genes using transgenic Drosophila melanogaster. We generated the transgenic flies containing candidate genes, and tried to evaluate their potential to rescue pattern defect mutants of Drosophila melanogaster. Simultaneously, we prepared samples for RNA sequencing to evaluate the role of candidate genes in tissue development. Using transgenic approach, these candidate genes were expressed in all the tissues of Drosophila, and samples for sequencing were collected at third instar larval (L3) stage. Interestingly, these newt-specific candidate genes have shown significant regulation of important eye and retinal developmental genes including, Pax-6 (ey), Eyes absent (eya), Sine oculus (six-4), Dachshund (dac), and Twin of eyeless toy (toy). In future we will test the regenerating potential of these newly identified genes in newt tissues as well as in other animals. The results from these studies will be presented.

Funding Source: National Institute of Health.

T4040

CELLULAR AND MOLECULAR MECHANISMS DURING SELF-ORGANIZATION OF MOUSE SKIN PROGENITOR CELLS INTO RECONSTITUTED HAIRY SKIN

Lei, Mingxing[1,2], Schumacher, Linus[3], Lai, Yung-Chi[1], Juan, Wen-Tau[1], Yeh, Chao-Yuan[1], Wu, Ping[1], Jiang, Ting-Xin[1], Baker, Ruth[1], Widelitz, Randall[1], Yang, Li[2] and **Chuong, Cheng-Ming**[1,4], [1]University of Southern California, Los Angeles, CA, U.S., [2]Chongqing University, Chongqing, China, [3]University of Oxford, Oxford, U.K., [4]China Medical University, Taichung, Taiwan

The transformation process from dissociated multipotent stem or progenitor cells into an organized tissue is a poorly understood and yet fundamental issue in regenerative medicine. Previously we developed a planar hair forming procedure that forms hairs robustly on the back of the nude mice. However, due to its in vivo nature, we do not know the cellular events during this morphogenetic process. To explore this process, we now developed an in vitro transwell 3-dimensional mixed culture, in which dissociated newborn mouse epidermal and dermal cells can self-organize to form planar hairy skin. With more than 500 time-lapse movie segments, to our surprise, we found dissociated cells go through a complex morphogenetic detour to become a planar skin, from small aggregates – dermal / epidermal cysts– fused cysts –hair-bearing planar skin. Collective cell behavior analyses showed a series of morphological phase transition. To analyze key molecular events between transitions of different phases, we use transcriptome analyses to profiling molecular expression at different time points. Non-biased clustering of molecular profiling data categorizes them into different phases. In the early phase, adhesion molecules, and morphogens such as PDGF, insulin-like growth factors are more involved. In the late phase, Wnts and extracellular matrix molecules are more involved. Functional perturbations show timely physical-chemical coupling of these molecular events with phase transition are essential for

tissue self-organization. When transplanted to the backs of nude mice, these reconstituted cultured skin grafts robustly formed hairs. While cells from adult mice do not form hairs easily, by comparing transcriptome analyses and cellular events, we made progress toward making adult cells to form new hairs. The results demonstrate the self-organizing process from dissociated progenitor cells to planar reconstituted skin with newly induced hair follicles. The in vitro model set up the platform to further analyze molecular process in detail, and can help us identify molecules that favor hair formation.

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T4042

THE GRAFTING OF MSC-SEEDED TISSUE ENGINEERED CONDUITS IN THE LEFT PULMONARY ARTERY OF NEWBORN PIGLETS SHOWS INTEGRATION AND PATENCY BUT VARIED DEGREE OF CALCIFICATION

Swim, Megan, Iacobazzi, Dominga, Zebele, Carlo, Albertario, Ambra, Holopherne-Doran, Delphine, Caputo, Massimo and Ghorbel, Mohamed, University of Bristol, Bristol, U.K.

Vascular repairs for children born with congenital heart defects often require replacement due to the lack of growth potential of the artificial structure. Autologous stem cell tissue-engineered grafts provide alternatives to current materials because they have the potential to grow, repair, and remodel. Non-differentiated stem cells were seeded on a scaffold and implanted into a piglet model to investigate their ability to integrate and acquire vessel cells-like phenotype. Stem cells were isolated from newborn piglet's thymus and expanded in vitro. Assessment of cell surface markers and functional characterization demonstrated the Mesenchymal lineage of thymus-derived stem cells. Mesenchymal stem cells (MSC) were seeded on commercially available and clinically used acellular porcine small intestine submucosa, shaped into conduits and grown in a bioreactor. In vitro examination of the resulting graft showed cell growth, engraftment and viability. Grafts were then tested in vivo by implantation into the left pulmonary artery of 12 kg piglets. All piglets recovered from surgery and grew at normal rate. At 3 months, piglets underwent echocardiography prior to termination. Echocardiograms showed graft patency. However, in some cases increased velocity was observed suggesting a narrowing. Inspection of the explanted grafts by histology demonstrated the integration of the graft within the neighboring structures. The histological stain von Kossa showed some evidence of calcification, which in one case was quite considerable. Scanning electron micrographs showed a layer of cells on the luminal side of the graft similar to the native neighboring artery. Immunohistochemistry using an endothelial cell marker demonstrated endothelialization of the luminal side of the graft. Immunostaining using smooth muscle cell (SMC) marker exhibited a multi-layer of smooth muscle-like cells indicating the development of a vessel-like phenotype. In conclusion, tissue-engineered grafts seeded with non-differentiated MSCs integrate within surrounding native tissue following in vivo implantation. However, evidence of calcification suggests that perhaps the non-differentiated cells should be differentiated into SMC-phenotype prior to implantation.

Funding Source: The Sir Jules Thorn Charitable TrustThe Enid Linder Foundation

T4044

RETINOID SIGNALING AND MIR-21 INVOLVEMENT IN REGENERATING NEWT CAUDAL SPINAL CORDS

Haj-Ahmad, Lila[1], Lepp, Amanda[1], Wlodarek, Lukasz[1], Rozema, David[2] and Carlone, Robert Leo[1], [1]Brock University, St. Catharines, ON, Canada, [2]McGill University, Montreal, QC, Canada

Adult urodele amphibians possess the ability to regenerate lost structures, including caudal spinal cord, following tail amputation. Ependymoglial cells, a stem cell population lining the central canal of the spinal cord in this species, are essential contributors to the formation of new axons and glial cells in this process. We have previously shown that this ependymal cell outgrowth and spinal cord regeneration are dependent upon retinoid signaling via the retinoic acid receptor beta (RARβ) subtype. Bath application of an inhibitor of retinoic acid synthesis (DEAB) or a selective antagonist of RARβ, LE-135, completely inhibit caudal spinal cord regeneration. To further ascertain the complex regulatory mechanisms underlying retinoid signaling in these cells, we identified, by a microarray, 61 microRNAs that were differentially synthesized in regenerates in which RA signaling had been inhibited with DEAB. In this study, we focused our attention on miR-21, which had previously been shown to be involved in limb regeneration in the axolotl by targeting *Jagged1*, a Notch ligand. Analysis of expression of both miR-21 by qPCR and Jagged-1 protein, by Western blotting, showed a reciprocal relationship, with miR-21 peaking between 21-35 days post amputation (dpa), during the redifferentiation phase of tail regeneration. This was balanced by a gradual decline in Jagged-1 protein. Injection and electroporation of a miR-21 mimic into ependymoglial cells early after tail amputation (0 and 2 dpa) significantly inhibited spinal cord outgrowth, accompanied by a decline in BrdU incorporation into ependymoglial cells and a decrease in Jagged-1 protein between 7-14 dpa in this tissue. Our results

highlight some of the complex interactions between various signaling pathways required to reconstruct the tail and spinal cord in a regeneration-competent vertebrate.

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T4046

AMNIOTIC FLUID COLLECTIONS FROM SCHEDULED C-SECTION DELIVERIES FOR CLINICAL APPLICATIONS

Jacobson, Pam, Pierce, Jan, Phibbs, Jessica, Benedetti, Eric, Preslar, Amber and Reems, Jo-Anna, University of Utah Cell Therapy and Regenerative Medicine Facility, Salt Lake City, UT, U.S.

Amniotic fluid is rich in nutrients and cytokines and possesses anti-inflammatory, anti-microbial and regenerative properties that make it attractive for clinical applications. The goals of this study were to assess the feasibility of collecting AF from full-term pregnancies and to characterize the AF and its cellular compartment for clinical applications. Donor informed consent, medical history and infectious disease testing were obtained from pregnant women scheduled for C-sections. AFs collected at C-section were evaluated for volumes, fluid chemistries, total protein, hyaluronic acid (HA) levels, cellular content and an ability to support angiogenesis. Cytokine composition of post-processed AF was also assessed using quantitative antibody array panels. The resulting cellular pellet from centrifugation of the fluid was cultured for the identification and characterization of a potential “stem-like” population.

During approximately a one year period, 62 individuals were approached for donation, 34 agreed to donate birth tissue and AF was successfully collected from 17 individuals. The median volume of AF was 70 mL (range: 10- 815 mL; n=17). HA levels averaged 311±75 ng/mL. Cytokine arrays revealed that an average of 304±20 of 400 proteins were present in AF with a majority of the cytokines associated with immunological defense. Fluid chemistries were similar among donors, but HA levels and cytokine profiles showed donor variability. The cellular elements of AF consisted predominately of epithelioid cells with minor populations of lymphoid cells and a plastic adherent, highly proliferative population of cells that produced therapeutic doses of mesenchymal stromal cells with tri-lineage differentiation potential.These findings indicate that AF can be routinely collected from full-term births and processed for storage. AF, a rich source of HA and cytokines, is associated with donor variability and requires further investigation to fully characterize these components for specific clinical applications.

T4048

COMPUTER-ASSISTED SCORING METHOD FOR HUMAN INDUCED PLURIPOTENT STEM CELL COLONY CHARACTERIZATION AND SELECTION

Lai, Hoyin[1], Kenyon, Zakary[1], Collins, Nicholas[2], Alworth, Samuel V.[1], Piloco, Louis[1], Daheron, Laurence M.[2,3] and Lee, James S.J.[1], [1]DRVision Technologies LCC, Bellevue, WA, U.S., [2]Harvard University, Cambridge, MA, U.S., [3]Harvard Stem Cell Institute, Cambridge, MA, U.S.

Induced pluripotent stem cells (iPSC) have become increasingly adopted for disease modeling, and have the potential to become a source of tissue for regenerative medicine. However, picking fully reprogrammed iPSC colonies can be unreliable, costly, and time consuming. 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.

We have created an iPSC scoring method to automate the scoring of reprogrammed colonies from label-free phase contrast images. To ascertain reliable scoring criteria on various imaging systems, we have developed innovative image normalization and robust measurement approaches to complement the scoring method. The image normalization routine uses structure guided image processing, which detects the image background automatically adjusts the image contrast to make images more similar. We have included robust measurements that are less sensitive to noise, and also self-normalized to reduce errors from inter-sample variability. The colony scoring algorithm is deployed in an analysis package called Colony Analyzer as part of the SVCell RS image analysis software (DRVision Technologies, Bellevue, WA). We used the SVCell RS software to characterize differences between patients in terms of reprogramming efficiency, and validate the proliferation of fibroblasts and reprogrammed colonies. In this study, fibroblast samples from healthy donors and several different types of disease patients were subject to Sendai virus-mediated reprogramming with Klf4, Oct3/4, Sox2 and c-Myc. Colonies were imaged on the Nikon BioStation CT, Thermo Fisher Scientific EVOS, and the Olympus IX71 and stored in an extensive image database. Here we show that SVCell software can be used to calculate the score for each colony, and visualize the score value and colonies in the image to intuitively assess the correspondence of the scores with quantitative morphological metrics and known gene expression and pluripotency data. The results show that Colony Analyzer package quantitatively characterizes iPSC colonies from

different microscopes, and select the best colonies for expansion.

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T4050

INVESTIGATION OF A GENETIC MODIFIER IDENTIFIED BY WHOLE GENOME SEQUENCING OF HUNTINGTON'S DISEASE PATIENTS IN INDUCED PLURIPOTENT STEM CELLS

Kaye, Julia A.[1], Dunlap, Mariah[2], Levén May, Hanna[3], Holloway, Alisha[4,5], Wyman, Stacia[6], Li, Hong[7], Roach, Jared[8] and Finkbeiner, Steven[6,9], [1]J David Gladstone Institute, San Francisco, CA, U.S., [2]Gladstone Institute for Neurological Disease, San Francisco, CA, U.S., [3]Umeå University, Umeå, Sweden, [4]Phylos Bioscience, Portland, CA, U.S., [5]University of California San Francisco, San Francisco, CA, U.S., [6]Gladstone Institutes, San Francisco, CA, U.S., [7]MPG Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, CA, U.S., [8]Institute for Systems Biology, Seattle, CA, U.S., [9]Taube/Koret Center for Neurodegenerative Disease and the Hellman Family Foundation Program in Alzheimer's Disease Research, San Francsico, CA, U.S.

While mutant huntingtin (mHtt) causes Huntington's disease (HD), other genes likely contribute to mHtt-induced neurodegeneration and disease onset. Our goal has been to identify genetic modifiers of age of onset of HD. Identifying HD genetic modifiers and determining their mechanisms of action can yield new insights into the disease process and may provide better druggable molecular targets for new HD therapeutics. We recently identified a number of novel potential genetic modifiers of HD using whole-genome analysis (WGA) of HD families. We observed the segregation pattern of variants in the genome from one generation of individual families to the next and tested for association with changes in HD symptoms. We found numerous putative modifiers. From one family with members with unusually late onset we identified thirteen variants associated with genes involved in proteasome function. Genetic variants in protein clearance pathways are potentially significant with regard to HD because the mechanisms that remove misfolded toxic mHtt, from neurons depend partly on proteasome activity. As the variants we identified are selectively expressed in late-onset HD patients, they may enhance mHtt clearance. In particular, we found a variant in the H3K27 acetylation site of a promoter of a gene that stimulates expression of the proteasome. We sought to investigate if this modifier's mechanism of action can slow neurodegeneration by increasing clearance of mHtt. To do this, we used a unique human neuron model of HD we developed from patient-derived induced pluripotent stem cells (iPSCs) that recapitulates key disease-relevant phenotypes. We monitored the effect of the potential genetic modifier on degeneration of the HD iPSC–derived neurons (i-neurons) by robotic microscopy (RM), an optical, high-throughput, high-content phenotyping technology that monitors gradual neurodegeneration and other cellular properties over time. We will report on our findings that show the use of a human iPSC-based neuronal model of HD as a validation platform and as a system to investigate mechanisms by which variants may affect mHtt-induced neurodegeneration. For the first time, we combine data from WGS studies of HD patients to produce candidates to test for effects on the progression of degeneration of HD patient human i-neurons.

T4052

STEM CELL BASED DRUG DISCOVERY IN GLYCOGEN STORAGE DISEASE 1

Lee, Jun Hyeog[1], Choi, Dong Kyu[2], Choi, Jae-Heon[2], Min, Sang Hyun[2] and Lee, Dong-Seok[3], [1]Kyungpook National University School of Life Science, Daegu, Korea, South, [2]DGMIF, DaeGu, Korea, South, [3]Kyungpook National University, School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Daegu, Korea

Glycogen storage disease type I (GSD I, also von Gierke's disease) is an inherited disorder which result from defects in the processing of glycogen and its storage. Generally, GSD has an incidence in the American population of approximately 1 in 50,000 to 100,000 births. When a person has GSD, a liver cannot control the use of glycogen or glucose and an abnormal amount of glycogen is stored in the liver. These abnormal glycogen storage lead critical metabolic disorders such as hyperglycemia, low insulin level and high glucose level in liver include lactic acidosis and hyperlipidemia. Unfortunately, there is no specific cure for GSD. High content screening is a phenotypic screening methods that is used for identifying effective substances in drug discovery. Compared with previous screening system, In HCS, cells are incubated with substances and structures and molecular components of the cells are automatically analyzed. It has advantages of simplicity, objectivity, and its high speed and, therefore, a large number of data points can be collected per cell and analyzed. To use HCS for our GSD drug-screening project, we set up GSD screening system based on lipid accumulation in the cells. As a positive control, we differentiated 3T3-L1 cells to adipocytes and stained its lipid contents using Bodipy dye. And its lipid content was successfully digitized using HCS. To use distinct advantages of patient derived hiPSC for use HCS, we established efficient iPS induction system using episomal vectors. We acquired fibroblast cells from

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GSD carrying 25 years old Caucasian male patient and generated iPS successfully. And we are establishing its differentiation toward hepatocyte for further screening.

After differentiation is established, we are planning to screen compounds from FDA approved compound library, which expected to decrease lipid accumulation of differentiated hepatocyte.

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T4054

EFFECTS OF RADIATION DOSE RESPONSE ON CELLULAR DYNAMICS IN SMALL AND LARGE INTESTINAL CRYPTSSE RESPONSE ON CELLULAR DYNAMICS IN SMALL AND LARGE INTESTINAL CRYPTS

Otsuka, Kensuke[1], Fujimichi, Yuki[1], Tomita, Masanori[1], Suzuki, Keiji[2] and Iwasaki, Toshiyasu[1], [1]Central Research Institute of Electric Power Industry, Tokyo, Japan, [2]Nagasaki University, Nagasaki, Japan

The protection of intestinal epithelial cells from the lethal effects induced by high-dose radiation is an important issue in radiotherapy and the treatment of acute radiation syndrome. However, the effects of middle or low-dose radiation on intestinal epithelial cells remain unknown. The accumulation of DNA damage in the intestinal stem cells may be crucial for the development of cancer initiating cells, and therefore, it is important to understand the kinetics of DNA repair and tissue response, which is involved in the elimination of damaged cells and tissue injury repair, in response to middle to low doses of radiation. In the present study, mice were exposed to different doses (0.1, 1, or 4 Gy) of X-rays, and the small intestine (duodenum and ileum) and colon were obtained from the mice after the X-ray treatments. DNA damage repair and the elimination of damaged cells were quantified by measuring the number of foci of 53BP1, a surrogate marker for DNA double strand breaks. Tissue proliferative response was evaluated by determining the number of Ki-67$^+$ and mitotic cells. Intra-crypt response was shown to be considerably different in the small intestine and colon. In the small intestine, 53BP1 foci were detected immediately after irradiation, but rapidly disappeared afterward, which was especially noticeable in Lgr5$^+$ stem cells. Cellular growth was temporally arrested, but the number of cells and mitotic cells in the crypt did not change. The kinetics of DNA damage repair in Lgr5$^+$ stem cells was shown to be similar to the small intestines, while the colon was more susceptible to radiation damage. Preferential cell loss in the lower crypt was obvious in the colon, and after low-dose X-irradiation, only colon showed considerable reduction of cell numbers and a dramatic induction of mitotic cells. Furthermore, we evaluated the cell cycle of colonic Lgr5$^+$ stem cells using flow cytometric ethynyl-deoxyuridine (EdU) / phosphorylated-Histone H3 (PH3) staining and found that it was affected by middle dose of radiation. These results suggest that dysregulation of colonic cells may be an important endpoint for considering radiation-induced cancer development.

T4056

PROPAGATION OF THE EARLY MURINE INNER CELL MASS STATE N CELL CULTURE

Treier, Mathias[1], Yin, Xiushan[2], Klasen, Christian[2], Roma, Guglielmo[3], Block, Franziska[2], Brouwer-Lehmitz, Antje[2], Treier, Anna-Corina[2], Salmon-Divon, Mali[4], Beibel, Martin[3], Anastassiadis, Konstantinos[5] and Bouwmeester, Tewis[3], [1]Max-Delbrück Center for Molecular Medicine (MDC), Berlin, Germany, [2]Max-Delbrück Center for Molecular Medicine (MDC) in the Helmholtz Association, Berlin, Germany, [3]Novartis Institutes for Biomedical Research (NIBR), Basel, Switzerland, [4]Ariel University, Ariel, Israel, [5]Technische University Dresden, Dresden, Germany

Mammalian reproduction out of cell culture would either require the propagation of functional germ cells, totipotent embryonic stem cells or the pre-implantation blastocyst state in vitro. Pluripotent murine embryonic stem cells (mESCs) however can only contribute to the embryo proper, extra-embryonic mesoderm and amnion of the mammalian embryo. In contrast cells of the inner cell mass are in addition able to contribute to primitive endoderm. We have previously shown that SALL4 is essential for epiblast formation and the establishment of mESCs in vitro. Delineation of the SALL4 proteomic interactome in ESCs has revealed beside established stem cell players functionally poorly characterized factors. To establish their expression pattern in vivo we generated GFP knock-in fusion proteins for most of them identifying one that is prominently expressed in the epiblast of the mature murine blastocyst. Gene knockout studies demonstrate that this factor is not essential for mouse development or reproduction. Unexpectedly, from this mouse strain it is possible to propagate the inner cell mass state in vitro which so far has not been possible. We furthermore show that conventional mESCs can acquire inner cell mass properties upon loss of this factor. Using an integrated omics approach we will present the molecular mechanism that explains why propagation of the inner cell mass state from this mouse strain is possible in vitro. Importantly, these ICM-like state cells can be co-cultured with trophoblast stem cells which for the first time allows now to co-culture all cell lineages of the pre-implantation embryo opening the door further for mammalian reproduction out of a dish.

FRIDAY

POSTER SESSION III ODD

PRE-CLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL CELLS

F1001

CONDITIONED MEDIUM OBTAINED FROM AMNION-DERIVED MESENCHYMAL STEM CELL CULTURE PREVENTS ESOPHAGEAL STRICTURE AFTER ENDOSCOPIC SUBMUCOSAL DISSECTION IN PIGS

Ohnishi, Shunsuke, Mizushima, Takeshi, Hosono, Hidetaka, Tsuda, Momoko, Onishi, Reizo and Sakamoto, Naoya, Hokkaido Univeristy, Sapporo, Japan

Endoscopic submucosal dissection (ESD) for esophageal cancer has been widely accepted in last decade; however, it often causes postoperative stricture when over three fourths the circumference of the esophagus is dissected, and lowers quality of life for patients. Although steroid and balloon dilatation is generally used to prevent the stricture, side effects and complications are of concern. Mesenchymal stem cells (MSCs) have been reported to be a valuable cell source in regenerative medicine, and large amounts of MSCs can be noninvasively isolated from human amnion, which is discarded after delivery. Moreover, conditioned medium (CM) obtained from MSCs has been reported to inhibit inflammation in several animal models. In this study, we evaluated whether CM obtained from amnion-derived MSC culture (MSC-CM) could prevent the esophageal stricture after ESD. We resected three fourths the circumference of pig's esophagus by ESD. We prepared MSC-CM gel by mixing MSC-CM obtained from amnion-derived MSC culture with 5% carboxymethyl cellulose, and 20 mL of MSC-CM gel was endoscopically applied onto the wound bed immediately after ESD (day 1), and on day 8 and 15 (MSC-CM group: n=3). Standard medium gel was used as a control group (n=3). We also injected triamcinolone acetonide (80 mg) into the remained submucosa immediately after ESD (steroid group: n=3). We euthanized the pigs on day 22 to measure the stricture rate and for histological analysis. Stricture rate in MSC-CM and steroid groups were significantly lower than control group (56.3±7.1% and 49.3±4.2% vs 80.0±2.0%, respectively). Moreover, histological examination demonstrated that MSC-CM and steroid attenuated the number of activated myofibroblasts (26.8±8.6 and 20.6±2.3 vs 68.3±5.7 cells/mm^2) and fiber thickness (832.9±26.1 and 944.3±250.8 vs 1,609±418.2 μm). There were no differences on capillary density and macrophage infiltration among three groups; however, MSC-CM and steroid tended to decrease the infiltration of macrophages compared with the control group. Myofibroblast activation causes fibrosis and contributes to the esophageal stricture after ESD, and MSC-CM gel prevents the esophageal stricture by suppressing the myofibroblast activation and fibrosis. MSC-CM gel would be a promising treatment for the prevention of post-ESD stricture.

F1003

LONG TERM BIOSAFETY OF HUMAN INDUCED PLURIPOTENT STEM CELLS AND THEIR MESENCHYMAL DERIVATIVES

Chen, Bertha[1], Zhu, Fuli[1,2], Sun, Bin[1,3], Wen, Yan[1], Green, Morgaine[1], Wang, Zhe[1,4], Wei, Yi[1], Zhang, Fan[1], Cabral, Elise[1] and Reijo Pera, Renee A.[5], [1]Stanford University School of Medicine, Department of Obstetrics & Gynecology, Stanford, CA, U.S., [2]Beijing University Third Hospital, Beijing, China, [3]Guangzhou Medical University, Guangzhou, China, [4]Southern Medical University, Guangzhou, Guangdong, China, [5]Montana State University, Bozeman, MT, U.S.

There is great interest in using stem cells (SCs) to regenerate damaged tissues. Stress urinary incontinence (SUI), a condition associated with a deficient urethral sphincter, is a common disorder in adult women. SC-based therapies for SUI using adult SC sources show promising effects. Induced pluripotent stem cell (iPSC) is a SC source with additional qualities, including easy accessibility from patients, ability to expand into large numbers, and capacity to differentiate into many cells types. However, a major concern for clinical translation of iPSC-based therapy is biosafety since pluripotent SCs (PSCs) can form tumors in vivo. It is thought that this tumorigenic risk can be reduced by differentiating the PSCs into the specific cell lineage before transplantation. We sought to study long term, in vivo tumorigenic potential of human PSCs (hESCs and iPSCs), and fibroblasts and smooth muscle cell precursors (pSMCs) differentiated from these PSC lines to provide a reference for future iPSC-based translational research in urological disorders. hESC, iPSCs, and fibroblasts and pSMCs differentiated from these PSC lines were transduced with dual reporter gene consisting of green fluorescent protein and firefly luciferase. Different doses of hESCs and hiPSCs (1×10^3 - 2×10^6 cells), and hPSC-differentiated fibroblasts (6.25×10^4-1×10^6 cells) and pSMCs ($1X10^6$) were injected intramuscularly into immune-deficient SCID mice (n=112). Bioluminescence imaging (BLI) was used to follow *in vivo* integration and migration over 12 months. Increase in BLI signal over time indicated tumor formation, confirmed by histology. The rate of teratoma formation with hiPSC injection (1×10^6 ,17% and 2×10^6, 17%) was significantly lower than with hESC injection (1×10^6, 71% and

2×10^{6}, 100%) ($p<0.05$). No teratoma was found in any of the iPSC- or hESC-differentiated fibroblast (12 months), or iPSC-differentiated pSMC groups (6 months). Our data show that iPSC is less tumorigenic than hESC when transplanted intramuscularly. Tumorigenesis was not detectable for hPSC-differentiated fibroblasts and for hiPSC-differentiated pSMCs, suggesting that tumorigenic risk is substantially reduced when hPSCs are differentiated into mesenchymal cell types needed for tissue regeneration.

Funding Source: California Institute of Regenerative Medicine (CIRM) ETA III 106180-TR3-05569, PI-B Chen

F1005

MESENCHYMAL STEM CELLS ATTENUATES KETAMINE-INDUCED INTERSTITIAL CYSTITIS

KIM, Yonghwan Heo, Jinbeom and SHIN, DONG-Myung, University of Ulsan College of Medicine, Seoul, Korea, South

Ketamine has been abused as a hallucinogenic drug and is increasingly being used by young people. Ketamine abuse promotes the development of lower urinary tract symptoms that resemble interstitial cystitis (IC). The patho-physiology of ketamine-induced cystitis (KC) is largely unknown and the effective therapy is also limited. Here, we show the therapeutic effect of human umbilical cord blood-derived mesenchymal stem cells (MSCs) in a KC rat model. Daily injection of ketamine in 10-week-old Sprague-Dawley rats induced the defective bladder function as indicated by irregular voiding frequency, increased maximum contraction pressure and decreased inter-contraction intervals. Furthermore, KC bladders were characterized with severe mast-cell infiltration, tissue fibrosis, and apoptosis, paralleled with up-regulation of tumor necrosis factor-a (TNFa) and transformation growth factor-b (TGFb) signaling genes. Of note, A single injection of MSCs significantly not only ameliorated most of aforementioned bladder voiding parameters but also reversed the histological and gene expression alternations characteristic for KC bladder. Coordinated with the mechanim underlying the benefits of MSC therapy is anti-fibrotic ability, the treatment of N-acetylcysteine (NAC), an anti-fibrosis compound alleviated the KC bladder, similarly to MSC therapy. Thus, this study for the first time demonstrate that MSC therapy is beneficial to cure KC through blocking tissue fibrosis using an animal model. Our findings provide the basis for clinical trials of MSC therapy to painful bladder conditions such as KC and IC.

F1007

REGENERATIVE MEDICINE IS “GOING TO THE DOGS”: TREATING NATURALLY OCCURRING CANINE DILATED CARDIOMYOPATHY WITH MESENCHYMAL STROMAL CELLS

Vulliet, Richard[1], Rosman, Pamela[2], Halloran, Mitch[1] and Tallon, Kelli[1], [1]University of California Davis, Davis, CA, U.S., [2]ReGena-Vet Labs, Davis, CA, U.S.

Veterinary patients with naturally occurring terminal diseases offer unique opportunities for stem cell trials. The clinical course and histological appearance of Doberman Pinscher dilated cardiomyopathy (DCM) closely parallels that of human familial idiopathic DCM. If left untreated, 50% of canine patients will die within three months and 95% of the canine patients are dead within six months following onset of congestive heart failure. We find that treatment of Doberman Pinschers with allogeneic mesenchymal stem cells (MSCs) reduces signs of their dilated cardiomyopathy in pilot patients. Dobes with DCM were treated systemically with allogeneic MSCs. With the exception of two severely affected dogs euthanized before the cells were able to improve the DCM, other patients demonstrated improved activity, attitude and/or cardiac parameters. Fractional shortening (used in veterinary medicine to monitor cardiac contractility instead of ejection fraction because of varying canine chest geometry) increased. Most treated canine patients survived longer than would be expected for their clinical stage of disease. One patient with over 17,000 PVCs per day had the frequency of PVCs reduced to less than 300 per day over a two month course of treatment. The temporal correlation with MSC administration suggests that the cells or cell products, decreased the frequency of PVCs. Thus, MSCs may be beneficial in treating DCM-associated arrhythmias, in addition to congestive heart failure. Observations from owners included “dog has more energy,” “is back to stealing toys from sibs,” or “is now dragging us around like he used to” substantiate our clinical data. Several adverse reactions were encountered during these studies that required modification to treatment protocols. We believe that MSCs are an effective treatment for DCM in Doberman Pinschers since we observed improved quality and quantity of life in the treated dogs. Canine patients with DCM are an ideal intermediary for translating stem cell therapies into human patients.

F1009

IMPACT OF INFLAMMATION ON THE IMMUNOGENICITY OF HUMAN SKIN-DERIVED PRECURSOR CELLS

De Kock, Joery[1], Buyl, Karolien[1], Raicevic, Gordana[2], Branson, Steven[1], Rodrigues, Robim Marcelino[3], Vanhaecke, Tamara[1] and Najar, Mehdi[2], [1]Vrije Universiteit Brussel, Brussels, Belgium, [2]Jules Bordet Institute, Brussels, Belgium, [3]Free University Brussels, Brussels, Belgium

Human skin-derived precursors (SKP) are somatic stem cells that reside in dermal skin throughout life harboring clinical potential. SKP have a high self-renewal capacity, the ability to differentiate into multiple cell types and low immunogenicity rendering them key candidates for allogeneic cell-based off-the-shelf therapy. However, potential clinical application of allogeneic SKP requires that these cells remain low immunogenic under all circumstances and in particular in the presence of an inflammation state. Therefore, in this study, we investigated the impact of pro-inflammatory stimulation on the biological properties, immunogenicity and immunosuppressive capacity of SKP. We found that SKP remain low immunogenic and maintain their immunosuppressive properties in the presence of inflammation. Furthermore, pro-inflammatory stimulation of SKP leads to changes in their cytokine and growth factor secretion. More specifically, the secretion of the chemokine ligands CCL2, 5, 7, 8 and 20, CXCL1, 5, 6 and 10, colony stimulating factor (CSF) 2 and 3, interleukin (IL) 6 and 8, leukemia inhibitory factor (LIF) and vascular endothelial growth factor (VEGF) was significantly increased. Most importantly, in the presence of inflammation we observed that SKP drastically increase the expression and secretion of hepatocyte growth factor (HGF), a soluble molecule that is mandatory for SKP to perform immunosuppressive functions. Finally, in-depth pathway analyses show that this observation is directly linked to the combined activation of CCL2, CSF2, IL6 and LIF. As a result, we believe that SKP could be a valuable and easily accessible cell source for cell therapeutic strategies requiring immunosuppression, high secretion levels of HGF and/or as a poorly immunogenic multipotent stem cell population for cellular replacement therapy.

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F1011

TOPICAL ADIPOSE-DERIVED MESENCHYMAL STEM CELLS PROTECT AGAINST STREPTOZOTOCIN-INDUCED DIABETES

PK Lam[1,2], Charing CN Chong [1,2], Don WC Ching [1,2], Cindy SW Tong[1,2], Kin KY Lo[1,2],Paul BS Lai[1,2]

Department of Surgery[1], ChowTai Fook-Cheng Yu Tung Surgical Stem Cell Research Center[2], Prince of Wales Hospital, The Chinese University of Hong Kong

Type 1 diabetes mellitus (T1DM) is characterized by damage of insulin-secreting islet β (ISIB) cells. To date, there is no effective therapy to reverse the sequels of T1DM. Lifelong insulin is the current treatment. Mesenchymal stem cells (MSCs) are multipotent and hold great promise for tissue regeneration and repair. We aimed to investigate the beneficial effects of MSCs which were directly applied onto the surface of pancreas with T1DM in a rodent model. T1DM was induced in SD rats (250-300 gm) by a single intraperitoneal injection of streptozotocin (STZ, 60 mg/kg). One day after STZ-induced hyperglycemia, a midline incision was made to expose the pancreas in all animals. 5 x 10^6 MSCs cultured from the adipose tissue of transgenic GFP SD rats were topically applied to the surface of pancreas (Group A, N=10). Then a layer of fibrin (Baxter) was used to fix the MSCs in position. Only fibrin was added in Group B (N=10). In Group C, neither MSCs nor fibrin was given (N=10). The blood glucose was measured daily and histology was examined. At day 5 after the induced T1DM, the blood glucose in Group B and Group C increased to 25.7+3.55 mmol/L and 27+6 mmol/L respectively. There was no intact ISIB cell in the pancreas. Group A had a significantly lower level of blood glucose (15.1+7.5mmol/L) ($p < 0.05$). Topical MSCs ameliorated the damaging effects on ISIB cells. Some islets of insulin-secreting cells were observed. They showed expression of insulin in immunohistochemistry staining. Although the underlying mechanism remains to be determined, topically applied MSCs showed beneficial effects on STZ-induced diabetes in this animal study.

F1013

QUANTITATIVE ASSAY OF CHONDROGENIC CONNECTIVE TISSUE PROGENITORS IN HUMAN KNEE ARTICULAR CARTILAGE – CORRELATION WITH HISTOLOGICAL GRADE AND STRUCTURE

Mantripragada, Venkata[1], Boehm, Cynthia[1], Chang, Neil[2], Wong, Van[2], Midura, Ronald[1], Alsberg, Eben[3], Sah, Robert[2] and Muschler, George F.[1], [1]Cleveland Clinic, Cleveland, OH, U.S., [2]University of California San Diego, La Jolla, CA, U.S., [3]Case Western Reserve, Cleveland, OH, U.S.

Our lab is exploring the relationship between in vivo tissue health and the underlying chondrogenic connective tissue progenitor (CTP-C) population. This process has three essential steps: Step1: define and optimize effective sampling and cell yield from clinically relevant human cartilage tissue; Step2: define systematic methods for quantitative histological and structural analysis; Step3: optimize colony forming efficiency using quantitative in vitro CTP-C assays to define CTP-C concentration, prevalence and biological potential. This report defines success in the first two steps. Human articular cartilage samples were obtained from 18 patients undergoing knee arthroplasty with informed consent. Discarded tissue cut from the distal lateral condyles was systematically cut into 4 x 4 mm cartilage capped "teeth". Three teeth were pooled for cell analysis, lateral (CL) and medial (CM). Cells were extracted from the "teeth" using collagenase2/dispase digestion and cultured in a 3D RGD modified alginate medium. Adjacent samples were qualitatively assessed using two established systems for histology scoring (HHGS and OARSI). Digital volumetric imaging (DVI) analysis provided quantitative measure of cells and chondrons in terms of (i) total number (ii) 3D distribution. The mean cellularity by tissue digestion in vitro was 6385 cells/mm^3 and 5245 cells/mm^3 from CL and CM respectively. The mean cellularity by DVI analysis was 20103 + 10105 cells/mm^3 and 14180 + 6331 cells/mm^3 from CL and CM respectively. Comparison of DVI cell data and cell yield demonstrated a cell harvest efficiency of 43% for both CL and CM samples. HHGS and OARSI scoring systems were moderately correlated, 0.66 and 0.53 for CL and CM respectively. Chondron formation in 3D RGD-modified alginate discs can be quantitatively assessed using a z-stacking algorithm to generate 3D image for quantitative characterization. CTP-C colonies are defined as clusters of 4 or more. Colony size ranges from 4 to 10 cells at 14 days. Understanding the changes in stem and progenitor populations that are associated with cartilage health and degeneration is essential to i) understand the pathogenesis of cartilage degeneration, and ii) optimize methods for cell sourcing for cartilage tissue regeneration.

F1015

THE EXPANSION POTENTIAL AND SECRETOME OF MSCs ARE ALTERED IN PROGRESSIVE MULTIPLE SCLEROSIS WITH IMPAIRED NEUROGLIAL PROTECTION IN VITRO

Redondo, Juliana, Sarkar, Pamela, Kemp, Kevin, Wilkins, Alastair, Scolding, Neil and Rice, Claire, University of Bristol, U.K.

Multiple sclerosis (MS) is an inflammatory demyelinating and neurodegenerative disease of the central nervous system; 80% patients develop progressive disease for which there are no proven disease modifying therapies. Multipotent mesenchymal stem cells (MSCs) have been shown to have neuronal and glial protective properties and can influence differentiation and maturation of oligodendrocytes. This, combined with their favorable safety profile, has made them attractive candidates for cell-based therapy and several clinical trials employing infusion of MSCs are now in progress for MS and other neurodegenerative conditions. We have isolated bone marrow-derived MSCs from patients with MS who are participating in the 'ACTiMuS' trial – a phase II trial of intravenous, autologous, unfractionated bone marrow as treatment for MS (NCT01815632). We have examined their phenotype and functional properties including cell surface markers, expansion potential, senescence, neuroprotective properties and secretome profile. With increasing duration of disease progression, we observed decreases in proliferation capacity and clonogenic efficiency as well as premature senescence. Neuroglial protection afforded by soluble factors secreted by MSCs in an in vitroassay, declined with duration of progressive disease. Using tandem mass spectroscopy, we have identified over 40 proteins whose secretion by MSCs is reduced in the context of progressive MS and we are currently exploring the relevance of these to the observed deficits in neuroglial protection. Our findings have significant implications for those developing cell-based therapy with MSCs as a treatment for MS and further work will explore the relevance of the altered MSC secretome in MS to disease pathogenesis with the aim of developing new drug candidates and optimisation of cell-based therapy for MS.

Funding Source: MRC (Medical Research Council)

F1017

IN VITRO AND IN VIVO STUDIES OF THE ASSOCIATION OF BACTERIAL CELLULOSE MEMBRANES AND DERMAL MESENCHYMAL STROMAL CELLS FOR THE TREATMENT OF SKIN WOUNDS

Trentin, Andrea[1], Machado, Rafaela Grecco[2], Jeremias, Talita da Silva[2], Heck, Diana[2], Rode, Michele Patrica[2], Silva, Maiara Marques[2], Silva, Camila Acordi[2] and Recouvreux, Derce[3], [1]Universidade Federal de Santa Catarina, Florianopólis, Santa Catarina, Brazil, [2]Universidade Federal de Santa Catarina, Florianópolis, Brazil, [3]Universidade Federal de Santa Catarina, Joinville, Brazil

Full-thickness skin injuries, such as extensive burns, chronic ulcers and deep wounds result in numerous physiological and functional problems. Therefore, new strategies for skin regeneration are needed. One approach is the association of biomaterials with mesenchymal stromal cells (MSCs) and subsequent grafting. MSCs have been shown to improve the regeneration of skin and other tissues. Bacterial cellulose (CB) is a natural polymer produced by Gluconacetobacter hansenii. Some features that make this biomaterial attractive for skin repair are: biocompatibility, tensile strength, good permeability and low solubility. The present work aimed to evaluate the potential of CB as a vehicle to delivery dermal MSCs (dMSCs) providing an improved environment for skin regeneration. The association of CB with human dMSCs was evaluated in vitro by the analysis of cell viability, morphology, adhesion, infiltration and immunophenotype. Human dMSCs, obtained from healthy patients undergoing facial lifting, were seeded on CBs and cultured under standard conditions. All procedures were approved by the Ethics Committee of the Federal University of Santa Catarina. MTS assay showed that dMSCs remained viable after 21 days of culture on CBs. Scanning electron, confocal and phase contrast microscopy revealed progressive adhesion of dMSCs on the CBs surface with the typical fibroblastic morphology. In addition, cells expressed the MSCs membrane markers as assessed by flow cytometry. These results showed that CBs support the viability and adhesion of dMSC. Next, the therapeutic potential of dMSCs associated with CBs was evaluated by in vivo assays of wound healing in mice. Wound closure rates, inflammation, vascularization, size of scar and neo-epidermis formation were evaluated. Results showed that although the wound closure rates were similar between treated (dMSCs + CBs) and non-treated groups, treated animals showed an increase of granulation tissue, neovascularization, and neutrophil infiltration accompanied by a decrease of scar length and epidermis thickness. Taken together, these results point to an improved tissue repair using the association of bacterial cellulose membranes and dMSCs that could be suitable for the treatment of skin wounds.

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F1019

CLINICAL APPLICATION OF HUMAN AMNION-DERIVED MESENCHYMAL STEM CELLS FOR THE TREATMENT OF ACUTE GVHD

Yamahara, Kenichi[1], Hamada, Akiko[1], Horikawa, Chisako[1], Ohnishi, Shunsuke[2], Fujimori, Yoshihiro[3], Soma, Toshihiro[3] and Ogawa, Hiroyasu[3], [1]National Cerebral and Cardiovascular Center, Suita, Japan, [2]Hokkaido Univeristy, Sapporo, Japan, [3]Hyogo Medical College, Nishinomiya, Japan

We have demonstrated that, similar to bone marrow-derived mesenchymal stem cells (BM-MSCs), fetal appendage-derived MSCs possess the differentiation, angiogenic and immunosuppressive property. Fetal appendage, normally discarded as medical waste, is a rich source of MSCs, which secret significant amounts of cytokines/growth factors, indicating a suitable cell source to perform a cell therapy at low-cost, faster, and with less MSCs. Among fetal appendage, amnion is anatomically easy to separate MSCs by enzymatic digestion, and we started a clinical trial to treat acute GVHD using amnion-derived MSCs. First we established a multicenter clinical team as follows: National Cerebral and Cardiovascular Center (amnion MSC manufacturing), Foundation for Biomedical Research and Innovation (quality control of amnion MSCs), and Hyogo College of Medicine (administration of amnion MSCs in patients with acute GVHD). We also established a method to isolate amnion MSCs with ease and high efficiency (International patent application number: PCT/JP2014/071546). After a consultation with the Pharmaceuticals and Medical Devices Agency (PMDA), we started to manufacture aminon MSCs adhering to GMP, and are planning to perform a phase I clinical trial within this year to assess the safety of amnion MSCs in patients with acute GVHD. In Japan, BM-MSCs have already received approval for the treatment of patients with acute GVHD. However, invasive procedure and long-term culturing are required to obtain an adequate number of BM-MSCs. Since a large number of MSCs could be obtained easily and non-invasively, amnion MSCs would be an ideal cell source for regenerative medicine.

MESENCHYMAL STEM CELL DIFFERENTIATION

F1021

TSH-TSHR INTERACTION MODULATES IGF-1 GENE EXPRESSION IN HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

Bagriacik, Emin Umit, Yaman, Melek and Oruklu, Nihan, Gazi University Faculty of Medicine, Ankara, Turkey

Previously, we demonstrated that human bone marrow-derived mesenchymal cells (MSCs) expressed a functional thyroid stimulating hormone receptor (TSHR). In the present study, we investigated effects of thyroid stimulating hormone (TSH)-TSHR interactions in gene expression of insulin-like growth factor 1 (IGF-1). MSCs were isolated from either human bone marrow of various donors or they were purchased from ATCC. After keeping MSCs starved for 24 hours in a supplement-free plain medium, they were incubated in TSH containing culture medium for four hours. Total RNA was isolated from untreated control cells as well as TSH-treated MSCs. IGF-1 gene expression was quantified by real time PCR. IGF-1 gene expression increased significantly in a dose-dependent way in the TSH-treated cells in comparison to those untreated control cells. Increased IGF-1 gene expression in TSH-treated cells was a common response for all of those MSCs obtained from either ATCC or from various donors. Based on these findings, we concluded that IGF-1 is an important growth factor for proliferation, migration, and differentiation of MSCs. TSH-TSHR interaction may be an important regulator of IGF-1 gene expression in human bone marrow-derived MSCs.

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F1023

SCALABLE BIOREACTOR-BASED PRODUCTION OF HUMAN MESENCHYMAL STEM CELL (HMSC) 3D AGGREGATES USING MICROCARRIERS WITH THERMO-REVERSIBLE SURFACES

Farrance, Iain K.[1], Yuan, Xuegang[2], Ma, Teng[3] and Rowley, Jon A.[1], [1]RoosterBio Inc., Frederick, MD, U.S., [2]Florida State University, Tallahassee, FL, U.S., [3]FAMU-FSU College of Engrg, Tallahassee, FL, U.S.

Mesenchymal Stem/Stromal Cells, or MSCs, will be key components of future therapeutics, engineered tissues, and medical devices. Recently, exciting research has shown that culture of MSCs as 3D aggregates (3D-hMSC) improves biological activity over MSCs grown as a monolayer. These advantages include greater differentiation, increased paracrine factor secretion, enhanced immunomodulatory activity, resistance to ischemia, smaller cell size, and therefore, improved pre-clinical results. Additionally, in tissue engineering applications, 3D-hMSC facilitate the assembly of microtissues. Clinical and tissue engineering application of 3D-hMSC require large cell numbers (100M to 10B cells). However, production techniques for 3D-hMSC are currently not scalable to levels consistent with industrial needs. For 3D-hMSC use to become widespread, 3D-hMSC production processes must be scalable, cost effective, and clinically-compatible, and be capable of generating billions of consistent, highly functional 3D-hMSC within a single manufacturing lot. Here we report on a scalable production technology for 3D-hMSC. hBM-MSC were expanded on microcarriers with thermo-reversible surfaces (TRM) and 3D-hMSC produced directly by temperature-dependent release of mini-cell sheets from the TRM. To test this scalable method, we hypothesized that hMSCs can be expanded on TRM in bioreactors and that 3D-hMSC with enhanced function, similar to AggreWell 3D-hMSC, can be produced by this method. hBM-MSC grew efficiently on the TRM and formed 3D-hMSC upon thermal release. We compared 3D-hMSC produced on the TRM (Bioreactor aggregates, BR-Aggs) to 3D-hMSC produced in AggreWells (Traditional aggregates, TR-Aggs) in assays for cell viability, immunomodulatory potential (IFN-γ induced indoleamine 2,3-dioxygenase (IDO) upregulation), and angiogenic cytokine secretion. BR-Aggs and TR-Aggs were similar, with high cell viability, activation of IDO under basal conditions and further induction by IFN-γ treatment, and displayed comparable angiogenic cytokine secretion levels (VEGF, HGF, TIMP-1 and -2, FGF2, and IL-8). These results confirm the hypothesis that the scalable TRM technology can be used to produce high-quality 3D-hMSC for translational researchers in Regenerative Medicine and Tissue Engineering.

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F1025

DIFFERENTIAL EXPRESSION OF NEDD4-1 DURING SKELETOGENESIS REGULATES BONE FORMATION BY PROMOTING OSTEOBLAST PROLIFERATION

Jeon, Seon-Ae[1], Lee, Ji-Hyun[2], Ryoo, Hyun-Mo[3], Kim, Hong-Hee[3] and Cho, Je-Yoel[4], [1]College of Veterinary Medicine, Seoul National University, Seoul, Korea, [2]Gacheon University, Incheon, Korea, [3]School of Dentistry, Seoul National University, Seoul, Korea, [4]College of Veterinary Medicine, Seoul National University, Seoul, Korea

Osteoblasts are specialized cells that are responsible for the bone formation and mineralization. Neural precursor cell expressed developmentally down-regulated protein 4-1 (Nedd4-1) is a well-known E3 ubiquitin-protein ligase which regulates animal growth and development. To investigate the in vivo function of Nedd4-1 in skeletogenesis, we established immature osteoblast-specific Nedd4-1 transgenic (TG) and conditional knock-out (cKO) mice. In vivo assays with these mice compared to each littermate wild type (WT) showed that Nedd4-1 enhanced bone mass accrual and up-regulated gene expression of osteogenic markers in bone tissue. Proliferation of calvaria-derived osteoblasts from Nedd4-1 TG mice were increased and osteoblast number and surface area in tibia of Nedd4-1 TG mice were higher than WT. The transforming growth factor-beta (TGF-β) is one of the most abundant cytokines in bone matrix and plays a major role in development and maintenance of the skeleton. The expression of Nedd4-1 and TGF-β were high at early stage of osteoblast maturation, but decreased at late stage which BMP2 expression level was increased. The opposite phospho-Smad1,5, and 8 which were classically activated by BMP2, were appeared transiently by TGF-β but ubiquitinated and degraded rapidly by Nedd4-1. In addition, Phosphatase and tensin homolog (PTEN) which is mutated in a large number of cancers at high frequency and acts as a tumor suppressor. was decreased in osteoblasts from Nedd4-1 TG mice. Furthermore, pERK signaling which regulates cell proliferation was enhanced in osteoblast from Nedd4-1 TG mice than WT. These all results suggested that Nedd4-1 positively regulates bone formation through promoting of osteoblast proliferation.

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F1027

MULTI-STAGE CONTROL OF OSTEOGENIC DIFFERENTIATION THROUGH A QUALITY-BY-DESIGN (QBD) APPROACH

Majumder, Mithu[1,2], Finney, Krystal[1,2], Gilkey, Michael[1,2] and Jensen, Jan[3,4], [1]Case Western Reserve University, Cleveland, OH, U.S., [2]Trailhead Biosystems, Cleveland, OH, U.S., [3]Cleveland Clinic Lerner Research Institute, Cleveland, OH, U.S., [4]Cleveland Clinic, Cleveland, OH, U.S.

Allogeneic bone marrow-derived messenchymal (BM-MSC) stem cells are evaluated in clinical trials for the effective treatment multiple diseases. BM-MSCs are multi-potent cells capable of differentiating into a cohort of fates. For instance, osteogenic differentiation of BM-MSC is used to evaluate the benefit of BM-MSCs in treatment of osseous defects. At current, FDA-approved BM-MSC-derived cell products for bone regeneration remains unavailable. Osteogenic differentiation of BM-MSCs remains inefficient. We have developed a QbD-compliant robotic platform that addresses multidimensional problems in cell culture and applied this technology to understand lineage control of BM-MSC. A DoE-based approach was utilized to simultaneously test 12 different signaling pathway agonists/antagonists known to substantially affect MSC lineage determination. Multi-lineage differentiation responses of BM-MSC to these different conditions were subsequently determined by TaqMan based qPCR using key fate tracking genes (>100). Software based mathematical modeling of each gene expression response was performed allowing predictive interrogation of the differentiation space. Each such experiment informed on combinatorial effects upon lineage control by the assembly of the tested compounds. We mapped known early, intermediate, and late markers for the osteogenic process, and assembled a serial signaling logic for the gradual induction of osteogenic fate. Altogether analyzing more than thirty compounds, we extracted a completely data driven protocol for osteogenic induction for mineralizing, BGLAP-expressing osteocytes in 6 days. The robotic DoE-based approach is a valuable tool in optimizing for maximal phenotype induction, allowing a rapid identification of the most effective conditions optimized within a multivariable input systems such as those represented by mammalian multi-potent cells.

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F1029

OSTEOBLASTIC DIFFERENTIATION OF HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS – MODEL SYSTEM TO DEVELOP ANTI-CANCER STRATEGIES

Sen, Sudip, Khera, Nupur, Saluja, Sumedha, Agrawal, Sandeep Kumar, Bhattacharya, Aditi, Manhas, Janvie, Bhat, Muzaffer Ahmed, Mridha, Asit Ranjan, Ansari, Mohd. Tahir and Mittal, Ravi, All India Institute of Medical Sciences, New Delhi, India

Introduction: Human Mesenchymal Stem Cells (MSCs) are multipotent cells that have the potential to differentiate into bone, cartilage and other tissues. They have a potential role in treating degenerative diseases. However, malignant transformation of MSCs has been reported and is a stumbling block. The objective of this study was to elucidate the relationship between osteogenic differentiation of MSCs and tumorigenesis and use it to develop anti-cancer strategies. Materials and methods: MSCs were isolated from human bone marrow and grown in vitro in DMEM (LG) with 15%FBS, Stempro SFM for MSCs at 37°C. MSCs in 3rd–5th passage were differentiated into osteoblasts using beta glycerophosphate, ascorbic acid and dexamethasone ±BMP-2. Flowcytometry was used to characterize MSCs (CD90, CD105) and differentiated osteoblasts (Osteopontin, Osteocalcin). qPCR was performed to check the mRNA expression of PCNA and Ki-67 in MSCs and osteoblasts. Microarray was done using Agilent whole genome 8x60K array slides to study transcriptomic changes between undifferentiated MSCs and differentiated osteoblasts. Data was analysed using Flow Jo, Gene Spring GX13 and GeneGO MetaCore. Validation was done using QPCR. Results: Morphology, H&E and Alizarin staining demonstrated MSCs and osteoblasts. Flowcytometry confirmed presence of MSCs and osteoblasts. MSCs differentiated into osteoblasts in 3 weeks with an efficiency of 50±11.1% (without BMP-2) and 82.6±3.24 (with BMP-2). Decrease in mRNA expression of PCNA and Ki-67 was observed as MSCs differentiated into osteoblasts. Gene expression analysis showed difference in expression of genes related to survival and proliferation that are currently being analyzed. Conclusion: Flowcytometry confirmed purity of MSCs (93-98%) isolated from human bone marrow by presence of characteristic markers. Appearance and quantification of bone matrix proteins *viz.* Osteopontin and Osteocalcin confirmed osteoblast formation. Decrease in mRNA expression of Ki-67 and PCNA is an interesting phenomenon observed during osteogenic differentiation and gives an insight into the possible association between differentiation and proliferation. Differential gene expression of genes related to survival and proliferation are being analyzed in an effort to develop anti-cancer strategies.

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F1031

CYTOSKELETAL MODULATION OF HUMAN PLURIPOTENT STEM CELL (PSC)-DERIVED MESENCHYMAL STEM CELLS (MSCS) INDUCES POTENT NEUROGENIC DIFFERENTIATION

Peng, Kai-Yen[1,2], Lee, Yu-Wei[3], Wang, Yun[4], Yen, B. Linju[3] and **Yen, Men-Luh**[5], [1]National Health Research Institutes, Taipei, Taiwan, [2]Department of Life Science, National Central University, Jhongli, Taiwan, [3]Regenerative Medicine Research Group, Institute of Cellular & System Medicine, National Health Research Institutes (NHRI), Zhunan, Taiwan, [4]Center for Neuropsychiatric Research, NHRI, Zhunan, Taiwan, [5]Department of Obstetrics/Gynecology, National Taiwan University (NTU) Hospital & College of Medicine, Taipei, Taiwan

Mesenchymal stem cells (MSCs) are paraxial mesodermal progenitors with potent immunomodulatory properties. Reports also indicate that MSCs can undergo neural-like differentiation, offering hope for use in neurodegenerative diseases. However, ex vivo expansion of these rare somatic stem cells for clinical use leads to cellular senescence. A newer source of MSCs derived from human pluripotent stem cells (PSCs) can offer the 'best-of-both-worlds' scenario, abrogating the concern of teratoma formation while preserving PSC proliferative capacity. PSC-derived MSCs (PSC-MSCs) also represent MSCs at the earliest developmental stage, and we found that these MSCs harbor stronger neuro-differentiation capacity than post-natal MSCs. PSC-MSCs express higher levels of neural stem cell (NSC)-related genes and transcription factors than adult bone marrow MSCs at baseline, and rapidly differentiate into neural-like cells when cultured in either standard neurogenic differentiation medium (NDM) or when the cytoskeletal modulator RhoA kinase (ROCK) is inhibited. Interestingly, when NDM is combined with ROCK inhibition, PSC-MSCs undergo further commitment, acquiring characteristics of post-mitotic neurons including nuclear condensation, extensive dendritic growth, and neuron-restricted marker expression including NeuN, β-III-tubulin and Doublecortin. Our data demonstrates that PSC-MSCs have potent capacity to undergo neural differentiation and also implicate the important role of the cytoskeleton in neural lineage commitment.

MESENCHYMAL CELL LINEAGE ANALYSIS

F1035

KERATIN-18 AND SECRETED FRIZZLED-RELATED PROTEIN 4 REGULATE PLURIPOTENCY VIA IL6 RESPONSE IN SYNOVIAL FLUID-DERIVED MESENCHYMAL STEM CELLS

Choi, Sung Sik[1], Lee, Eui-Jin[2], Yang, Yun-Jung[3], Lee, Dong-Seok[4], Kim, Da Hee[1], Kim, So Hui[1], Lee, Daniel[5] and **Lee, Hong Jun**[1], [1]Chung-Ang University, Seoul, Korea, [2]Incheon St. Mary's Hospital, Incheon, Korea, [3]International St. Mary's Hospital, Incheon, Korea, [4]Kyungpook National University, School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Daegu, Korea, [5]Seonbu High School, Ansan, Korea

Individual stem cells derived from synovial fluid show more variation in their gene expression, morphology, and proliferation than other adult stem cells. SFSCs were obtained from 16 patients with osteoarthritis, and transcripts of pluripotent markers were investigated by qPCR and microarrays. The most notable differences were found in the transcript levels of the SFRP4, PDGFRD, C3, KRT18, and LEP genes which were closely related with the Wnt pathway and Akt pathway and were associated with IL6. In SFSCs, SFRP4, antagonist of the Wnt signaling pathway, induce differentiation and KRT18 decrease cell proliferation by the downregulation of OCT4A and KRT18 is regulated by IL6. Individual variation in SFSCs may be caused by SFRP4 and KRT18 with IL6, or inflammatory response and differentiation and proliferation of SFSCs may be regulated by Akt pathway and Wnt pathway via SFRP4 and KRT18. These genes can be further studied as possible regulators of the transition between stemness/differentiation, and may be useful markers for studies of SFSC cell-mediated therapy.

F1037

MESENCHYMAL STEM CELL DEPENDENT PATHOGENESIS OF FANCONI ANEMIA SYNDROME

Boregowda, Siddaraju V.[1], Krishnappa, Veena[1], Strivelli, Jacqueline[1], Cappelli, Enrico[2] and Phinney, Donald G.[1], [1]Scripps Florida, Jupiter, FL, U.S., [2]Laboratorio di Ematologia, Genova, Italy

Fanconi anemia (FA) is an autosomal recessive genetic disease caused by mutations in the FANC gene complementation group. FA is characterized by progressive myelodysplastic syndrome and acute myeloid leukemia in more than 60% of affected individuals, and terminal bone marrow failure by the age 40 in 98% of patients. There is extensive research on hematopoietic stem cell (HSC) dependent pathogenesis of FA. However, an understanding of the possible role of mesenchymal stem cells (MSCs), which contribute to the HSC niche in bone marrow, in disease pathogenesis of FA is completely lacking. MSCs physically associate with HSCs and adrenergic nerve endings in bone marrow. They also express a cadre of factors that maintain HSCs and promote restoration of hematopoiesis after bone marrow transplantation. Given that bone marrow transplantation is the only long term treatment available for FA, understanding the role of MSCs in FA pathogenesis may lead to better therapeutic outcomes. Our studies demonstrate that MSCs isolated from FA patients harboring a mutation in the FANCH gene demonstrate reduced growth, undergo rapid senescence, and also exhibit altered differentiation potential. These functional changes are accompanied by changes in expression of genes belonging to the FANC/BRCA/P53 signaling axis. Furthermore, MSCs from FA patients exhibit reduced expression of genes that function in HSC maintenance, lymphopiesis, and that exhibit myelosuppressive activity including KITLG, HGF, GDNF, PGF, CFB and IL1B. In contrast, these cells exhibit enhanced expression of genes that drive myelopoiesis and are involved in anemia, such as IL6, GDF15, and CFI. These findings demonstrate that FANC gene mutations alter normal MSC function, and this altered function likely contributes to FA pathogenesis We propose further study to examine how FANC mutations alter the function of human MSCs using animal models of FA.

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HEMATOPOIETIC CELLS

F1039

THE CYTOTOXIC EFFECT OF RNA-GUIDED ENDONUCLEASE CAS9 ON HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS (HSPCS)

Yu, Kyung-Rok[1], Corat, Marcus[1], Metais, Jean-Yves[2] and Dunbar, Cynthia[1], [1]National Institutes of Health, Bethesda, MD, U.S., [2]Hematology, St. Jude Children Research Hospital, Memphis, TN, U.S.

The CRISPR (clustered regulatory interspaced short palindromic repeats) – CRISPR-associated protein 9 (Cas9) system (CRISPR/Cas9) has recently emerged as an efficient and powerful approach widely used for targeted genetic engineering. Although CRISPR/CAS9 system very efficiently disrupts target locations in the genome of multiple model organisms, editing in some primary cell types, especially hematopoietic stem and progenitor cells

(HSPCs) has been more challenging, with lower efficiencies particularly in functional engrafting HSPC. However, the exact mechanisms have not been elucidated. To optimize editing via CRISPR/Cas9 in human HSPCs, we generated several different lentiviral vectors; a negative control GFP only non-editing vector (LeGO-GFP), U6 promoter-AAVS1 targeting sgRNA-EF1a promoter-GFP, U6-EFS promoter-Cas9-EF1a-GFP, U6-AAVS1 targeting sgRNA-EFS-Cas9-EF1a-GFP, U6-EFS-human codon-optimized Cas9-EF1a-GFP, and U6-AAVS1 targeting sgRNA-EFS-human codon-optimized Cas9-EF1a-GFP. Lentivirus-transduced HSPCs were analyzed by flow cytometry based on their GFP expression in a time-dependent manner (3, 6, 9, 12 days). All vectors resulted in a gradual decrease in the percentage of GFP+ cells over time, however, Cas9 containing vectors showed significant decreases, whereas the vectors without Cas9 showed the least decrease in GFP+ cells. These results led us to investigate the cytotoxicity of Cas9 on HSPCs, using Vivid and Annexin V staining. Cas9 expressing HSPCs showed strong Annexin V positivity and a gradual increase in Vivid+ cells, explaining the loss of GFP+ cells with Cas9-expressing vectors. On the other hand, HSPCs transduced with the non-Cas9-expressing vectors remained Vivid and Annexin V low until through 12 days. Our findings suggest that sustained expression of Cas9 in human HSPCs, for instance via an integrating vector, has significant toxicity, and that an alternative Cas9 delivery method for HSPCs is required. We have tried integrase-defective lentivirus (IDLV) and mRNA transfection to deliver Cas9 into HSPCs, and data will be presented. This approach will facilitate the use of CRISPR/Cas9 system to engineer genes of clinical significance in HSPCs with a therapeutically meaningful efficacy.

F1041

EPIGENOMIC ANALYSIS OF BONE MARROW ALDH BRIGHT CELLS ISOLATED FROM PERIPHERAL ARTERIAL DISEASE PATIENTS

Cabreira, Maria da Graca[1], Estecio, Marcos Roberto[2], Gong, Ting[2], Wang, Xiaohong[1], Azares, Alon R[1], Critsinelis, Andre Cormack[1], Li, Ke[1], Taylor, Doris[1], Resende, Micheline[1], Willerson, James[1] and Perin, Emerson Carvalho[1], [1]Texas Heart Institute, Houston, TX, U.S., [2]MD Anderson Cancer Center, Houston, TX, U.S.

Epigenetic mechanisms are closely related to cellular differentiation and disease states. We are screening genome-wide DNA methylation patterns in bone marrow ALDH^{bright} cells, a population enriched for undifferentiated cells that comprise stem/progenitor bone marrow compartments (hematopoietic, mesenchymal and endothelial). We hypothesized that age and disease-associated changes in the epigenetic landscape of stem cells affect the regenerative capacity ALDH^{bright} cell population. Samples of marrow harvested from patients enrolled in a clinical trial named PACE (Patients with Intermittent Claudication Injected with ALDH^{bright} Cells; IRB: HSC-GEN-13-0138) have been used for cell isolation and analysis. The PACE trial is testing the ability of autologous cells to regenerate blood flow in affected limbs. As part of an ancillary study, we are analyzing methylation patterns using global and cellular senescence-focused platforms. In addition, we integrate methylation data with patient's cells phenotypic and functional characterization obtained from *ex vivo* expanded mesenchymal progenitor cells. So far, we have collected data from a small cohort of patient's cells (N=10). Collection and analysis has been done in a blinded fashion. We used Reduced Representation Bisulfite Sequencing to evaluate nearly 420K CpG sites, representing gene promoter, gene body and intergenic regions. We identified the top 1% most variable CpG sites to perform an unsupervised hierarchical clustering, as a means to form subgroups based on their differential methylation; the clustering generated two groups (N=5 per group). These groups also differed in proliferative capacity measured as population doubling time (36.6 hours +/-10.02 versus 75.08 +/-18.81; p=0.016) in mesenchymal progenitor cell cultures. In addition, proteome analysis of revealed that PCNA and phosphorylated Rb1 at Ser807/Ser111 are significantly more elevated in the group showing faster doubling (respectively 1.1 versus 0.98; p=0.008 and 1.29 versus 0.96, p=0.003). More data will be shown at the meeting. These preliminary results support the idea that DNA methylation reflects cell functional characteristics in these patient samples. The study of larger populations will likely identify markers relevant to the regenerative capabilities of these cells

F1043

ROLE OF LONG NONCODING RNAS IN NORMAL MURINE HEMATOPOIESIS AND MALIGNANT TRANSFORMATION

Delas, M Joaquina[1,2], Sabin, Leah R[1], Dolzhenko, Egor[3], Zhou, Meng[3], Knott, Simon RV[1,2], Wild, Sophia A[2,4], Lee, Emily[1], Kelley, David R[5], Rinn, John L[5], Smith, Andrew D[3] and Hannon, Gregory J[1,2], [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, U.S., [2]CRUK Cambridge Institute, Cambridge, U.K., [3]University of Southern California, Los Angeles, CA, U.S., [4]German Cancer Research Center, DKFZ, Heidelberg, Germany, [5]Harvard University, Department of Stem Cell and Regenerative Biology, Cambridge, MA, U.S.

Noncoding RNAs are known to play essential roles in the regulation of gene expression. While different types of small RNAs have well-studied functions in cellular processes such post-transcriptional gene silencing and transposon repression, our knowledge of long noncoding RNAs (lncRNAs) is still very sparse. For that reason, we set out to characterize the function of long noncoding

RNAs in the hematopoietic compartment, both in normal hematopoiesis and in malignant transformation. We used high throughput sequencing to sample the transcriptome of nine normal hematopoietic cell types, as well as in vivo models of murine acute myeloid leukemia (AML) and B cell lymphoma. Based on this data, we have built a catalog that encompasses the lncRNA repertoire for both normal blood and their transformed counterparts. Using differential expression analysis we have identified lncRNAs that are highly regulated during differentiation from hematopoietic stem cell to myeloid/lymphoid cell types or with lineage specific expression. We have also used this resource to study expression changes between normal myeloid progenitors and AML, a progenitor-derived cancer. In order to ask whether lncRNAs play a role in the hematopoietic system, we selected a subset of relevant lncRNAs, including AML-specific lncRNAs or shared lncRNAs, with ranging levels of expression as targets for functional studies. We performed an shRNA screen in an in vivo model of AML, identifying several lncRNAs required for leukemia progression. We identified a number of lncRNAs that were essential for leukemia maintenance. Of these, several were essential for maintenance of leukemia stem cell signatures, and their loss of expression led to myeloid differentiation. Our current efforts are focused on identifying lncRNA interaction partners in order to elucidate the molecular mechanisms that mediate this phenotype.

F1047

ADAR1-DEPENDENT REGULATIONOF MICRORNA REGULATES LEUKEMIA STEM CELL SELF-RENEWAL AND PROVIDES NOVEL BIOMARKERS OF DISEASE PROGRESSION

Zipeto, Maria Anna[1], **Jiang, Qingfei**[1], Crews, Leslie A[2] and Jamieson, Catriona H.M.[3], [1]University of California San Diego, La Jolla, CA, U.S., [2]University of California, San Diego, La Jolla, CA, U.S., [3]Moores Cancer Center University of California San Diego, La Jolla, CA, U.S.

Cumulative research by ourselves and others revealed that microenvironmental activation of adenosine deaminase acting on RNA (ADAR) editing of adenosine to inosine (A-to-I) is an early and robust predictor of cancer progression and therapeutic resistance in cancer stem cell (CSC)-driven human malignancies. Of particular relevance to non-coding RNA, emerging evidence suggests that ADAR1 alters microRNA (miRNA) biogenesis at the level of primary or precursor and triggers their degradation. We have recently shown that blast crisis (BC) Chronic Myeloid Leukemia (CML) stem cells (LSCs) harbor high levels of inflammation-responsive ADAR1 transcripts that regulate both LSC differentiation and self-renewal as demonstrated by *in vivo* humanized xenograft CML BC models. However, edited miRNA profiles and the effect of increased-ADAR1 on miRNA regulation in LSC generation are still unclear.

To directly determine the impact of ADAR1 on miRNA regulation, we developed a lentiviral human overexpression vector and transduced CD34$^+$ cells from both CML chronic phase (CP) and normal cord blood (CB) samples. Initial screening of ADAR1-dependent miRNA expression in CB and CP was performed by profiling 1008 and 420 candidates respectively. Lentivirally transduced CD34$^+$ CP and CB cells harbored decreased expression of 79 and 155 mature miRNAs, and increased expression of 34 and 38 miRNA respectively. Pathway analysis revealed that significant percentage of differentially regulated miRNAs in both CP and CB was involved in stem cell regulatory pathway, including let-7 family of miRNAs and miR-26a. Interestingly, primary BC CD34$^+$ cells have decreased level of miRNA that are regulated by ADAR1 in both CB and CP, suggesting ADAR1-mediated miRNA biogenesis may play a role in BC LSC generation. Moreover, we observed reduced self-renewal capacity upon overexpression of let-7 and miR-26a in CB and BC CD34+ cells. To determine if ADAR1 affects miRNA biogenesis in an RNA-editing dependent manner, we developed an RNA editing deficient ADAR1-E912A overexpression vector by site-directed mutagenesis. Expression in CD34$^+$ CB reveals that at least 10% of miRNA are regulated by A-to-I editing of ADAR1. These data confirm a role for ADAR1-dependent miRNA regulation in self-renewing LSC generation and provide novel biomarkers of CML progression.

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F1049

MODELING SEVERE CONGENITAL NEUTROPENIA WITH IPSCS

Krutein, Michelle C[1], Tidwell, Timothy[1], Trump, Lisa[2], Nayak, Ramesh[2] and Horwitz, Marshall[1], [1]University of Washington, Seattle, WA, U.S., [2]Cincinnati Children's Hospital, Cincinnati, OH, U.S.

Neutropenia refers to abnormally low number of peripheral blood neutrophils, which predisposes to bacterial and fungal infection. Hereditary neutropenia exists in two forms, cyclic (CN) and severe congenital neutropenia (SCN). SCN exhibits a block in granulopoiesis at the promyelocytes stage paired with an increase in cell death. Additionally, SCN can progress to myelodysplasia (MDS) and acute myeloid leukemia (AML). Although genetically heterogeneous, CN and SCN are most frequently attributed to heterozygous mutations in the gene ELANE, encoding neutrophil elastase (NE). A serine protease with multiple substrates, NE is stored in primary granules. Two hypotheses have been suggested to explain how NE mutations facilitate disease. In one, mutations disrupting NE's subcellular trafficking mislocalize proteolytic activity. In the other, disruption of NE protein folding induces

ER stress and an unfolded protein response. Our group recently identified novel ELANE mutations that disrupt the translational start site, supporting a new 'alternative peptide' hypothesis that may explain a subset of NE mutants. It is notable that during normal granulopoiesis, ELANE expression is controlled in a temporal manner at the promyelocyte stage. However, analysis of CN and SCN bone marrow does not detect mutant ELANE transcript; therefore observing the direct effects of these mutations on granulopoiesis is difficult. Currently, ELANE-associated neutropenia mouse models fall short of recapitulating the disease phenotypes observed in humans. We and others have successfully generated induced pluripotent stem cells (iPSCs) from patients with ELANE mutations, which upon myeloid differentiation corroborate cellular phenotypes observed in vivo. We are pairing ELANE-iPSC models with live cell, RNA imaging of ELANE transcript, in order to help deduce the direct effects of wild type and mutant ELANE expression during myeloid differentiation, and apply single-cell technologies to better detail the pathogenesis of ELANE-associated neutropenia.

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F1051

ADIPOCYTE-DERIVED ADIPONECTIN ENHANCES ACTIVATION OF HEMATOPOIETIC STEM CELLS BY POTENTIATING MTORC1 ACTIVATION AFTER MYELOTOXIC INJURY

Masamoto, Yosuke[1,2], Arai, Shunya[2], Sato, Tomohiko[1,2], Takamoto, Iseki[3], Kubota, Naoto[3], Kadowaki, Takashi[3] and Kurokawa, Mineo [2], [1]University of Tokyo Hospital, Department of Transfusion Medicine, Tokyo, Japan, [2]University of Tokyo Hospital, Department of Hematology/Oncology, Tokyo, Japan, [3]University of Tokyo Hospital, Department of Metabolic Diseases, Tokyo, Japan

Myelotoxic injury unlocks the vigorous power of hematopoietic stem cells (HSCs) to replenish the hematopoietic system, making quiescent HSCs enter the cell cycle. Microscopically, adipose tissue replaces cellular components in bone marrow (BM) after myeloablation. Considering both HSC-intrinsic and –extrinsic mechanisms enforce quiescence of HSCs in a steady-state, the drastic change in BM environment by myeloablation might trigger and promote the cell cycle entry of HSCs. We have reported that adiponectin, adipocyte-derived hormone, indirectly enhances proliferation of immature myeloid progenitors in the setting of infection by suppressing TNF-α production from macrophages, however, its direct effect against HSCs in vivo is needed to be elucidated. Given that BM adipose tissue is an endocrine organ and adipocytes are the major cellular component in ablated marrow, we hypothesized that adiponectin derived from adipocytes might be implicated in HSC activation. Adiponectin-null (adipo-/-) mice showed normal hematopoiesis in a steady state, but significantly delayed hematopoietic recovery after 5-fluorouracil (5-FU) administration. In 5-FU-treated BM, adipo-/- HSCs were more quiescent than adipo+/+ counterparts. Strikingly, adipo-/- HSCs were shown to be defective in mTORC1 activation, phosphorylation of S6 and mitochondrial activation. mTORC1 inhibition by rapamycin cancelled the effect of adiponectin. Recombinant adiponectin enhanced not only 5-FU-induced HSC activation in vivo but also cytokine-induced activation in vitro, shortened the time to first division, without affecting subsequent proliferation of HSCs. The adiponectin level in BM had a 4-fold increase after 5-FU treatment while the plasma level remained unchanged. Though various BM cell components expressed adiponectin mRNA, adipocytes had the highest. Furthermore, reciprocal transplants with adipo+/+ and adipo -/- mice demonstrated that adiponectin from BM environment of recipient mice plays a major role in the activation of HSC. These data reveal that adiponectin, produced mainly from adipocytes, positively regulates HSC activation and subsequent hematopoietic recovery. Our data also highlight adipocytes as a source of adiponectin to ensure the proliferative burst of hematopoietic cells in ablated marrow.

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F1053

PSC-DERIVED HEMATOPOIETIC SYSTEM TO ELUCIDATE THE COOPERATION BETWEEN GENE ALTERATIONS AND ORIGINAL CELL LINEAGES IN LEUKEMOGENESIS

Niwa, Akira, Saito, Megumu and Nakahata, Tatsutoshi, Center for IPS Cell Research and Application, Kyoto University, Kyoto, Japan

Onset of acute myeloid leukemia (AML) has been accounted for by accumulated genetic mutations including chromosomal abnormalities. For example, AML1-ETO fusion (AE) by t(8;21) (q22;q22) and MLL-AF9 (MF9) by t(11;9) (q23;q22), both of which have been thought to impair cell differentiation are among the common causes of pediatric AML. However, although those alterations are thought to occur in very immature stages, leukemia cells often show phenotypes similar to specific later-stage progenitors such as myeloid, erythroid and megakaryocytic cells. Until today, it remains unclear how those lineage specifications and mutations are cooperatively involved in disease pathogenesis. To address this issue in reproducible manner, we generated the lines of human pluripotent stem cells (hPSCs) harbouring doxycycline (Dox) inducible AE and MF9 expression cassette respectively, and applied them for hematopoietic differentiations in a step-wise manner. First, when cKIT(822K) mutation was additionally transduced, both AE and MF lines showed reinforced potential in growth and prolonged colony forming efficacies in serial replanting assays in methylcel-

lulose-containing semisolid media as well as liquid culture. In addition to in vitro assays, in vivo transplantation of subjected line-derived hematopoietic cells into immunodeficient NOG mice also revealed the increased engraftment with leukemia-like phenotypes. More interestingly, both lines showed significantly less cytokine-dependencies in proliferation and colony-forming potential when committed into myeloid lineages compared with erythroid lineages. We are now searching cooperating mechanisms associated with specific cell stages or lineages in AML transformation related to AE and MF9 mutations, respectively. In conclusion, we succeeded in establishing the way to dissect the leukemogenesis from the view of relationships between cell stages and gene alterations using PSCs. We believe that our model will enable us to better understand the pathogenesis of leukemia.

F1055

DECIPHERING THE PROXIMAL AND DISTAL CIS-REGULATORY LOGIC OF CEBPA ENHANCERS DURING MYELOPOIESIS USING PREDICTIVE TRANSCRIPTIONAL MODELING

Repele, Andrea, Krueger, Shawn, Tuineau, Michelle and Manu, Manu, University of North Dakota, Grand Forks, ND, U.S.

The specification and reprogramming of cell fate are largely driven by changes in gene regulation resulting from the modulation of transcription factor (TF) expression levels. The gene regulatory logic of most TFs and target genes remains unknown. We have developed a hybrid experimental-computational methodology to infer cis-regulatory logic of distal enhancers and silencers. Our approach integrates multiple datasets, genome-wide gene expression, reporter activity, TF position weight matrices (PWMs), and DNA sequence, using a mathematical model capable of predicting regulatory activity. Using this approach, we have identified 4 enhancers of Cebpa (which encodes a TF necessary for neutrophil development) predicted to be activated by C/EBP family TFs, Egr1, and PU.1. Here, we describe the experimental validation of these predictions utilizing a high-throughput experimental pipeline. The computational model has been used to design mutations in predicted binding sites, allowing us to avoid off-target effects. We adopted the restriction-free "stitching" method Gibson assembly to synthesize the mutant reporter plasmids. These plasmids are nucleofected™ into Sfpi1-/- PU.1-inducible estrogen receptor (PUER) cell line, and assayed for Luciferase activity in 96-well formats. Using this combination of synthetic and high-throughput approaches, we have verified that PU.1 activates a novel enhancer located 30kb downstream of Cebpa. The validation of the other enhancers is ongoing. Our data suggest that Cebpa is organized into a far more complex system than previously realized, with multiple enhancers and silencers spread across the gene locus. More generally, our results demonstrate that decoding gene-regulatory logic is feasible on a large scale with the aid of transcriptional modeling.

F1057

ANGIOPOIETIN-2 PROMOTES DEFINITIVE HAEMATOPOIETIC STEM CELL MATURATION FROM THE MOUSE EMBRYONIC AGM REGION

Tamagno, Sara[1], Rybtsov, Stanislav[2] and Medvinsky, Alexander[2], [1]University of Edinburgh, Edinburgh, U.K., [2]MRC - Centre for Regenerative Medicine, The University of Edinburgh, U.K.

Definitive haematopoietic stem cells (dHSCs) are capable of self-renewing and multi-lineage reconstitution of the haematopoietic system of irradiated recipient mice. In the mouse embryo, dHSCs originate in a step-wise manner from the haematogenic endothelium, a common ancestor between the endothelial and the haematopoietic lineage. The first HSC precursor has been detected at E9.5 in the dorsal aorta, while dHSCs emerge in the aorta-gonad-mesonephros (AGM) region around E11-E12. To date, the molecular mechanisms regulating these events are poorly characterized. Angiopoietin-1 (Ang1) plays a critical role in dHSC maintenance in the adult bone marrow niche by binding to the tyrosine kinase receptor Tie2 and promoting its activation. Angiopoietin-2 (Ang2) is described as being a Tie2 antagonist, however its role in dHSC regulation processes is still unknown. In this study, we identified Ang2 as a regulator of dHSC formation in the mouse embryo. We used our ex vivo reaggregation system to culture E9.5 cells derived from the aorta and its microenvironment in presence of Ang1 and/or Ang2. We found that Ang2-treated cells were able to reconstitute the peripheral blood of recipient mice to a higher extent compared to untreated cells. Concurrently, these cells showed an impaired ability to form endothelial colonies in vitro. In all our experiments, Ang1-treated cells behaved like the untreated controls. All together, these data suggest an implication for Ang2, but not Ang1, in promoting haematopoietic specification from the haematogenic endothelium.

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F1059

TOLL-LIKE RECEPTOR 5 SIGNALING IN BONE MARROW NICHE ENHANCES HEMATOPOIETIC STEM CELLS PROLIFERATION

Zhang, Benyue and Gewirtz, Andrew T., Georgia State University, Atlanta, GA, U.S.

Bacterial flagellin, acting via toll-like receptor 5 (TLR5) and Nod-like receptor 4 (NLRC4), activates an array of innate immune signaling pathways that confer protection against a range of challenges including infection, toxic chemicals, and g-radiation. Survival after radiation largely depends on renewal of peripheral blood system by hematopoietic stem cells (HSC). We here show that flagellin treatment results in an expansion of the HSC, that mediate long-term repopulation (LTR) of all blood lineages. Murine HSC are contained in the lineage-negative, stem cell antigen 1 (SCA1)-positive and KIT-positive subset ($LIN^-SCA1^+KIT^+$, termed LSK cells) and comprises about 0.1~0.5% of bone marrow cells. LSK cells are further classified as short-term HSC, multi potential progenitors (MPP) and long term HSC (LT-HSC), the latter of which has long term renewal potential. We've observed that flagellin induces a dramatic TLR5-dependent expansion of LSK cells both in vivo and in bone marrow cell cultures. Moreover, such flagellin-induced HSC expansion predominantly reflects increased levels of LT-HSC, which are sought for many clinical applications. Flagellin induces a 10-fold increase in LT-HSC associated with a 3-fold increase in the percentage LT-HSC in S-phase. LT-HSC hold great therapeutic promise to treat a wide range of injuries and diseases but attaining sufficient numbers of these cells is often a limiting hurdle to these use. Hence, there is great interest in defining ways to boost production of these cells in vivo and ex vivo.

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F1061

INDUCTION OF HEMATOPOIETIC STEM AND PROGENITOR CELLS FROM HUMAN PLURIPOTENT STEM CELLS VIA IN VIVO SCREENING OF DEFINED FACTORS

Sugimura, Ryohichi and Daley, George Q., Boston Children's Hospital, Boston, MA, U.S.

Clinically relevant cell types have been generated from human pluripotent stem cells (hPSCs) by two approaches; one strategy attempts to mimic conserved mechanisms of embryonic development by supplementing cultures of differentiating hPSCs with morphogens in a sequential and temporally defined fashion ("evo-devo"); the other strategy employs exogenous lineage-determining master transcription factors (TFs) to drive cell fates, a form of synthetic biology. To date, neither approach has achieved induction of functional hematopoietic stem cells (HSCs) from hPSCs. We endeavored to combine morphogen-directed differentiation to definitive hemogenic endothelium with exogenous expression of TFs known to be selectively expressed in HSCs. In vivo screening revealed a combination of TFs that conferred "intermediate"-term transplantable hematopoietic progenitor cells that contributed to mutilineage cells of monoclonal origin in primary recipients for 14 weeks and 8 weeks in secondary, but diminishing thereafter likely due to constitutive cycling. Terminally differentiated cells included enucleated erythrocytes expressing adult beta globin genes, neutrophils, IgM/CD19+ B-cells and αβ T-cells. The functionality of those cells was validated by the production of myeloperoxidase, IgM and IgG in serum, and IFNγ respectively. In conclusion, a combinatorial approach exploiting both the evo-devo strategy and synthetic biology has induced functional hematopoietic stem and progenitor cells from hPSCs that mediate multilineage hematopoiesis in vivo.

CARDIAC CELLS

F1065

PHYSICAL FREQUENCY WAVES BY REAC TECHNOLOGY IMPROVE CHEMICALLY-INDUCED CARDIAC DIFFERENTIATION IN UIPSCS

Basoli, Valentina[1], Santaniello, Sara[1], Pigliaru, Gianfranco[1], Matthias, Wieser[2], Strajeriu, Agata[2], Castagna, Alessandro[3], Fontani, Vania[3], Rinaldi, Salvatore[3], Redl, Heinz[4], Ventura, Carlo[5,6], Grillari, Regina[2,7] and Maioli, Margherita[1,8], [1]University Of Sassari, Sassari, Italy, [2]Evercyte GmbH, Vienna, Austria, [3]Rinaldi Fontani Institute, Florence, Italy, [4]Ludwig Boltzmann Institute for Experimental & Clinical Traumatology, Vienna, Austria, [5]Ettore Sansavini Health Science Foundation, Lugo, Italy, [6]National Institute of Biostructures and Biosystems, Bologna, Italy, [7]University of Natural Resources and Life Sciences, Vienna, Austria, [8]Consiglio Nazionale delle Ricerche (CNR), Monserrato, Cagliari, Italy

Regenerative medicine based on stem cells technology offers intriguing tools for both chronic and genetic-based heart disease. Induced pluripotent stem cells (iPSCs) allow derivation of pluripotent progenitors from somatic sources, offering the opportunity to generate the own-patient stem-cells based therapy. Other authors demonstrated that iPSCs represent a valid source, able to yield de novo heart tissue. Within this context iPSCs derived from urine can be very easily obtained through a totally non-invasive procedure. Unfortunately, this process exhibit a variable yield. In previous works we demonstrated that stem cells could be committed toward cardiac phenotype us-

ing physical energy. We used an electromagnetic field delivered through a radio electric asymmetric conveyor (REAC) a device that emit frequency waves around 2.4 GHz directly on cells in culture. In the present work we decided to induce cardiogenesis in urine iPS by the exposure to both a conditioned medium (a chemical stimulus) and a physical stimulus (electromagnetic fields). In particular, the chemical treatment included Bmp4, recombinant Activin A, and the Wnt inhibitor IWR-1, physical stimulus was delivered by REAC. Cells treated to both stimuli exhibited higher levels of the cardiogenic genes GATA4, and Nkx-2.5 and of the cardiac specific transcripts MHC, TBX5, cTnT, MEF2C and ACT2. Protein analysis performed by confocal microscopy, further inferred this molecular data. In addition also the number of iPS-derived beating clusters of myocardial cells were higher in iPS exposed to both stimuli, as compared to cells exposed to one stimulus alone. We investigated also the effect of the combined stimuli on the expression of three dimetiltransferase proteins (DMNT3A, DMNT3B and DMNT1) involved in new DNA methylation. Interestingly DMNT3B gene expression was higher in cells exposed to both stimuli in combination compared to the cells exposed to the chemical stimulus alone, suggesting an action of physical energy waves involved directly on epigenetic regulation. On the whole, we have developed a new method based upon the combination of chemical and physical stimuli means to afford a high-throughput yield of cardiac differentiation from human urine-derived iPS, paving the way to future developments in cardiovascular regenerative medicine.

F1067

ELECTROTAXIS OF MOUSE CARDIAC PROGENITOR, CARDIAC FIBROBLAST, AND HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIAC PROGENITOR CELLS REQUIRES SERUM AND IS DIRECTED VIA PI3K

Frederich, Bert Joseph[1], Timofeyev, Valeriy[1], Thai, Phung[1], Lau, Victor[1], Sirish, Padmini[1] and Chiamvimonvat, Nipavan[1,2], [1]UC Davis, Davis, CA, U.S., [2]Department of Veterans Affairs, Mather, CA, U.S.

Cardiovascular disease is the largest cause of mortality in the world. Specifically, in the United States, 1 in 6 people suffer from coronary artery disease, which increases their risk of heart attacks. The limited regenerative capacity of cardiac tissue has long been an obstacle to treating damaged myocardium. Cell-based treatment offers an enormous potential as an alternative therapy. However, the efficacy of cell-based therapies remains limited by inefficient delivery and engraftment. Electrotaxis, electrically guided cell movement, has been clinically utilized to improve recovery in a number of tissues but has not been investigated for treating myocardial damage. The goal of the study is to test the electrotactic behaviors of several types of cardiac cells. Cardiac Progenitor Cells (CPCs) and Cardiac Fibroblasts (CFs) electrotax towards the anode of a direct current electric field, while human induced Pluripotent Stem cell-derived Cardiac Progenitor Cells (hiPS-CPCs) electrotax toward the cathode. The voltage-dependent electrotaxis of CPCs and CFs requires the presence of serum in the media. Moreover, addition of soluble vascular cell adhesion molecule (sVCAM) to serum-free media restores directed migration. We further present evidence that CPC and CF electrotaxis is mediated through phosphatidylinositide 3-kinases (PI3′K) signaling. In addition, Very Late Antigen-4 (VLA4), an integrin and growth factor receptor, is required for electrotaxis and localizes to the anodal edge of CPCs in response to direct current electric field. HiPS-CPCs do not express VLA4, migrate toward the cathode in a voltage-dependent manner, and similarly to CPCs and CFs require media serum and PI3′K activity for electrotaxis. The electrotactic behaviors of these therapeutic cardiac cells is a novel finding and could be utilized for improving the state of therapy for recovering function in damaged myocardium.

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F1069

AUTOPHAGY IS A NOBEL AND CRITICAL MECHANISM DEVELOPING HEART FAILURE OF DUCHENNE MUSCULAR DYSTROPHY

Hirata, Takuya, Baba, Shiro, Yoshinaga, Daisuke and Heike, Toshio, Department of Pediatrics, Kyoto University, Kyoto City, Japan

In recent progress of medicine, the main cause of death of Duchenne Muscular Dystrophy (DMD) have switched from respiratory failure to heart failure. However, the mechanism of developing the heart failure in DMD has not been revealed clearly. Therefore, we cannot optimize effective therapies. Although a report, published in 2015, demonstrated that apoptosis was one of the mechanisms of pathogenesis assessed by human induced pluripotent stem cells (iPSC), the report did not conclude that apoptosis was the main mechanism of DMD heart failure. In our study, we investigated cell death using human iPSC and mdx mice (DMD model mice) to discover a new possible targeting therapy. First, we generated DMD patient specific and control iPSC, and differentiated the iPSCs into cardiomyocytes (iPSC-CMs). Size and shape of DMD iPS-CMs were almost same as those of control iPS-CMs. However, the DMD iPS-CMs were more vulnerable than the control iPS-CMs. Thus, we studied cell death using isoproterenol (Iso) load. Even after Iso load to iPS-CMs, there was no significant difference between DMD and control iPSC-CMs in analysis of Caspase 3 and TUNEL staining. In an experiment using GFP-mRFP-LC3, the number of au-

tophagosome in DMD iPS-CMS increased significantly after Iso load. The same phenomenon was observed in mdx mice cardiomyocytes. In addition, mdx mice cardiac function decreased significantly assessed by echocardiography, and their cardiac fibrosis area increased after 0.5mg/kg/day continuous Iso medication. Immunostaining of LC3 showed an increase number of autophagosome. After chloroquine, which was an inhibitor of autophagy, was administered 1.2mg/day orally to mdx mice, their cardiac function became better and the fibrosis area reduced remarkably. In conclusion, heart failure of DMD was associated with autophagy rather than apoptosis. Therefore, chloroquine could be a new and effective medicine for DMD patients.

F1071

ESTABLISHMENT OF AN IPSC DISEASE MODEL TO CHARACTERIZE VASCULAR SMOOTH MUSCLE CELLS IN MARFAN SYNDROME

Klein, Sandra, Yarrabothula, Akshitha and Schaniel, Christoph, Icahn School of Medicine at Mt. Sinai, New York City, NY, U.S.

Marfan syndrome (MFS) is a connective tissue disorder with an estimated prevalence of 1:3000-5000 men, women and children worldwide. The disease is caused by mutations/ deletions in the fibrillin-1 (FBN1) gene that encodes for an extracellular matrix glycoprotein involved in the regulation of TGFβ signaling. The major pathologic MFS manifestations affect the skeletal, ocular and cardiovascular system; the latter is the most dominant and life threatening condition. Approximately 90% of patients develop a thoracic aortic aneurysm (TAA) with an onset at an early age. The TAA in MFS is exclusively located in the ascending aorta. Even though the aortic wall is composed of a variety of cell types, it is suggested that its weakening in MFS originates from a defect in the vascular smooth muscle cell (VSMC) population. In order to establish a disease model that allows the characterization of potential molecular abnormalities in MFS-VSMCs, we have generated induced pluripotent stem cells (iPSCs) from skin fibroblasts of patients that carry FBN1 mutations/ deletions. We have subsequently differentiated MFS-iPSCs into VSMCs with an intermediate step that converts iPSCs into neural crest cells, the developmental origin of the ascending aorta. The neural crest cells expressed the markers Pax6 and Nestin and we have furthermore observed the morphology of neural rosettes. VSMCs generated from those neural crest cells passed essential quality controls: (a) They transformed into the spindle shaped morphology typically associated with VSMCs, (b) expressed the VSMC markers ACTA2 and TAGLN and (c) possessed contractile function. Furthermore, preliminary RNA seq data indicated that neural crest cells and VSMCs activated signaling pathways specific to those cell types. The established iPSC model will provide a patient-specific in vitro system to investigate molecular characteristics of MFS-VSMCs to ultimately identify potential targets for TAA treatment.

F1073

EFFICIENT CARDIOMYOCYTE DIFFERENTIATION AND STABLE ELECTROPHYSIOLOGICAL SENSOR REPORTER FROM NON-HUMAN PRIMATE IPSCS

Lin, Yongshun[1], Liu, Huimin[2], Hong, So Gun[2], Linask, Karri[2], Titus, Steve[3], Zheng, Wei[3], Boehm, Manfred[2], Dunbar, Cynthia[2] and Zou, Jizhong[2], [1]NIH, Bethesda, MD, U.S., [2]NHLBI/NIH, Bethesda, MD, U.S., [3]NCATS/NIH, Bethesda, MD, U.S.

Recent advances in cardiomycocyte (CM) differentiation methods for human induced pluripotent stem cells (hiPSCs) prompt a wave of pre-clinical testing of hiPSC-CM in animal models for treating cardiomyopathies. Given their strong physiological similarity to humans, non-human primates (NHPs) are considered better large animal models than rodents for this purpose, although species-specific barriers still complicate the results. Alternatively, allogeneic or autologous cell therapy model can be established using NHP to NHP transplantation that requires large-scale production of NHP iPSC-derived CMs and evaluation of their physiological functions in vitro and in vivo. Although multiple serum-free protocols have been developed to differentiate hiPSCs, efficient NHP iPSC-CM differentiation has been rarely reported. Here we described the development of a method to robustly differentiate cardiomyocytes from rhesus macaque iPSCs (RhiPSCs) in fully chemically defined media. By optimizing the combination of recombinant growth factors and chemical inhibitors that modulate signaling pathways such as Wnt, BMP, Activin, and FGF, we demonstrated that beating cTNT+ cardiomyocytes could be efficiently derived from rhesus macaque iPSCs with up to 90% purity in one week under a serum-free and albumin-free chemically defined condition. 1-2x10^7 RhiPSC-CMs can be derived from one 6-well plate of RhiPSCs (~1x10^6), and they recover efficiently from cryopreservation and mature under in vitro culture. To further facilitate in vitro and in vivo applications of these rhesus iPSCs-CMs, we also applied gene editing to knock-in genetically encode calcium sensor indicators at Rhesus safe harbor locus in these iPSCs, which enables the visualization of intracellular calcium flux in RhiPSC-CMs and chemical compounds screening. In summary, our approach provides a powerful tool for generating desired NHP iPSCs-derived cardiomyocytes that could be utilized in basic research and pre-clinical translation studies.

F1075

IMPROVING MATURATION OF HPSC-DERIVED CARDIOMYOCYTES VIA MICROPATTERNS ON SOFT SUBSTRATES

Napiwocki, Brett N.[1,2], Salick, Max R[3], Ashton, Randolph S.[1,4] and Crone, Wendy C.[1,2], [1]University of Wisconsin-Madison, Madison, WI, U.S., [2]University of Wisconsin-Madison, Wisconsin Institutes for Discovery, Madison, WI, U.S., [3]Novartis Institute for Biomedical Research, Cambridge, MA, U.S., [4]Wisconsin Institutes for Discovery, University of Wisconsin-Madison, Madison, WI, U.S.

With the advent of well-established protocols to derive cardiomyocytes from human pluripotent stem cells (hPSC-CMs), researchers are now able to generate an unlimited supply of heart cells which will redefine regenerative medicine therapies, disease modeling, drug testing, developmental and cardiotoxicity studies; however, hPSC-CMs derived from these protocols are immature and more similar to fetal CMs than they are to primary adult CMs which limits their usefulness. For example, both hPSC-CMs and fetal CMs grown in vitro are pleomorphic in shape and have misaligned myofibrils which disrupts the contractile activity of the heart muscle cells. In comparison, micropatterned hPSC-CMs and adult CMs are rod-shaped and display a highly organized internal cytoskeletal structure when our lab used microcontact printing on glass slides to control the cell shape of hPSC-CMs. To further enhance the level of maturation seen in hPSC-CMs, it is believed multiple cues will be necessary to create a more physiologically-relevant model of cardiac tissue. Our new work has focused on the combination of patterning methods with softer substrates using both microcontact printing, as well as a sacrificial polyvinyl alcohol (PVA) film. The use of multiple stimuli better recapitulates native human heart tissue and is shown to augment the maturation status of hPSC-CMs, thus bringing them one step closer to modeling mature, human cardiomyocytes.

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F1077

REPRODUCIBLE CARDIOTOXIC RESPONSE IN CARDIOMYOCYTES ANALYZED WITH MEA TECHNOLOGY

Runeberg, Kristina, Takara Bio Europe AB, Gothenburg, Sweden

Failure to correctly predict adverse cardiotoxic effects of new pharmaceuticals is the major cause of compound attrition during the drug development process, as well as the main reason for the withdrawal of approved drugs already on the market. This is partly due to the lack of relevant human models for pre-clinical testing. However, recent developments in reprogramming adult human somatic cells into induced pluripotent stem cells (hiPSC) and differentiating them into a desired cell type have made it possible to access physiologically-relevant, human material with consistent and known genetic backgrounds. At present, human iPS cell-derived cardiomyocytes (hiPS-CM) are being actively investigated for their ability to accurately predict cardiotoxic responses arising from new chemical drug entities. In this study, three different lots of iPS cell-derived cardiomyocytes (from iPS cell line, ChiPSC22) were validated by multi-electrode array (MEA) for their ability to predict cardiotoxic effects from a selection of reference compounds directed towards sodium, potassium and calcium channels. These hiPS-CMs, cultured in a monolayer without genetic engineering or selection, displayed spontaneous beating and expressed >80% cardiac troponin T. The cells were plated on MEA probes and stable signals were recorded over several days. Upon exposure to increasing concentrations of the hERG channel blocker E-4031, the cells' field potential duration (FPD) was prolonged in an expected dose-dependent manner. Similarly, FPD prolongation was also displayed when treated with Mexiletine, a non-selective, voltage-gated sodium channel blocker. Nifedipine, an L-type calcium channel blocker, caused a shortened FPD, whereas DMSO (used as a vehicle control), did not affect the FPD. In addition, the beat frequency of the hiPS-CMs was, as expected, increased in response to beta-adrenergic stimulation with Isoproterenol. By analyzing three separate lots of hiPS-CMs, we were able to reproduce all of the data described above, indicating low lot-to-lot variability. Taken together, this data illustrates the potential for our hiPS cell-derived Cardiomyocytes to be used in combination with the MEA methodology and provides promising possibilities for further investigation of cardiac liabilities.

F1079

TRANSCRIPTIONAL PROFILING OF HUMAN EMBRYONIC STEM CELLS DURING MESODERMAL- AND CARDIAC DIFFERENTIATION

Synnergren, Jane Marie[1], Ameén, Caroline[2], Åkesson, Karolina[2], Karlsson, Alexander[1], Riveiro, Maria[1], Andersson, Christian X[2] and Sartipy, Peter[1], [1]University of Skovde, Lidkoping, Sweden, [2]Takara Bio Europe, Gothenburg, Sweden

Human pluripotent stem cells can provide an unlimited source of functional cells, such as cardiomyocytes, which are challenging to obtain from primary sources. However, a better understanding of the early specification processes is needed to improve the selective differentiation of clinically relevant and functional human cardiomyocytes, for use in applications such as drug discovery, toxicity

testing, and cell replacement therapy. In this study, we used human embryonic stem cells that were differentiated via mesoderm to cardiomyocytes with an EB protocol. Cells were harvested daily from day 0 to day 10 during the differentiation process, as well as at day 21, which represent more mature cardiomyocytes. Global gene expression profiling using microarrays was performed in order to study regulatory mechanisms involved in mesoderm- and cardiac differentiation. Clustering analysis was applied and groups of genes with similar expression patterns were identified and further investigated for putative co-regulation. Transcription factors that are known from previous studies to be critical during mesoderm differentiation (e.g., brachyury) showed expected expression profiles, but also other transcription factors with similar patterns were identified as potential key players in these early cardiac specification processes (e.g., NKX2-5, GATA4, YY1, SP1 and TBX5). Furthermore, several signal transduction pathways (e.g the Calcium-, TGF-beta-, Wnt-, and Notch signaling pathways) that are known to be involved in differentiation processes were selected and profiles from genes in these pathways were clustered to reveal putative modules of co-regulation. For example, a cluster of 20 genes were identified from genes in the TGF-beta-signaling pathway and 25 genes involved in the calcium-signaling pathway, including two well-known cardiac related genes (RYR2 and PLN), that show a peak in gene expression at day 1 and 2, down-regulation at day 3-5, and then up-regulation again at day 6 with continuous increased expression until the end of differentiation at day 21. Taken together, the results from this study revealed interesting transcriptional patterns during early mesoderm- and cardiac differentiation, and provide further insights into the molecular machinery regulating cell specification and differentiation.

F1081

GENETICALLY ENGINEERED HUMAN INDUCED PLURIPOTENT STEM CELLS TO STUDY CRONOS TITIN IN SARCOMERE FORMATION AND FUNCTION

Zaunbrecher, Rebecca, Klaiman, Jordan M., Pabon, Lil, Reinecke, Hans, Regnier, Michael and Murry, Charles, University of Washington, Seattle, WA, U.S.

The giant protein titin plays numerous important roles in the cardiomyocyte, including providing passive tension and facilitating sarcomere formation. Recently an internal promoter was identified in the titin gene (TTN) indicating the presence of a previously unstudied isoform, Cronos. Although the function of Cronos titin is unknown, the majority of disease-causing mutations in TTN are found downstream of this internal promoter suggesting an important role in health and disease. To create a cell line to study the role of Cronos titin we have introduced homozygous frameshift mutations in exon 2 of the TTN gene in human induced pluripotent stem cells (Ex2 KO hiPSCs) using the CRISPR/Cas9 system to prevent translation of full-length titin while leaving the Cronos isoform intact. Directed differentiation of Ex2 KO hiPSCs into cardiomyocytes (Ex2 KO hiPSC-CMs) yields contracting cells, and immunofluorescence studies indicate the formation of short, dispersed myofibrils in Ex2 KO hiPSC-CMs compared to isogenic wildtype controls. Additionally, staining Ex2 KO hiPSC-CMs with antibodies specific to the MIR and M8-M10 regions of titin downstream of the Cronos internal promoter demonstrates incorporation of these domains into the sarcomere. Staining of Ex2 KO hiPSC-CMs with antibodies specific to the Z1Z2 and PEVK regions of titin upstream of the internal Cronos promoter suggests these domains are not present in the sarcomeres. We conclude that the Ex2 KO hiPSC-CMs present a system in which to study Cronos titin and could provide important insights into the role of this isoform in sarcomere formation and function.

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MUSCLE CELLS

F1085

MATURATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO FETAL OR ADULT LIKE SKELETAL MUSCLE SATELLITE CELLS

Hicks, Michael R.[1], Hiserodt, Julia[1], Fujiwara, Wakana[1], Paras, Katrina[1], XI, Haibin[2], Jan, Majib[1] and Pyle, April[2], [1]Department of Microbiology, Immunology and Molecular Genetics, Center for Duchenne Muscular Dystrophy, and Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles, CA, U.S., [2]UCLA, Los Angeles, CA, U.S.

Satellite cells (SCs) are the endogenous muscle stem cells capable of repairing damaged skeletal muscle. Cell based strategies that harness the regenerative potential of SCs could significantly improve the disease course for several muscle wasting conditions including the most prevalent pediatric muscle disorder Duchenne Muscular Dystrophy (DMD). However, obtaining SCs is difficult and ex vivo manipulation or expansion results in loss of SC self-renewal. Recent reports have successfully directed the differentiation of human induced pluripotent stem cells (hiPSCs) to skeletal muscle progenitor cells (SMPCs) without lentiviral-mediated overexpression. We have developed DMD-hiPSCs and a platform to generate “reframed’ SMPCs using CRISPR/Cas9 gene editing to restore dystrophin expression and protein function. Translation of this platform using stem cell delivery has

the potential for a lifelong cure for patients with DMD. However, hPSC-SMPCs are morphologically and functionally different than fetal or adult SMPC/SCs and their therapeutic potential is unclear. To define the developmental status of hPSC-SMPCs, hPSC, fetal (week 9-17), and adult SMPC/SCs were evaluated using in vitro fusion assays, in vivo engraftment assays, and profiled by RNA-SEQ. HPSC-SMPCs fused to form significantly fewer myofibers in vitro and in vivo compared to fetal or adult SCs. RNA-SEQ identified differential expression of surface receptors and signaling pathways between developmental stages of SMPC/SCs as potential regulators of diminished myogenic potential. HPSC-SMPCs enriched for fetal receptors significantly improved fusion over unsorted/passaged or NCAM-sorted SMPCs in vitro and in vivo. Further, inhibition of over activated embryonic signaling pathways improved myogenesis from all hPSC lines tested to levels of fetal and adult SC-derived myotubes. We are currently developing a nanoparticle platform to co-deliver small molecule inhibitors with SMPC subpopulations, which we expect will enable robust engraftment in vivo. These discoveries are currently being applied to CRISPR/Cas9 'reframed' DMD hiPSCs and tested for engraftment and dystrophin restoration in preclinical mouse models of DMD.

Funding Source: CureDuchenne Fellowship

F1087

RESCUE OF DIAPHRAGM FUNCTION UPON INFUSION OF MYOGENIC PROGENITORS IN DYSTROPHIC MICE

Olivera, Nelio AJ[1], Magli, Alessandro[2], Zhan, W-Z[3], Oliveira, Vkp[2], Selvaraj, S[2], Mantilla, CB[3] and Perlingeiro, Rita C.R.[1], [1]University of Minnesota, Minneapolis, MN, U.S., [2]University of Minnesota, Minneapolis, MN, U.S., [3]Mayo Clinic, Rochester, MN, U.S.

The Fukutin related protein (*FKRP*) gene encodes for a protein involved in the O-glycosylation of α-Dystroglycan (α-DG), one of the components of the Dystrophin Glycoprotein complex (DGC). Because of the importance of O-glycosylated α-DG in connecting the cytoskeletal components to the extracellular matrix, alteration of this process by mutations in the FKRP coding region results in muscular dystrophy (MD). FKRP associated muscular dystrophies are characterized by a broad spectrum of phenotypes and, similarly to other MDs, cardiac and respiratory failures are the main causes of mortality. Our group has previously shown that both ES- and iPS-derived myogenic progenitors are ideal candidates for the development of cell therapy approaches to efficiently treat MDs. To develop an appropriate cell therapy protocol for this disease, we used a previously published animal model, in which a point mutation was introduced in the FKRP coding sequence ($FKRP^{mut}$). Importantly, these mice recapitulate the muscle pathology observed in FKRP-associated MDs, in particular the diaphragm, which is totally absent of α-DG glycosylation. To test whether disease phenotype could be reversed upon cell therapy, we took advantage of the ability of pluripotent-derived myogenic progenitors to home to affected skeletal muscles upon systemic delivery. Our results show that intravenous (IV) injection of GFP-labeled myogenic progenitors from inducible-Pax3 mouse ES cells into FKRP-mutant mice resulted in extensive engraftment in the diaphragm. Immunohistochemistry analysis revealed positive sites of α-DG glycosylation that co-stained with GFP solely in mice that had received cell transplantation. Importantly, engraftment was accompanied by improvement in diaphragm contractility. Engraftment was also observed in other skeletal muscles, but no donor cells were detected in other organs like lung, liver, brain and heart, attesting for tissue specificity and safety of these cell preparations. These findings demonstrate for the first time the feasibility of targeting the diaphragm upon systemic stem cell transplantation in dystrophic mice, which is extremely relevant for the potential future treatment of patients affected by muscular dystrophy.

Funding Source: LGMD2ifund

F1089

ROTATOR CUFF TEAR STATE MODULATES THE DIFFERENTIATION AND SELF-RENEWAL OF HUMAN SKELETAL MUSCLE PROGENITOR CELLS

Thomas, Kelsey[1], Gibbons, Michael[1], Lane, John[1], Singh, Anshuman[2], Ward, Samuel[1] and Engler, Adam[1], [1]University of California, San Diego, La Jolla, CA, U.S., [2]Kaiser Permanente, San Diego, CA, U.S.

Human skeletal muscle progenitors (hSMPs) are critical for muscle growth and repair but are limited in both number and ex-vivo expansion capabilities, which currently limits their potential in cell-based therapies. Muscle tissue source and age are crucial factors in proliferation and differentiation ability. Given limited donor supply, we determined if hSMPs isolated from aged patients undergoing rotator cuff (RC) repair could be expanded into a clinically viable cell population. To model in vivo niche characteristics which were assessed by mass spectrometry, polyacrylamide (PA) gels of physiological stiffness were created with collagen type IV and laminin-111 ECM protein coatings. hSMPs were then culture-expanded using several different growth factors and subsequently differentiated in myogenic medium to assess the ability of these niches to promote expansion without phenotype loss. Data show that the addition of FGF2 significantly improves proliferation of hSMPs whereas oxytocin suppresses it, despite mouse evidence to the contrary. Furthermore, hSMPs isolated from muscles from torn RCs differentiate into myosin heavy chain-positive myotubes at higher rates than hSMPs isolated from no tear RC mus-

cles, consistent with prior observation that hSMPs are more activated in muscles from RC tears; FGF2 and IGF appear most effective. Together these data suggest that ECM composition and stiffness in conjunction with specific growth factors aid in expanding hSMPs from aged donors with specific combinations resulting minimal loss of self-renewal and differentiation capacity. However significant disagreement with mouse literature and other human muscle types and injury states imply that expansion and therapeutic use will be highly specific to the niche.

PANCREATIC, LIVER, LUNG, OR INTESTINAL/GUT CELLS

F1093

ACTIVATING STAT3 MUTATION CAUSING NEONATAL DIABETES CORRECTED WITH CRISPR IN PATIENT-DERIVED IPS CELLS

Balboa, Diego[1], Saarimäki-Vire, Jonna Marianne[1], Eurola, Solja[1], Grym, Heli[1], Ustinov, Jarkko[1], Valensisi, Cristina[2], Andrus, Colin[2], Saarikettu, Juha[3], Varjosalo, Markku[4], Silvennoinen, Olli[3], Hawkins, David[2] and Otonkoski, Timo[1], [1]Research Program of Molecular Neurology and Biomedicum Stem Cell Center, University of Helsinki, Helsinki, Finland, [2]Division of Medical Genetics, Department of Medicine, Department of Genome Sciences, Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Seattle, WA, U.S., [3]Laboratory of Molecular Immunology, School of Medicine and Institute of Biomedical Technology, Biomeditech, University of Tampere, Finland, Tampere, Finland, [4]Molecular Systems Biology Research Group, Institute of Biotechnology, University of Helsinki, Finland, Helsinki, Finland

Permanent neonatal diabetes is usually caused by mutations impairing the correct development or function of the pancreatic beta cells. A recent exome sequencing screening of neonatal diabetes cases found de novo germline activating mutations in STAT3. The patient with the most activating STAT3 mutation K392R presented hypoplastic pancreas and high beta-cell autoantibody levels already at birth. We hypothesize that the mutation may cause pancreatic developmental failure due to altered STAT3 signaling. iPS cells were derived from patient fibroblasts and differentiated towards pancreas using a 17-day stepwise protocol leading to efficient specification of endocrine progenitors. Expression of pancreatic progenitor markers Pdx1 and Nkx6.1 did not differ between STAT3^{K392R} cells and healthy controls. Instead, endocrine differentiation master regulator NEUROG3 expression was upregulated prematurely in STAT3^{K392R} cells, together with significantly higher expression levels of its downstream targets Nkx2.2 (4-5 fold,) Ins (10-fold) and GCG (5-fold). RT-qPCR results were confirmed by immunocytochemistry, with more NEUROg3-positive nuclei after 13 days and markedly more ins-positive area after 17 days, and by RNA-seq, showing robust upregulation of all NEUROG3 downstream targets. To demonstrate this phenotype is caused by STAT3^{K392R} mutation, we corrected it using CRISPR with guide RNAs targeting next to the mutation site and a 202 bp double stranded DNA repair template. Corrected isogenic cells differentiated similarly to control cells, showing a complete reversion of the disease phenotype. STAT3^{K392R} activating properties are not a result of increased DNA-binding affinity or changes in its phosphorylation status. Further studies on the mechanism leading to upregulation of NEUROG3 by STAT3^{K392R} are ongoing. Our results show that overactive STAT3 leads to abnormally early NEUROG3 activation and consequent premature endocrine differentiation. This is expected to result in reduction of the pancreatic progenitor pool, leading to pancreatic hypoplasia. Patient-specific iPSC in combination with CRISPR-based genome editing are a valuable tool for recapitulating pancreatic developmental defects, enabling the study of pathogenetic mechanisms leading to monogenic diabetes.

F1095

THE ROLE OF HNF1B IN HUMAN PANCREAS AND LIVER DEVELOPMENT AND DIABETES

El Khairi, Ranna[1,2], Hattersley, Andrew[3] and Vallier, Ludovic[1,2], [1]Wellcome Trust Sanger Institute, Cambridge, U.K., [2]Cambridge Stem Cell Institute, Cambridge, U.K., [3]University of Exeter Medical School, Exeter, U.K.

Diabetes mellitus is a heterogeneous disorder with multiple aetiologies. Monogenic diabetes accounts for an estimated 2–5% of cases and is often associated with impaired pancreas development and beta-cell dysfunction. Heterozygous mutations in the transcription factor, HNF1B, result in multisystem disease including diabetes due to beta-cell dysfunction, hepatic insulin resistance and pancreatic hypoplasia. However, the mechanisms that underlie development of diabetes in HNF1B mutation carriers are still not fully understood due to lack of an appropriate model system. Human pluripotent stem cells (PSCs) can be differentiated along the pancreatic or hepatic lineages and present a unique tool to model human pancreatic and liver development and disease. The aim of this project is to use a human PSC based model system to determine the molecular mechanisms by which HNF1B mutations cause pancreatic hypoplasia and diabetes. Human PSC models of HNF1B-associated diabetes were created through knockout of HNF1B in human PSCs and generation of induced PSCs (iPSC) from patients. HNF1B deficient PSCs were differentiated along pancreatic and hepatic lineages to investigate the effect of HNF1B mu-

tations on human pancreas and liver development and function. Preliminary experiments analysed the normal expression pattern of HNF1B and showed upregulation of HNF1B at the foregut stage, and during pancreatic and liver specification. Homozygous knockout of HNF1B resulted in failure of foregut and pancreatic and hepatic progenitor development, while heterozygous knockout of HNF1B resulted in impairment of pancreatic progenitor specification. This in vitro model will allow further understanding of the molecular mechanisms by which HNF1B regulates human pancreas and liver development and function, as well as potentially identifying new genes and pathways that contribute to diabetes pathogenesis and providing novel therapeutic targets.

Funding Source: Wellcome Trust, UK

F1097

LAMININ-BASED MATRICES FOR HEPATIC-LINEAGE SPECIFICATION OF HUMAN PLURIPOTENT STEM CELLS

Kanninen, Liisa[1], Harjumäki, Riina Maria[1], Peltoniemi, Pasi[1], Porola, Pauliina[1], Niklander, Johanna[1], Smutný, Tomáš[1,2], Malinen, Melina[1], Urtti, Arto[1,3], Yliperttula, Marjo[1] and Lou, Yan-Ru[1], [1]University of Helsinki, Helsinki, Finland, [2]Charles University in Prague, Prague, Czech Republic, [3]University of Eastern Finland, Kuopio, Finland

There is an extensive need of functional human hepatocytes in drug development to investigate biotransformation pathways and possible hepatotoxicity of a new drug candidate. Human pluripotent stem cells (hPSC) offer an alternative source to primary hepatocytes, golden standard cell type, used in in vitro liver models. Current hepatic differentiation protocols mainly rely on the use of growth factors even though cell-matrix interactions are also known to play an important role in the complex cell differentiation process. In vitro culture systems can be improved by applying tissue-specific extracellular cues to the cultured cells by using extracellular matrix (ECM) proteins. In hepatic differentiation, the definitive endoderm (DE) cells first differentiate into hepatic progenitors. However, the optimal culture matrix for in vitro hepatic differentiation of the DE cells is poorly understood. We hypothesized that ECM produced by human liver progenitors would induce efficient hepatic specification of the DE cells and, thus, we aimed to create a stage-specific matrix to assist the hepatic lineage differentiation. We created acellular matrix (ACM) from human HepaRG cells that is a well-known liver progenitor cell line. We showed that HepaRG-ACM supports the attachment of DE cells derived from three hPSC lines and, more importantly, their hepatic-lineage differentiation. Next, we characterized the ECM proteins secreted by HepaRG cells and identified fibronectin and laminin α5. The identified ECM proteins were used for plating DE cells individually and in combinations. We noticed that both fibronectin and laminin α5-based matrices can reproduce the effect of HepaRG-ACM. The hPSC-derived DE cells were successfully differentiated towards hepatic cells expressing typical liver markers and their hepatic functions were upregulated during the culture period. In turn, matrices including Matrigel, laminin-111, and collagen types I and III failed to support the attachment or growth of DE cells. In conclusion, laminin α5 is a promising defined matrix for hepatic differentiation.

F1099

USING PATIENT SPECIFIC IPSCS TO MODEL LIVER DISEASE IN ZZ-MUTATIONS OF ALPHA-1 ANTITRYPSIN (AAT)

Marsolais, Renee Parker[1,2], Taketani, Tamara N.[3,4], Ordonez, Paulina M.[3,4] and Goldstein, Lawrence S.B.[4], [1]California Institute for Regenerative Medicine, Oakland, CA, U.S., [2]California State University, San Marcos, San Marcos, CA, U.S., [3]Rady Children's Hospital San Diego, San Diego, CA, U.S., [4]University of California San Diego, La Jolla, CA, U.S.

AAT deficiency is a potentially fatal disorder characterized by the accumulation of AAT in the liver. The most severe type (the ZZ mutation) affects an estimated 1 in 3,000, although it remains severely under-diagnosed. This disorder shows large clinical heterogeneity: despite having the same mutation, the severity of liver disease experienced by patients will vary significantly; some will require liver transplants early on, while others may experience no liver issues whatsoever. Therefore there must be some modifier, either environmental or genetic that predisposes only some patients to develop severe liver disease. We hypothesized that abnormalities of the autophagy pathway may lead to abnormal clearance and buildup of mutant AAT in affected hepatocytes. A related prediction is that abnormal autophagy correlates with degree of AAT-related liver disease, therefore supporting a role of autophagy as a clinically relevant genetic modifier in AAT deficiency. To test our hypothesis, we reprogrammed three different types of patient fibroblasts: AAT-ZZ patients with liver disease, AAT-ZZ patients without liver disease, and normal MM genotype cells as a control. We differentiated patient-specific induced pluripotent stem cells to hepatocyte-like cells (HLCs) that resemble immature hepatocytes by a standard protocol. We have evidence that AAT-ZZ patients with liver disease show a lower baseline level of autophagy when compared to AAT-ZZ patients without liver disease. Our data also raises the important possibility that ZZ HLCs from patients with severe liver disease have a blunted response to drugs that are known to induce the autophagy pathway. We are currently testing drugs that enhance the autophagic process with a focus on those that are being used or proposed as a therapy

for AAT-related liver disease and measuring proteins such as LC3 that will help us define autophagy level and progression. Additionally, we are interested in learning what other environmental or genetic factors influence the presentation and progression of AAT-related liver disease in affected individuals. The results of our experiments will provide a more thorough understanding of how mutant AAT and autophagy interact and influence each other and could eventually lead to the development of new treatments for affected individuals.

F1101

EPIGENETIC AND TRANSCRIPTIONAL REGULATION OF DRUG METABOLIZING ENZYMES IN HUMAN PLURIPOTENT STEM CELL-DERIVED HEPATOCYTE-LIKE CELLS

Park, Han-Jin, Korea Institute of Toxicology, Daejeon, Korea, South

Hepatocyte-like cells derived from human pluripotent stem cells (hPSC-HLCs) have emerged as the most attractive source for hepatotoxicity prediction. However, their application has been restricted due to the low expression and activity of drug metabolizing enzymes (DMEs). To determine the mechanism underlying limited DME expression in hPSC-HLCs, we investigated transcriptional regulation of DME genes by epigenetic control and transcription factors. Firstly, we analyzed epigenetic regulation in terms of DNA methylation and histone modifications of CYPs, including CYP1A1, CYP1A2, CYP1B1, CYP2D6, and CYP2E1, in human embryonic stem cell (hESC)-HLCs. Repressive histone modification and DNA hypermethylation was associated with diminished expression of CYP genes in hESC-HLCs. Next, we investigated the transcriptional activities of xenobiotic receptors, especially constitutive androstane receptor (CAR), pregnane X receptor (PXR), and aryl hydrocarbon receptor (AHR), that are capable of regulating transcription of a series of DMEs and transporters. We found that low expression of xenobiotic receptors (CAR and PXR) contributes to low activity of DMEs in hPSC-HLCs. Most DMEs and transporters that are regulated by CAR and PXR were transcriptionally down-regulated in hPSC-HLCs. Transcriptional expression of CAR and PXR was highly repressed in hPSC-HLCs, whereas AHR mRNA level was comparable to that of adult liver. Bisulfite sequencing analysis demonstrated that promoter hypermethylation of CAR and PXR was associated with diminished transcriptional activity in hPSC-HLCs. Treatment of AHR-selective ligands enhanced transcriptional level of AHR-dependent target genes by direct AHR-DNA binding at the xenobiotic response element. Thus, AHR seems intrinsically to function as xenosensor as well as ligand-dependent transcription factor in hPSC-HLCs. Our findings indicate that transcription of DMEs and transporters in hPSC-HLCs is modulated by epigenetic factor as well as xenobiotic receptors. This implies that better understanding of DME regulation including epigenetic and transcriptional control may lead to generation of HLCs exhibiting functionally matured phenotypes in drug metabolism.

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F1103

MODELLING NEONATAL AND MODY DIABETES IN VITRO USING IPS CELLS-DERIVED HUMAN PANCREATIC BETA CELLS

Soares, Filipa[1], Santos, Rodrigo[2], **Kalpesh, Jhaveri**[1], Schofield, Christine L[2], McRunnel, Tina[1], Vallier, Ludovic[1,3] and Yeo, Marcus[1], [1]DefiniGEN Ltd, Ltd, Babraham Research Campus, Babraham, Cambridge, U.K., [2]Horizon Discovery Group Plc, Cambridge Research Park, Waterbeach, Cambridge, U.K., [3]Wellcome Trust - Medical Research Council Stem Cell Institute, Anne McLaren Laboratory, Department of Surgery, University of Cambridge, UK, Cambridge, U.K.

Human induced Pluripotent Stem Cells (iPSCs) offer a unique in vitro platform for the generation of large quantities of cells for disease modelling, drug screening and ultimately cell based therapy. Our studies have demonstrated that functional pancreatic cells can be generated on a single directed differentiation platform from both patient and healthy donor derived iPSCs. Here we show how iPSCs and genome engineering technologies can be applied to develop valuable disease models for neonatal, and MODY diabetes. Some of the largest healthcare problems result from pancreatic dysfunction most notably diabetes, making the pancreas a key "metabolic organ" to study. In a five-stages protocol iPSCs undergo differentiation and functional maturation into beta cell-like cells, fetal characteristics diminish, and functions associated with adulthood increase to reach a maximum level at day 25. A significant proportion of these pancreatic cells expressed key beta cell markers and exhibited glucose-responsive C-peptide production at physiologically relevant levels comparable to primary human islets. The cells also respond to key reference drugs like GLP-1 and Exenatide in a similar manner to human islets. Genome–editing of iPSC lines to generate the disease models has been achieved using CRISPR technology which has been utilized to transfect the cells. The platform has generated iPSC disease models for neonatal and MODY diabetes which have been validated at both the genotypic and phenotypic levels using an array of biochemical methodologies. The neonatal model contains a mutation in KCJN 11 (RC201H) whilst the MODY3 form has an O291fsinsC frameshift mutation caused by the insertion of C in the 290 codon. Future work will focus

on combined directed differentiation and genome-editing approaches to generate disease models for complex diseases such as diabetes type 2. These activities will take advantage of GWA (genome-wide association) studies which have identified dozens of disease implicated loci, a subsection of which are amenable for investigation as key disease causative mechanisms and novel drug discovery targets.

F1105

DIFFERENTIATION OF FUNCTIONAL ISLETS FROM HUMAN IPS CELLS AND HIPS-ENDOCRINE PROGENITOR CELLS IN VITRO.

Watanabe, Ami[1], Tanaka, Anna[1], Otsubo, Hiroyuki[1], Ohta, Masahiro[1], Yamashita-Sugahara, Yzumi[2], Mitani, Kohnosuke[3], Nakanishi, Mahito[4], Okazaki, Yasushi[2] and Miyajima, Atsushi[1], [1]University of Tokyo, Bunkyo-ku, Japan, [2]Division of Functional Genomics and Systems Medicine, Research Center for Genomic Medicine, Saitama Medical University, Saitama, Japan, [3]Gene Therapy Division, Research Center for Genomic Medicine, Saitama Medical University, Saitama, Japan, [4]Biotechnology Research Institute for Drug Discovery, National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki, Japan

Pancreatic islets are clusters of endocrine cells, including insulin-producing β-cells, a-cells,δ-cells, PP-cells, and ε-cells. Transplantation of pancreatic islets is a promising therapeutic option for severe insulin-dependent diabetic patients. However, donor shortage is a major problem for this therapy. In order to secure a sufficient quantity of islets, large efforts have been made to generate β-cells from pluripotent stem cells, human ES or iPS cells. While some β-cells or immature pancreatic cells were differentiated from human ES/iPS cells, it has been difficult to produce islets with proper functions and structure in vitro. Based on our previous findings that functional islets with a three-dimensional (3D) structure consisting of multiple endocrine cells can be formed in vitro from mouse fetal pancreatic bud cells and mouse iPS cells, we have developed a culture system to generate functional islets with proper 3D structure and function from human iPS cells. In this differentiation system, cell clusters of endocrine cells were formed on adherent cells derived from human iPS cells. Moreover, these cell clusters exhibited a 3D structure similar to human islets. Those islet-like cell clusters secreted human c-peptide in response to glucose concentrations in vitro. Furthermore, when these clusters were transplanted into the kidney capsule of strepotozocin-induced diabetic mice, the blood glucose level was reduced to normal levels within 5 days. Furthermore, we found that sorted NGN3+ endocrine progenitor cells efficiently differentiated into the insulin-expressing islet-like cell clusters in vitro.

Funding Source: Japan agency for Medical reserch and Development (AMED)

ENDOTHELIAL CELLS/ HEMANGIOBLASTS

F1109

THE NECTIN-2 IS A NOVEL MARKER AND REGULATES CELL PROLIFERATION AND ANGIOGENIC FUNCTION OF OUTGROWTH ENDOTHELIAL CELLS

Son, YeonSung[1], Lee, BomNaeRin[1], Choi, Young-jin[1], Jeon, Sun-Ae[1], Kim, Ju-Hyun[1], Lee, Hoo-Keun[2], Kwon, Sang-Mo[3] and Cho, Je-Yoel[1], [1]Seoul National University, Seoul, Korea, [2]Gachon University, Incheon, Korea, [3]Pusan National University, Yangsan, Korea

Outgrowth endothelial cells (OECs) have been defined as a subpopulation of Endothelial progenitor cells (EPCs) that have capacity for proliferation and ability to promote angiogenesis. Despite extensive preclinical trials, thus far, OEC-based cell therapy studies have shown inconsistent results. The lack of surface markers specific for OECs limits the therapeutic success and impedes its clinical use. In this study, by unbiased quantitative mass spectrometry analysis, we identified the Nectin-2 as a surface marker of OECs. Using immunocytochemistry and flow cytometry, we confirmed that the Nectin-2 is highly expressed on OECs. Nectin-2 expression was limited or lower on mononuclear cells (MNCs) and mature tube forming endothelial cells (ECs), which inversely display promising feature for isolation and evaluation of OECs. Moreover, blocking Nectin-2 by neutralizing monoclonal antibody significantly increased OEC's trans-well migration and tube forming capacity. Similarly Nectin-2 knockdown by short hairpin RNA resulted in much enhanced tube formation, wound healing, cell migration and proliferation. These results indicate that Nectin-2 is a surface marker and an important regulator of OECs and has significant implication for preparation and evaluation of OECs for clinical application.

F1111

FOXO1A REGULATES ARTERIAL AND VENOUS IDENTITY IN HUMAN PLURIPOTENT STEM CELLS-DERIVED ENDOTHELIAL CELLS, 3D CULTURE FOR VASCULAR TISSUE ENGINEERING

Gara, Edit[1], Kiraly, Szilvia[1], Kiszler, Gábor[2], Skopal, Judit[1], Pólos, Mikós[1], Merkely Prof, Béla[1], Harding Prof, Sian E.[3] and Földes, Gábor[1,3], [1]Semmelweis University Heart and Vascular Center, Budapest, Hungary, [2]Department of Pathology and Experimental Cancer Research, Budapest, Hungary, [3]Imperial College National Heart and Lung Institute, London, U.K.

Endothelial derivatives of human pluripotent stem cells may offer regenerative treatments in ischemic cardiovascular diseases. We aimed to investigate the regulatory role of PI3K/FOXO1A signalling pathway on arterial and venous identity of endothelial subpopulations and the fate of generated cells in 3D cultures. Human embryonic stem cells (hESC) were differentiated via either embryoid body (EB) or monolayer method under normoxic and hypoxic conditions. CD31-positive endothelial cells (EC) were sorted by FACS and compared with human induced pluripotent stem cell-derived endothelial cells (hiPSC-EC). Both hESC-EC and hiPSC-EC showed mature phenotype *in vitro:* cobblestone pattern, ac-LDL uptake and tube formation. Proteome profiling revealed high abundance of angiogenesis-related proteins both in cell lysates and supernatant. Expressions of arterial (EphrinB2, Notch1-2) and venous (EphB4) endothelial markers were increased during differentiation, suggesting the presence of mixed endothelial population. Transfection of hESC-EC/hiPSC-EC with plasmids encoding FOXO1A-eGFP or pmaxGFP was carried out by electroporation. Human ESC-EC and hiPSC-EC with high FOXO1A showed downregulated expressions of universal (CD31, angiopoietin-2 and ve-cadherin) as well as arterial and venous markers. Indeed, arterial index (EphrinB2/EphB4 mRNA ratio) decreased in response to FOXO1A overexpression (hESC-EC 8.16±3.22 vs. 2.24±0.49, $p < 0.01$; hiPSC-EC 6.46±2.75 vs. 1.67±0.72, $p < 0.05$; n=3 biological replicates). This suggests a key role of PI3K/FOXO1A pathway in the modulation of arterial and venous phenotype. For engineering 3D vascular constructs decellularised human aortic slices (300µm) were repopulated with hESC-EC and hiPSC-EC. Cells remained viable on engineered matrices. Imaging with Calcein AM live staining and 3DHistech analysis proved recellularisation with CD31-positive, viable endothelial cells. We found that PI3K/FOXO1A signalling pathway has strong effects on arterial and venous endothelial identity. Human ESC-EC and hiPSC-EC remained viable on 3D vascular matrices. In-depth analyses of phenotype and functional characteristics of hESC-EC and hiPSC-EC may enhance their application for vascular tissue engineering.

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F1113

CXCR2-SPECIFIC PEPTIDE ACETYLATED PRO-GLY-PRO (AC-PGP) STIMULATES WOUND HEALING BY PROMOTING NEOVASCULOGENESIS

Kwon, Yang Woo, Pusan National University, Yang San-si, Korea, South

Wound healing is mediated by multiple steps, including inflammation, epithelialization, angiogenesis, and granulation. Many therapeutic approaches to treat wound healing using endothelial progenitor cells (EPCs) have been developed. EPCs can integrate into blood vessels and stimulate neovascularization of the wound tissues. However, therapeutic potential of EPCs is limited due to low homing and engraftment efficiency of transplanted EPCs. Chemokine receptor 2 (CXCR2), a receptor of interleukin 8 (IL-8), mediates neutrophil migration to the site of inflammation. The angiogenic effects of IL-8 in intestinal vascular endothelial cells are mediated by this receptor. Our hypothesis is that CXCR2 is involved in the regulating growth and survival of endothelial cell and EPCs through the mechanism similar to IL-8-regulated angiogenesis. We explored the role of CXCR2 in angiogenesis and tissue regeneration by using Acetylated Pro-Gly-Pro (Ac-PGP), which is the endogenous degradation product of extracellular collagen and binds to CXCR2. Ac-PGP stimulated chemotactic migration, tube formation ability of human EPCs in vitro. The silencing of CXCR2 abrogated Ac-PGP-induced migration and tube formation of EPCs. Treatment of Ac-PGP into the wound area resulted in the acceleration of the wound closure and the stimulation of angiogenesis in the dermal wounds. In addition, re-epithelialization was accelerated by treatment with Ac-PGP and infiltration of immune cells could be detected on early phase during wound healing and Ac-PGP treatment acutely augmented infiltration of CD68-positive macrophages. CXCR2 knockout mice showed the attenuation in Ac-PGP-induced in vivo wound healing. These results suggest that Ac-PGP has therapeutic effects by stimulating neovascularization through CXCR2-dependent mechanism.

F1115

EFFECT OF PHYSICAL EXERCISES IN CAROTID ARTERIAL THROMBOSIS: INFLUENCE OF ENDOTHELIAL PROGENITOR CELL LEVELS IN THIS PROCESS

Pedrosa, Denise[1], Terra, Maiara[2], Werneck, Claudio[2] and Vicente, Cristina Pontes[2], [1]State University of Campinas, Paulinia, Brazil, [2]State University of Campinas, Brazil

Thrombosis in coronary and cerebral arteries is the main cause of death among cardiovascular diseases. Endothelial progenitor cells (EPCs) originate from hemangioblast in bone marrow and can be mobilized to peripheral blood promoting new blood vessel formation, and also helping in the recovery of the damaged endothelium. Previous studies demonstrated that physical exercises can stimulate the release of hematopoietic and vascular stem cells from bone marrow to peripheral blood. The objective of this study is to analyze the influence of physical exercises in the thrombus formation time after ferric chloride induced arterial injury and in the EPCs blood levels in mice. C57bl6 male mice with 7-8 weeks were divided into 4 groups: (1) control non exercised; (2) exercised for 21 days; (3) non exercised, injured + 3 days; and (4) exercised, injured + 3 days. The animals were exercised on a treadmill for 5 days per week at 12m/min, 30 min for 21 days before surgery. Arterial injury was made in the right carotid using ferric chloride and blood flow measured using a transonic 0.5 VB ultrasound probe. We quantified the number of $CD34^+$, $CD31^+$, $VEGFR2^+$ and $CD45^-$ cells by flow cytometry in peripheral blood. Physical exercises prolonged occlusion time after injury and reduced thrombus area 3 days after lesion when compared to the non-exercised group. Flow cytometry analysis showed an increase in CD34+ cells and CD34/CD31 positive cells in exercised mice and a decrease after arterial injury. Exercised and injured mice presented an increase in CD34/CD31 positive cell when compared to injure non-exercised mice. Our results indicate that exercises alter thrombosis time and promote faster thrombus resolution after injury, also increasing the number of progenitor cells in peripheral blood.

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EPITHELIAL CELLS (NOT SKIN)

F1117

MODULATION OF HUMAN LIMBAL STEM CELL EXOANSION UISNG WNT SMALL MOLECULE INHIBITORS

Deng, Sophie[1], Mei, Hua[1], Zheng, Jie[2] and Zhang, Chi[1], [1]Stein Eye Institute, UCLA, Los Angeles, CA, U.S., [2]UCLA, Los Angeles, CA, U.S.

Purpose: To study the possible function of small molecules of Wnt inhibitors in regulating the in vitro expansion of limbal stem/progenitor cells (LSCs). Methods: Two Wnt inhibitors (MFH, ND) were tested in this study. Single cell suspension of limbal epithelial cells were cultured on 3T3 cells in the SHEM medium containing 1, 2 or 5 μM of small Wnt molecules for up to 2 weeks. Cells cultured in medium containing corresponding concentrations of DMSO served as the control. Medium were refreshed every 2-3 days and single cells were collected at the end of culture. Cells were analyzed for morphology, proliferation rate, and percentages of keratin (K) 14^+, $K12^+$, p63α-bright cells and small cells (cell diameter ≤ 12μm). Results: At 1μM, MFH did not affect cell proliferation or cell morphology but significantly reduced the percentage of small cells by 56% ($p<0.05$). At 2 μM, MFH increased cell proliferation by 19% ($p<0.05$) and induce vacuoles formation. At 5μM, MFH decreased the proliferation by 51% ($p<0.05$) and majority of cells showed big cytoplasmic or secreted vacuoles. MFH had no effect on percentage of small cells at 2 or 5 μM and no effect on percentage of $K14^+$, $K12^+$, or p63α-bright cells at all tested concentrations. Cells cultured with ND had comparable epithelial morphology to the control at all concentrations but showed different proliferation rate and stem-cell phenotype. At 1μM, ND decreased the percentage of small cells by 63% ($p<0.05$) and did not alter the proliferation rate or expressions of markers. ND increased the percentage of K14-positive cells by 4% at 2μM ($p<0.05$) and did not affect the proliferation rate or percentages of p63a-bright, $K12^+$, or small cells. At 5μM, ND decreased cell proliferation rate by 18% ($p<0.05$) but increased the percentage of p63a-bright cells by 2 folds ($p<0.05$). It had no effect on percentages of $K14^+$, $K12^+$ or small cells at 5μM. Conclusions: MFH differentially regulated cell proliferation and change the morphology at different concentrations without affecting the expression of K12, K14 and p63a. ND enhanced the population of the undifferentiated LSCs at higher concentrations although it decreased the proliferation rate. Small Wnt molecules targeting different components of Wnt signaling pathway might exert different effects on cultured LSCs within a narrow window of concentration.

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F1119

THE REGULATION OF ADULT INCISOR STEM CELLS BY YAP/TAZ AND HIPPO SIGNALING.

Hu, Jimmy, UCSF, San Francisco, CA, U.S.

Tissue homeostasis and injury repair depend on the correct regulation of somatic stem cells, which have the capacity to self-renew and differentiate into specialized cell types. However, the mechanisms controlling stem cell proliferation and differentiation remain poorly understood. The mouse incisor is an ideal system for this experimentation as it maintains a group of epithelial stem cells that first proliferate and then differentiate along the length of the incisor epithelium in order to continuously replenish distal matrix secreting cells in a conveyor belt fashion. Here we show that YAP, an effector of the Hippo pathway, accumulates in the nucleus of proliferating transit-amplifying cells prior to their differentiation. Conditional deletion of Yap and its homolog Taz then demonstrates that they are required for maintaining cell proliferation and survival, as well as inhibiting precocious differentiation in the incisor epithelium. These processes may be in part controlled through the mTor pathway, as YAP/TAZ regulate the expression of Rheb, which encodes the mTorc1 activator, and mTor activities diminish in the absence of Yap/Taz. The regulation of YAP localization is therefore critical for determining stem cell proliferation and differentiation. To that end, we have identified FAK signaling through CDC42 and LATS1/2 as a key mechanism for promoting nuclear YAP. Additional experiments are currently underway to further dissect the pathway and to investigate the potential involvement of mechanotransduction.

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F1121

NEUREGULIN-1/ERBB3 SIGNALING IS ESSENTIAL FOR MOUSE SALIVARY GLAND DEVELOPMENT AND ACINAR CELL FORMATION

Mattingly, Aaron Joshua, University of California San Francisco, San Francisco, VA, U.S., Cruz-Pacheco, Noel, Program in Craniofacial Biology, University of California San Francisco, San Francisco, CA, U.S. and Knox, Sarah, University of San Francisco, San Francisco, CA, U.S.

Acinar cells are essential components of multiple exocrine organs including the salivary glands (SG). Saliva is synthesized and secreted by salivary acinar cells through an interconnected ductal network into the oral mucosa where it serves a plethora of functions critical to oral health and whole body well being. Despite their importance to organ function, mechanisms by which acinar cells form and the factors that drive their maturation are poorly understood, thus impairing therapeutic strategies aimed at tissue replacement/regeneration. Here we demonstrate that the EGF receptor ERBB3 is robustly expressed on early KRT14+KIT+ acinar progenitor cells of mouse embryonic salivary glands where it serves in the regulation of acinar cell formation. We show that genetic ablation of ErbB3 in KRT14+ cells of the developing salivary gland severely reduced pre-acinar cell production and acinar maturation. Furthermore, removal of Erbb3 or inhibition of ERBB3 signaling in salivary rudiments cultured ex vivo increased apoptosis of pre-acinar cells but not ductal cells, suggesting that ERBB3 is necessary for acinar cell survival. These changes also coincided with increased Wnt signaling, a positive modulator of duct formation, providing evidence that ERBB3 negatively regulates the ducts to promote acinar cell development. Thus, ERBB3 signaling is necessary for mouse salivary gland development and acinar cell formation during embryogenesis.

F1123

MODELLING PRIMARY SJOGREN'S SYNDROME USING SALIVARY GLAND STEM CELLS

Pringle, Sarah[1], Bootsma, Hendrika[1], Verstappen, Gwenny[1], Vissink, Arjan[2], Spijkervet, Frederik[2], Coppes, Robert P.[3,4] and Kroese, Frans[1],
[1]Rheumatology and Clinical Immunology, UMCG, Netherlands, Groningen 9713 GZ, Netherlands, [2]Oral and Maxillofacial Surgery, UMCG, Groningen 9713 GZ, Netherlands, [3]Cell Biology, UMCG, Groningen, Netherlands, [4]Radiation Oncology, UMCG, Groningen, Netherlands

Primary Sjögren's Syndrome (pSS) is an autoimmune disease characterized by lymphocytic infiltration of exocrine glands, particularly the salivary gland (SG). Through as yet unclear interactions between infiltrating cells and the epithelial cells of the SG, SG function is dramatically reduced (hyposalivation). Hyposalivation confers many problems, including dental caries, sleeplessness and difficulties eating, and results in a large decrease in quality of life for pSS patients. We have recently demonstrated isolation of salivary gland stem cells (SGSCs) from human SG biopsies, and have characterized their self-renewal and differentiation capabilities. SGSCs reside in the epithelial compartment in the SG, and are therefore likely to be complicit in pSS manifestation. We sought to elucidate any intrinsic defects in SGSCs from pSS patients representing potential therapeutic targets, through comparison of their regenerative capacity (self-renewal and differentiation), transcriptome and proteome with healthy control biopsies. Yield of SGSCs from pSS biopsies was markedly lower than from healthy control tissues. When exposed to a self-renewal assay, SGSCs from pSS biopsies generated fewer SGSCs. Expression of matrix metalloprotease-2 and -9, associated with pSS, was greater in SGSCs isolated from pSS biopsies. In addition, proteins involved in both pro-inflammatory (polymeric immuno-

globulin receptor, immunoglobulin kappa constant) and anti-inflammatory (annexin A1) immunereactions were detected in conditioned medium from pSS SGSC cultures at greater levels than controls, using mass spectrometry. Taken together, our preliminary data demonstrates that SGSC isolation and characterization can be used to model epithelial cell involvement in pSS, and furthermore that there is an innate difference in regenerative potential and immune-reactive nature of SGSCs from pSS biopsies, compared to controls. To our knowledge this is the first use of stem cells to model an autoimmune disease. We anticipate that full characterization of SGSCs from pSS biopsies will reveal mechanisms underpinning SG epithelial cell involvement in pSS, and permit development of new therapeutic strategies for the treatment of primary Sjögren's' Syndrome.

F1125

SINGLE CELL RNA-SEQ OF HUMAN PROSTATE BASAL STEM CELLS REVEALS CELLULAR HETEROGENEITY AND LINK TO AGGRESSIVE CANCER

Smith, Bryan A.[1], Chronis, Konstantinos[1], Bonora, Giancarlo[1], Sabri, Shan[2], Sokolov, Artem[3], Uzunangelov, Vladislav[3], Cheng, Donghui[1], Wei, Wei[1], Stuart, Joshua[3], Plath, Kathrin[1] and Witte, Owen N.[4], [1]UCLA, Los Angeles, CA, U.S., [2]UCSC, Los Angeles, CA, U.S., [3]UCSC, Santa Cruz, CA, U.S., [4]UCLA/Howard Hughes Medical Inst, Los Angeles, CA, U.S.

Evidence from numerous cancers suggests that aggressive phenotypes often possess molecular and functional properties shared by tissue stem cells. We have shown that histological subtypes of advanced prostate cancer vary in their enrichment of a normal prostate basal stem cell signature with the highly aggressive, small cell neuroendocrine carcinoma being the most stem cell-like. It is unclear if the prostate basal stem cell population is composed of multiple subpopulations and if a specific subpopulation is driving this similarity with aggressive prostate cancer. Here, we performed high-throughput, single cell RNA-seq on over 150 uncultured basal stem cells from human prostates obtained after radical prostatectomy. Unsupervised clustering identified multiple subpopulations within the bulk basal stem cell population. Bioinformatic analyses revealed each subpopulation to be associated with distinct gene networks, transcription factors, and cell surface markers. Surface marker protein expression on over 750 single basal stem cells from 2 human prostates confirmed the existence of subpopulations within the bulk population. Molecular interrogation of human prostate cancer tissue samples showed that small cell neuroendocrine carcinoma was enriched for cell surface markers associated with one specific subpopulation. These results suggest that the prostate basal stem cell population is composed of molecularly distinct subpopulations. Further, normal stem cell surface markers may be potential targets for treating advanced prostate cancer.

EPIDERMAL CELLS

F2001

SINGLE CELL ANALYSIS OF HUMAN AGE-RELATED ALTERATIONS OF SKIN EPIDERMAL PRECURSORS: INTRINSIC AND ENVIRONMENTAL MODIFICATIONS

Dimitrov, Ariane, Riesterer, Hélène and **Paris, Maryline**, L'Oreal R&I, Aulnay-sous-Bois, France

Keratinocytes of the basal layer (the niche of epidermal stem cells and precursor cells) are involved in cutaneous regeneration process. Growth capacity of keratinocytes shows that most of the colony-forming cells are classified as holoclones, meroclones or paraclones when analyzed in a clonal assay. Their function is to maintain tissue homeostasis in physiological situation and to process to skin repair in response to an environmental aggression such as wound. In human, clinical observations of the skin indicate that regenerative potential, preservation of tissue homeostasis and more widely skin's functions are altered with ageing. The decline of tissue regenerative potential of skin and hair is a hallmark of physiological ageing and may be associated with age-related changes in tissue-specific stem cells and/or their environment. It is agreed that the stem cells are affected by the ageing process in their capacity to produce precursors and to differentiate. The consequences of ageing on the number of epidermal stem cells and their capacity to self-renew are still debated. Molecular and cellular changes that affect these cells during ageing are still poorly described. If the cutaneous regenerative potential decreases with age, it is still unclear whether this is related to intrinsic stem cell ageing or due to a functional defect of the surrounding tissue. The present study aimed at assessing the modification of individual human keratinocytes clone-forming efficiency independently of the modification of their environment. Using keratinocytes isolated from 21 donors from 18 to 73 years old in the clonal microculture test (a single cell analysis of the clonogenic potential), we demonstrated that 1) ageing affects individual human keratinocytes clone-forming efficiency; 2) this phenotype can be reversed by a modification of the environment (growth factors supplementation). This study allows to better understand the impact of intrinsic and environmental age-related alterations of epidermal precursors on mecanisms involved in cutaneous regeneration.

F2003

EPIDERMAL PATTERNING AND CLONALITY DURING MAMMALIAN DEVELOPMENT AND HOMEOSTASIS

Leung, Eva, Jacob, Fadi, Sada, Aiko, Zhang, Ying and Tumbar, Tudorita, Cornell University, Ithaca, NY, U.S.

The mammalian epidermis develops from surface ectoderm which consists of a single layer of multipotent epithelial progenitors. A Hoxb7-Cre transgene was active prior to the onset of skin developmental program, as judged by expression of Keratin 8 at embryonic E9.5. Hoxb7-Cre marked cells generated a subset of K8+ progeny, which developed in a striped pattern accross the back skin that persisted to adulthood. To further investigate this heterogeneity in the early skin population, we employed a multi-color reporter that allows single-cell tracking and compared the clones derived from Hoxb7-Cre with clones generated by an ubiquitously expressed CreER transgene. The data suggested that all clones contributed broadly to all epithelial structures of the skin, including hair follicles, sebaceous glands, and epidermis. These structures were polyclonal, although the intial labeling occured at very early stages of development. Unexpectedly, the Hoxb7-Cre derived clones showed both a different distribution of clone sizes as well as a different average clone size when compared to clones generated via ubiquitous activation of Cre at the early embryonic stages. These data suggest heterogeneity in the clonal behavior of early epidermal progenitors that were previously presumed equipotent. In addition, the Hoxb7-marked lineage produces a persistent polyclonal striped pattern resembling "Lines of Blaschko," a pattern identified in humans with certain skin diseases. The Hoxb7-Cre can be employed as a valuable transgenic tool for studying this pattern and mosaicism in mice.

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F2005

AGING OF HAIR FOLLICLE STEM CELLS IS LINKED TO A WNT5Aa-CDC42 SIGNALING AXIS AND IS REVERSIBLE

Tiwari, Rajiv Lochan[1], Martin, Nicola[1], Sakk, Vadim[1], Soller, Karin[1] and Geiger, Hartmut[1,2], [1]Institute for Molecular Medicine and Aging Research Center Ulm, Ulm, Germany, [2]Division of Experimental Hematology and Cancer Biology, Cincinnati, OH, U.S.

Aging of skin results in a failure of tissue homeostasis. Somatic stem cell aging is supposed to be one of the underlying causes of attrition of skin with age. Skin is a complex organ and composed of various distinct kinds of stem cells that together contribute to skin homeostasis. Alpha-6-Integrin^{high} CD34^{+} hair follicle (HF) stem cells reside in the bulge of hair follicle and contribute to the formation of new hair follicle during anagen onset. Upon aging the onset of anagen is delayed and one underlying cause might be aging of HF stem cells. Wnt signaling is one signaling pathway contributing to anagen onset in mouse skin during normal homeostasis. Our data demonstrate that upon aging there is decrease in canonical Wnt signaling and a shift towards non-canonical Wnt signaling in HF stem cells, which is reminiscent of changes in signaling in aged hematopoietic stem cells. Further, we demonstrate that there is increase in Cdc42 activity in Sca1$^{-/low}$ keratinocyte of the aged mouse which correlates with an apolar distribution of the Cdc42 protein in the cytoplasm, which is a hallmark of aging of hematopoietic stem cells. A high percentage of aged HF stem cells are apolar for Cdc42 distribution, while in young most HF stem cells present with a polar distribution. In-vitro treatment of aged HF stem cells with a specific inhibitor for Cdc42 activity (CASIN) changes the frequency of aged cells polar for Cdc42 distribution to a youthful level. Further, in-vivo treatment of aged mice with CASIN at a dose of 2.4mg/kg induces the onset of anagen in aged mice, and the percentage of back skin area with anagen hair follicle was significantly increased. The expression of Wnt5a is elevated in HF stem cells upon aging. Upon aging of hematopoietic stem cells, an elevated level of Wnt5a drives elevated activity of the small RhoGTPase Cdc42. In vitro treatment of young HF stem cells with Wnt5a increased Cdc42 activity and induced an aging-like higher percentage of cells with apolar Cdc42 distribution. In summary, aging of hair follicle stem cells in linked to changes in a Wnt5a-Cdc42 signaling axis with elevated non-canonocial signaling upon aging and elevated activity of the RhoGTPase Cdc42. Aged phenotypes of hair follicle stem cells like elongated telogen time can be reverted to a youthful phenotype by inhibition of the activity of RhoGTPase Cdc42.

EYE OR RETINAL CELLS

F2009

MISLOCALISATION OF BESTROPHIN1 IN IPSC-RPE CELLS DERIVED FROM A FAMILY WITH AUTOSOMAL DOMINANT VITREORETINOCHOROIDOPATHY

Carr, Amanda-Jayne[1], Carter, David[1], Letton, William[1], Smart, Matthew[1], da Cruz, Lyndon[1,2], Moore, Anthony[2,3] and Coffey, Peter[1], [1]UCL Institute of Ophthalmology, London, U.K., [2]Moorfields Eye Hospital, London, U.K. of Great Britain and Northern Ireland, [3]UCSF School of Medicine, San Francisco, CA, U.S.

Autosomal dominant vitreoretinochoroidopathy (ADVIRC) is a rare, early-onset retinal dystrophy characterised primarily by the presence of distinct bands of cir-

cumferential pigmentary degeneration in the peripheral retina and developmental eye defects, such as microcornea and nanophthalmos. Although ADVIRC belongs to a group of diseases collectively known as Bestrophinopathies, it is clinically distinct from these dystrophies since the primary area of pathogenesis resides in the periphery rather than the central macula region. Bestrophinopathies are caused by mutations in Bestrophin1 (Best1), a transmembrane protein thought to function as an ion channel in retinal pigment epithelial (RPE) cells. Previous studies have suggested that the distinct ADVIRC phenotype results from alternative splicing of Best1 pre-mRNA. Here, we have used induced pluripotent stem cell (iPSC) technology to investigate the effects of an ADVIRC mutation on Best1 expression in patient derived iPSC-RPE. We have reprogrammed fibroblast cells taken from a patient family expressing the c.704T>C (p.V235A) Best1 missense mutation. Pluripotency was confirmed by teratoma formation assay and hPSC Scorecard analysis. Patient and control iPSCs were allowed to spontaneously differentiate by removal of bFGF from the culture medium. Pigmented foci were isolated from the iPSC cultures by manual dissection and seeded to form a monolayer of RPE. ADVIRC iPSC-RPE cells were pigmented, polarised and formed a monolayer with the classic cobblestone-like appearance. The cells expressed a panel of RPE cell markers, including Best1, at the RNA and protein level. We found no evidence of alternate splicing of the Best1 transcript1 as a result of the ADVIRC mutation; however, immunocytochemical analysis demonstrates that p.V235A missense alteration results in mislocalisation of BEST1 within the RPE cell. Immunohistochemistry of the developing human eye (Week 7-11) shows that Best1 protein is expressed more abundantly in the periphery compared to the central macular area. Together, these data suggest that the mislocalised expression of Best1 within the RPE, from an early developmental stage, may lead to the distinct clinical phenotype observed in ADVIRC patients.

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F2011

ASSESSING THE FEASIBILITY OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO GENERATE RETINAL PIGMENTED EPITHELIUM PROVIDING A DISEASE MODEL FOR AGE RELATED MACULAR DEGENERATION

Hallam, Dean Mark[1], Bojic, Sanja[2], Kavanagh, David[2], Armstrong, Lyle[2], Steel, David[2] and Lako, Majlinda[2], [1]Newcastle University, Gateshead, U.K., [2]Newcastle University, Newcastle, U.K.

Age related macular degeneration (AMD) is the commonest cause of blindness in the over 50's. AMD presents in two forms, dry and wet. Dry AMD is caused by a build-up of debris in the macular, while wet is caused by the abnormal growth of blood vessels. Treatments which target these abnormal blood vessels help to slow down the progress of the disease, however the underlying causes are still poorly known. Drusen comprise of many different proteins, however a number of those are associated with the immune system. The complement system triggers a collection of proteins called the membrane attack complex to help destroy invading organisms. The complement system is in a state of constant readiness and has a tendency to trigger into action to all sorts of stimuli including products of ageing and light damage. The body keeps this in check with a series of regulatory proteins to prevent the complement system from activating inappropriately. Complement factor H (CFH) is an important component of this regulatory system. Recently genetic factors have been identified which increase the chance of developing AMD. A single base pair polymorphism (SNP) can change the gene and consequent protein ability to function correctly, as is the case with the CFH. Different, but commonly occurring genetic forms of complement factor H (CFH) have been identified and could be a major risk factor for developing dry AMD. We derived dermal fibroblasts from two individuals with low risk, two medium risk and two high risk for Y402H polymorphism of the CFH gene. The high risk individuals were clinically diagnosed with wet AMD and presented with drusen. Induced pluripotent stem cells were derived from all six fibroblast samples and differentiated towards RPE lineages together with a medium risk and high risk genotype human ESC. In this poster we will present our molecular, structural and functional data which suggest that RPE derived from high risk individuals display profound differences to low and medium risk which are likely to underlie the development of AMD phenotype.

Funding Source: Macular Society

F2013

PROTEOMIC ANALYSIS OF CHRONIC OXIDATIVE STRESS IN RETINAL PIGMENT EPITHELIUM CELLS DERIVED FROM PLURIPOTENT STEM CELLS

Meyer, Jesse, Garcia, Thelma, Schilling, Birgit, Ramanathan, Arvind, Lamba, Deepak and Gibson, Bradford, Buck Institute for Research of Aging, Novato, CA, U.S.

Dysfunction of the Retinal Pigment Epithelium (RPE) cell layer is associated with age-related macular degeneration (AMD) due to chronic oxidative stress and/or loss of antioxidant capacity. Several reports detail how tissues generated from patient-specific induced pluripotent stem cells (iPSCs) reflect the clinical status of individuals. Due to the poorly understood and heterogeneous aspects of AMD progression, we assert that eye tissues derived from

human pluripotent stem cells are an ideal surrogate model for understanding AMD. We have previously assessed transcriptional changes associated with chronic oxidative stress in RPE cells (Garcia et al. 2015). Here, we compare proteomic alterations associated with chronic stress in human RPE. Towards this goal, we first chronically stress RPE cells differentiated from embryonic stem cells with a low dose of either paraquat (PQ) or 4-hydroxynonenal (4-HNE) for up to 3 weeks. PQ promoted intracellular ROS accumulation while 4-HNE is a lipid peroxidation by-product that is known to promote RPE cell dysfunction. We implemented a method for combined extraction of the proteome and metabolome from a single sample. We first analyzed the changes in proteome composition upon chronic stress using a quantitative label free mass spectrometry approach. This method allows us to identify and quantify nearly 5,000 proteins from a single sample, or over 8,000 proteins total. A large number of statistically significant changes in protein levels were found after 1 week or 3 weeks of either stress. Pathway and network analysis of changing proteins revealed several perturbed pathways (e.g. lipid metabolism and membrane maintenance), as well as several interesting transcriptional relationships. We also examined the correlation between proteins that change with either stress to understand which proteins represent stress-specific responses or general oxidative stress response. To build on the insight gained from proteomic analysis, we plan to follow up with same-sample metabolic profiling, especially of lipid quantities, as well as compare several RPE cell lines differentiated from either AMD patient iPSCs or control iPSCs, which will further our understanding of how RPE cells change in patients with AMD.

Funding Source: Foundation for Retinal Research

F2015

HUMAN RETINAL GANGLION PROGENITOR CELL INTEGRATES INTO RETINAS IN A MURINE MODEL

Qin, Yu[1], Chan, Ann M[1], Luo, Chenmei[2], Lu, Shi-Jiang[2], Kwong, Jacky[1], Lanza, Robert[2], Levinson, Ralph[1] and Gordon, Lynn[1], [1]University of California Los Angeles, Los Angeles, CA, U.S., [2]Ocata Therapeutics, Marlborough, MA, U.S.

Purpose: Retinal ganglion cells (RGC) are retinal neuronal cells that form the inner layer of retina and the axons of these cells form the optic nerve. Loss of RGC is a significant cause for vision loss in glaucoma and optic neuropathies. Optic nerve crush (ONC) is an acute optic nerve damage animal model. We investigated whether human RGC progenitor cells could be integrated into the appropriate retinal layer post ONC in a murine model. Methods: Unilateral ONC was performed surgically on 6-8 weeks old C57BL/6 mice. After mice were anesthetized, a conjunctival incision was made on temporal side and optic nerve was exposed following blunt dissection. Crush was applied approximately 2 mm behind the globe for 3 seconds with a self-closing forceps. RGC progenitor cells (GCH9) were obtained from Ocata Inc. and cultured in ultra-low attachment dish for 3 days prior to cell transplant. Immediately after ONC, 1x10^{5}RGC progenitor cells in 1 μl saline were injected intravitreally using a 33-gauge needle. Vehicle was injected as control. Mice were treated with cyclosporine in drinking water to prevent rejection of cell transplant from 2 days prior to transplant through the duration of the experiments, from 2- 6 weeks post cell transplant. At the end of each study retinas were dissected and wholemount retinas and frozen vertical sections were analyzed for incorporation of the cells into the retina using immunofluorescent staining with RBPMS (RGC marker) and an antibody to reveal cells of human origin. Results: Staining of wholemount retinas showed the presence of human cells within the retina at 2 (n=23 mice) and 4 weeks post cell transplant (n=10 mice). Confocal microscopy identified colocalization of human cells with RBPMS positive cells, indicating that some of the GCH9 cells are in the RGC lineage. Quantification of cells of human origin in the mouse retinas showed a decrease in cells at the 6 weeks (n=10 mice) compared with 2 or 4 weeks. Conclusion: RGC progenitor cells (GCH9) integrate into retina and survive at least 4 weeks, some of which gain the RGC transcription factor RBPMS, suggesting differentiation towards the RGC lineage. Additional work is needed to identify factors to examine the long-term survival and functionality of GCH9 in the retina as well as define the features that would allow for axonal regrowth.

F2017

GENERATION OF HUMAN EMBRYONIC STEM CELLS CARRYING MUTATIONS IN RB1 USING CRISPR/CAS9

Steenpass, Laura, Hiber, Michaela and Kanber, Deniz, University Hospital Essen, University Duisburg-Essen, Germany

Retinoblastoma is the most common eye tumor of early childhood. For the development of retinoblastoma inactivation of both alleles of the retinoblastoma gene (RB1) is necessary and sufficient. However, it is still unclear how the type of mutation relates to penetrance, aggressiveness and number of tumor foci. To answer these questions, a good model for retinoblastoma is indispensable. In the mouse, additional genetic aberrations are needed to induce retinoblastoma, excluding it as good model organism. We aim to establish a human cell-based model for retinoblastoma combining CRISPR/Cas9 mutagenesis for the introduction of mutations into RB1 in hESCs and their subsequent differentiation into neural retina. Using the CRISPR/Cas9 system we introduced random mutations into the RB1 gene by targeting the 3' end of its exon 3 in the human embryonic stem cell line H1. Initially, 192

clones were screened for mutations by Genescan fragment length analysis, identifying aberrations in 66 clones. This represents an efficiency of CRISPR mutagenesis of 34%. Of those, 48 clones were selected for expansion and cryopreservation. For characterization of the introduced mutations, high-throughput amplicon sequencing on the Roche Junior 454 platform was performed. Due to a single nucleotide polymorphism present in RB1 intron 3 in H1 hESCs (C/T; rs520342), the mutations could be assigned to one of the two alleles. We observed about 50% of clones to show unequal reads for the two alleles, indicating that they consist of two or more cell populations. These mixed clones were excluded from further analysis. For the pure clones we identified four clones having the wildtype sequence, 13 clones carrying a heterozygous deletion (group A), four clones carrying a homozygous deletion (group B) and three clones carrying compound heterozygous deletions (group C). From each group we chose clones for further analysis on DNA, RNA and protein level. Having completed these analyses, we will conduct comparative differentiation into neural retina using wildtype H1 hESCs and one clone each carrying a heterozygous, homozygous or compound heterozygous deletion in RB1.

NEURAL CELLS

F2019

GENERATION OF NESTIN+ AND PAX-6+ NEURAL PROGENITOR CELLS USING INDUCED PLURIPOTENT STEM CELLS (IPS) CREATED FROM HUMAN CORD BLOOD UNRESTRICTED STEM CELLS (USSC)

Kamath, Anant, Ternes, Sara J. and Moy, Alan B., Cellular Engineering Technologies Inc., Coralville, IA, U.S.

Human Cord Blood Unrestricted Stem Cells (USSC) are rare cells that can be isolated and scaled up from human cord blood. Unlike mesenchymal stem cells, USSC show a more cuboidal morphology, much faster doubling time and the capacity to significantly scale up (up to $1x10^{15}$ cells). These characteristics, along with their multipotent nature, make these cells attractive candidates for future therapeutic interventions. Moreover, USSC are MHC Class II negative and express lower levels of MHC Class I. IPS cells created using USSC as the target cell showed a lack of MHC Class I expression while remaining Class II negative. Neural Progenitor Cells (NPC) were created from USSC-IPS using a differentiation pathway that involved serial induction with Epidermal Growth Factor (EGF) and Fibroblast Growth Factor-2 (FGF-2) using Poly-ornithine/Laminin as the substrate. These NPC also showed rapid doubling time (< 48 hours), significant scale up (1000x), and cells were positive for Nestin and Pax-6 expression. Taken together, USSC-IPS represent an attractive candidate for the creation of bulk NPC, which could then be used to treat both traumatic and chronic neuronal loss and injury.

F2021

THE MECHANISTIC BASIS BY WHICH THE BONE MORPHOGENETIC PROTEINS DIRECT SENSORY INTERNEURON IDENTITY IN THE DORSAL SPINAL CORD

Andrews, Madeline Gail, Del Castillo, Lorenzo Moises, Sivalingam, Daniel and Butler, Samantha J., University of California, Los Angeles, Los Angeles, CA, U.S.

Inductive signaling by the Bone Morphogenetic Protein (BMP) family is reiteratively required to direct stem and progenitor cells towards different cell fates throughout embryonic development. This family of growth factors is thus a critical reagent in the development of many stem cell replacement therapies. As part of the effort to rebuild damaged or diseased spinal cords, we are studying how the BMPs direct neuronal identity in the developing spinal cord. The roof plate (RP), located at the dorsal midline of the spinal cord, expresses many different BMPs and the collective activity of these BMPs are required to specify the different classes of sensory interneurons (INs) that reside in the dorsal spinal cord. Surprisingly, the mode by which the BMPs direct IN identity remains unresolved and thus there is no current protocol to differentiate these spinal sensory IN populations *in* vitro. A better understanding of how the BMPs direct embryonic stem cells (ESCs) toward INs will provide insight into the therapeutic potential for implantation of these cells after spinal damage to replace the lost endogenous sensory INs. Previous studies have suggested that the BMPs direct neural identity as concentration-dependent morphogens, largely by analogy with the gradient of Sonic hedgehog (Shh) that patterns the ventral spinal cord. However, it is unclear how multiple BMPs would cooperate to establish a single morphogen gradient. Moreover, our recent studies have suggested that different BMPs have specific effects on the induction of particular IN fates. Using both in vitro and in vivo methods, we are assessing the extent to which the different BMPs act in a concentration dependent manner, or have distinct activities in directing cellular identity. Additionally, we are evaluating how the canonical BMP second messengers, the Smad proteins, translate the activities of the different BMPs into the specification of particular neural identities. Through these studies, we will better understand the mechanism by which the BMPs direct IN identity in development and utilize this knowledge to develop an ESC differentiation protocol as a first step toward restoration of the disrupted sensory circuitry after spinal damage.

F2023

ANALYSIS OF SEMAPHORIN 3A NEUROTOXICITY ON HESCS-DERIVED MOTOR NEURONS

Birger, Anastasiya, The Hebrew University of Jerusalem, Israel

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons (MNs) in the brain and spinal cord. A mutated human superoxide dismutase 1 (G93A-hSOD1) gene has been found in about 20% of cases of the inherited form of ALS. Recent experiments have shown that human and mouse astrocytes expressing mutant hSOD1, co-cultured with human embryonic stem cells (hESCs) derived MNs, induce MNs cell death. It was also reported that G93A-hSOD1 transgenic mice, in comparison to control mice, display a marked increase of semaphorine 3A (Sema3A) mRNA expression in terminal Schwann cells located in neuromuscular junctions. Since it has become clear that Sema3A is up-regulated in many human neurodegenerative diseases, we hypothesized that abnormal expression or function of Sema3A may have a role in the pathogenesis of ALS and in particular in MNs survival. We derived MNs from HB9-GFP hESCs and found that hESCs-derived spinal cord MNs expressed the semaphorins receptors PlexinA1-4 and Neuropilin1-2. To study the effect of Sema3A on MNs Sema3A was obtained from conditioned media of HEK293 cells, which were transiently transfected with a plasmid expressing Sema3A or Fc-only. We confirmed protein functionality in an impedance based label-free platform that allows for dynamic monitoring of morphological and adhesive changes of cells caused by Sema3A. We next incubated enriched cultures of hESC-derived MNs with Sema3A over night. Analysis of secreted LDH has not shown any cytotoxic affect of Sema3A. Co-staining for CHAT and cleaved caspase-3 detected absence of apoptosis activation in MNs incubated with Sema3A. To further test the effect of Sema3A on survival of MNs we applied the protein on MN- enriched cultures overnight, and examined total cell survival 24 and 48 hours later. Alamar/Blue analysis has not detected differences in the number of living cells between non-treated and treated cultures. Specific monitoring of HB9-GFP+ MNs by microscopy did not show differences in MNs numbers between non-treated and treated cultures. Our results do not support a cytotoxic effect of Sema3A on hESC-derived spinal cord MNs.

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F2025

DEVELOPMENT OF AN IMAGE-BASED HIGH-CONTENT SCREENING ASSAY FOR TAU CLEARING DRUGS IN A HUMAN IPSC-DERIVED NEURONAL CELL MODEL OF FRONTOTEMPORAL DEMENTIA

Cheng, Chialin[1], Reis, Surya A.[1], Adams, Emily T.[1], Silva, M. Catarina[1], Hennig, Krista M.[1], Fass, Daniel M.[1], Feldman, Danielle A.[2], Sur, Mriganka[2], Dickerson, Bradford C.[3], Kosik, Kenneth S.[4] and Haggarty, Stephen J.[1,3], [1]Massachusetts General Hospital, Harvard Medical School, Boston, MA, U.S., [2]Massachusetts Institute of Technology, Cambridge, MA, U.S., [3]Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, U.S., [4]Neuroscience Research Institute, University of California, Santa Barbara, CA, U.S.

Autosomal dominant mutations in the microtubule-associated protein gene (MAPT) encoding the protein tau cause frontotemporal dementia spectrum disorders (FTD-s). These MAPT mutations are associated with pathologically abnormal tau phosphorylation levels and intracellular accumulation of aggregated protein predominantly in neurons (“tauopathy”). Recently, a rare variant of tau, Tau-A152T, located N-terminal of the microtubule-binding domain has been described. This variant of tau has decreased affinity for binding microtubules in vitro, and has been shown to increase the risk for FTD-s, Alzheimer's disease, and synucleinopathies. Here we used human induced pluripotent stem cells (iPSC) from a FTD-s subject diagnosed with progressive supranuclear palsy carrying this Tau-A152T variant as a genetically accurate cell model of tauopathy. To utilize these cells to create a rapid and robust biological cellular assay system capable of supporting the discovery of novel therapeutics for tauopathies, we have adapted strategies for the inducible expression of the pro-neural transcription factor Neurogenin 2 in stably transduced iPSC-derived neural progenitor cells (iNgn2-NPCs). We demonstrate the ability to efficiently and reproducibly generate nearly limitless numbers of excitatory, glutamatergic-like neurons from these iNgn2-NPCs in a 96-well plate format with abundant expression of tau with enhanced polarized distribution to axonal processes. In order to monitor potential mutation-induced aberrant subcellular tau distribution, as well as drug-induced tau clearance, we developed a high-content image-based screen utilizing automated confocal microscopy and an advanced image-processing pipeline optimized for analysis of morphologically complex neuronal cultures. We summarize the results of a pilot screen for tau clearing compounds targeting autophagy and protein homeostasis pathways with an emphasis on clinically used FDA-approved drugs with potential for repurposing. This strategy will aid in expediting the translational research in

elucidating novel targets for therapeutic intervention for neurological diseases involving tauopathy such as FTD-s.

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F2027

SINGLE CELL TRANSCRIPTOMIC ANALYSIS OF YOUTHFUL AND AGING ADULT MURINE NEURAL STEM CELLS DEFINES TRANSCRIPTIONAL DYNAMICS AND REVEALS HETEROGENEITY IN THE AGING PROCESS

Dulken, Ben[1], Leeman, Dena[1], Boutet, Stephane[2], Hebestreit, Katja[1] and Brunet, Anne[1], [1]Stanford University, Stanford, CA, U.S., [2]Fluidigm Corporation, South San Francisco, CA, U.S.

Neural stem cells (NSCs) in the adult mammalian brain serve as a reservoir for the generation of new neurons, oligodendrocytes, and astrocytes throughout life. However, NSCs exhibit a decline in their proliferative capacity and function during aging. The molecular identities and heterogeneity of in vivo NSC populations in the youthful and aged states have been incompletely characterized. Here we use single cell RNA-sequencing to characterize adult NSC populations in young and aged mice. We show that cells in the NSC lineage exist on a continuum through the processes of activation and differentiation, and that rare intermediate states with distinct molecular profiles can be identified. Our analysis also identifies putative surface markers and key intracellular regulators for these new subpopulations of NSCs. We subsequently extended this analysis to define the aging adult NSC lineage at single cell resolution. We found that while the majority of aged NSCs were highly similar to their young counterparts, a subset of aged NSCs exhibit the marked upregulation of a module of genes associated with interferon signaling. We go on to validate the presence of this population of aged NSCs by staining in vivo for markers of this putative population as suggested by their RNA-sequencing profiles. These data help refine the understanding of the youthful NSC lineage, and are an important step to understanding and ameliorating the functional decline of adult NSCs during the aging process.

F2029

RESTORATION OF HUMAN APP TRANSGENIC MOUSE COGNITIVE DYSFUNCTION AFTER TRANSPLANT OF HUMAN IPS CELL-DERIVED NEURAL STEM/PRECURSOR CELLS

Fujiwara, Naruyoshi[1], Kenji, Takai[2], Chieko, Hirotsu[2], Erika, Takada[2], Nagisa, Arimitsu[2], Jun, Shimizu[2] and Noboru, Suzuki[2], [1]St. Marianna University School of Medicine, Kawasaki-shi Kanagawaken, Japan, [2]St. Marianna University School of Medicine, Kawasaki-shi Kanagawa-ken, Japan

Cell replacement is one of the radical treatments on regenerative medicine. We have already reported that transplantation of neural precursor cells derived from human iPS (hiPS) cells restored the spatial memory learning of PDAPP mice last year's ISSCR. On this occasion, we are going to show several experimental findings which contributed to restore the spatial memory learning. The hiPS cell lines, 253G1 (RIKEN, Tsukuba, Japan), were used in this study. We first developed embryoid bodies (EB). Then EB were cultured on fibronectin (FN)-coated dishes and we added retinoic acid (RA), noggin-Fc (NOG) and sonic hedgehog (SHH). We transplanted the neuronal precursors into the PDAPP mice at day 8. Neuronal precursors derived from hiPS cells (2×10^5 cells per 2µl of saline; n=21) and PBS (n=19) were stereotaxically transplanted into hippocampus. MWM test was conducted 14 days before and 15 days after the transplantation to assess the spatial memory learning of PDAPP mice. We generated neuronal precursors with cholinergic and GABAergic neuron phenotype. The cells expressed ChAT (82.0 ± 1.5 %), VGAT (2.5 ± 0.2 %) and a7nAChR (79.0 ± 4.0 %) at day 8. Thus, cholinergic and GABAergic neurons emerged from hiPS cells in vitro. (Transplantation and MWM test) After the transplantation, platform escape latency of the transplanted PDAPP mice was shorter compared with that of vehicle injected PDAPP mice (P=0.04). We conducted histological analysis of serial brain sections. We found that transplanted neural cells differentiated into ChAT positive neurons and VGAT positive neurons. Moreover, transplantation of neuronal precursors induced differentiation of recipient neurons, which expressed a7nAChR and GABAR. The grafted cells differentiated into both cholinergic neurons and GABAergic neurons in the cortex and hippocampus. The recipient neurons expressing receptors for the neurotransmitters emerged after the transplantation. The restoration of both acetyl choline/a7nAChR interaction and GABA/GABAR interaction may be importantly associated with the improvement of cognitive dysfunction in the dementia model mice.

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F2031

ESTABLISHMENT OF IN VITRO FUS-ASSOCIATED FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS MODEL USING HUMAN INDUCED PLURIPOTENT STEM CELLS

Ichiyanagi, Naoki[1], Fujimori, Koki[1], Yano, Masato[2], Fujisaki, Chikako[3], Sone, Takefumi[1], Akiyama, Tetsuya[4], Okada, Yohei[5], Akamatsu, Wado[6], Matsumoto, Takuya[7], Ishikawa, Mitsuru[6], Nishimoto, Yoshinori[1], Ishihara, Yasuharu[8], Sakuma, Tetsushi[9], Yamamoto, Takashi[9], Tsuiji, Hitomi[10], Suzuki, Naoki[4], Warita, Hitoshi[4], Aoki, Masashi[4] and Okano, Hideyuki[11], [1]Keio University, Tokyo, Japan, [2]Keio University, Tokyo, Japan, [3]Keio University School of Medicine, Tokyo, Japan, [4]Tohoku University Graduate School of Medicine, Sendai, Japan, [5]Aichi Medical University School of Medicine, Aichi, Japan, [6]Department of Physiology, Keio University, School of Medicine, Tokyo, Japan, [7]Keio University, Shinanomachi, Japan, [8]Keio University School of Medicine, Tokyo, Japan, [9]Hiroshima University, Hiroshima, Japan, [10]Nagoya City University, Aichi, Japan, [11]Keio University School of Medicine, Tokyo, Japan

Amyotrophic lateral sclerosis (ALS) is a late-onset motor neuron disorder. Although its neuropathology is well understood, the cellular and molecular mechanisms that lead to the initiation and progression of this disease are yet to be elucidated due to limitations in the currently available human genetic data. In this study, we generated induced pluripotent stem cells (iPSC) from two familial ALS (FALS) patients with a missense mutation in the fused-in sarcoma (FUS) gene carrying the heterozygous FUS H517D mutation, and the isogenic iPSCs with the homozygous FUS H517D mutation obtained by genome editing from the healthy control iPSCs. These cell-derived motor neurons mimicked several neurodegenerative phenotypes. A part of the mutant FUS protein was localized outside the nucleus and co-localized with stress granules under stress conditions. Moreover, FALS motor neurons showed more apoptotic activity than did control motor neurons. Exon array analysis using motor neuron precursor cells (MPCs) combined with CLIP-seq data sets revealed aberrant gene expression and/or splicing pattern in FALS-MPCs. These results suggest that iPSC-derived motor neurons are a useful tool for analyzing the pathogenesis of human motor neuron disorders.

F2035

GENERATION OF LARGE NUMBERS OF FLOOR PLATE DERIVED, MIDBRAIN-SPECIFIED DA NEURONS FROM HUMAN PCS FOR SCALED APPLICATIONS

Kuninger, David[1], Derr, Michael[1], Yan, Yiping[1], Vedvik, Kevin[2], Sangenario, Lauren[1] and Shin, Soojung[1], [1]Thermo Fisher Scientific, Frederick, MD, U.S., [2]Thermo Fisher Scientific, Madison, WI, U.S.

Stem cell derived midbrain dopaminergic (DA) neurons provide an excellent cell source for disease modeling and drug screening for Parkinson's disease. To expedite this effort, we have developed culture system which can make authentic midbrain DA neurons starting from human pluripotent stem cells. Our system was designed to simplify and standardize the whole differentiation process while compressing timelines and adding increased flexibility in to this complex differentiation workflow. The process has 3 distinct steps: (1) specification of hPSC to midbrain floor plate (mFP) cells, (2) expansion and cryopreservation of derived mFP cells, and (3) maturation to DA neurons. Our system has the unique feature of enabling the generation of expanded banks at the midpoint, with mFP cells expanded at least 200 fold (2 passages) prior their cryopreservation (optional step). However, we were challenged to further increase the size of the cell bank to facilitate certain applications requiring large numbers of cells and standardization, such as high through put drug screening. To achieve this goal we developed an alternative workflow enabling stable growth of mFP cells up to passage 10 (p10). The cryopreserved mFPp10 were able to be directly recovered on assay format and after 7 days in maturation, phenotype marker of TH was examined using high throughput imaging system. The standardized work flow resulted in consistent differentiation of our reference PSC line over time. However we have noticed significant variation in maturation efficiency can occur across PSC lines and looked for ways to reduce this variability. To improve the differentiation efficiency, we tested a number of conditions and ultimately incorporated an extended differentiation procedure for low performing lines. As a result, we could get comparable differentiation efficiency from this line. Thus, we could use our system successfully across multiple cell lines. For Research Use Only.

F2037

EVALUATION OF HUMAN EMBRYONIC STEM CELL DERIVED DOPAMINERGIC PROGENITORS AND NEURONS FOR CELL TRANSPLANTATION IN PARKINSON MOUSE MODEL

Liao, Mei-Chih[1], Qui, Lifeng[2], Chen, Allen[1], Wei, Shunhui[3], Reuveny, Shaul[1], Tan, Eng King[2], Zeng, Li[2] and Oh, Steve[1], [1]Bioprocessing Technology Institute, Singapore, [2]National Neuroscience Institute, Singapore, [3]Institute of Molecular and Cell Biology, Singapore, Singapore

Parkinson's disease (PD) is second most prevalent neurodegenerative disease in the world. It is a progressive disease causing complicated motor disorders. Studies show that the main cause of PD is a significant loss of dopaminergic (DA) neurons in the Substantia Nigra, a region of the midbrain. At present, there is no cure for PD, but a few medical treatments could alleviate PD symptoms. We believe cell replacement therapy is a promising strategy to treat PD. With recent advances, we can generate functional midbrain dopaminergic neurons from human pluripotent stem cells. Several research groups have transplanted either DA progenitors or neurons into animal models and demonstrated functional improvement in symptoms. However, none of them have compared the effects of transplanting progenitors versus neurons. Here, we applied the floor plate- based protocol to produce midbrain DA progenitors and neurons from human embryonic stem cells. We obtained a high percentage of Foxa2 and Lmx1a –expressing cells in culture (indicating differentiation into DA progenitor cells), and subsequently DA neurons that expressed Nurr1, TH, and beta-tubulin. Furthermore, differentiated DA neurons release dopamine, and display multiple action potentials, hence demonstrating their functional behavior. These derived DA progenitors and neurons were transplanted into the striatum of adult NOD-SCID IL2Rgc null mice. Cells were delivered as neural spheroids instead single cells to increase cell survival and functionality. The result showed that animal transplanted with early stage of DA neuron (cells started to express Nurr1) exhibited better behavior recovery. This could be correlated to higher proportion of grafted DA neurons integrated with the host brain tissues. In conclusion, the identification of the stage of differentiated cells in DA neurogenesis plays an important role in future cell replacement therapy for Parkinson's disease.

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F2039

MATERNAL IMMUNE RESPONSES EVOKED BY TOLL-LIKE RECEPTOR ACTIVATION RESULT IN NEOCORTCAL PROGENITOR CELL DYSFUNCTION LEADING TO ABNORMAL NEURODEVELOPMENT IN MICE

Moon, Hyang Mi[1], Saravanapandian, Vidya[1,2], Babineau, Brooke[1], Su, Jennifer[1], Subramanyam, Geetha[3], Narayan, Aditi[1], Cisneros, Trinidad[4], Carpentier, Pamela[1], Rivera, Moises[1] and Palmer, Theo D.[1], [1]Stanford University, Department of Neurosurgery, Institute for Stem Cell Biology and Regenerative Medicine, Stanford, CA, U.S., [2]UCLA, Interdepartmental PhD program for Neuroscience, Los Angeles, CA, U.S., [3]San Jose State University, CIRM Bridges Program, Graduate Program in Biological Sciences, San Jose, CA, U.S., [4]Stanford University, Immunology Graduate Program, Stanford, CA, U.S.

Neocortical progenitor cells (NPCs) play crucial roles in fetal brain development by generating neurons, astrocytes, and oligodendrocytes. Mid-gestation is a critical timepoint of fetal/placental development and coincides with the peak of NPC-derived neurogenesis, which makes NPCs vulnerable to environmental immune insults. Autism spectrum disorder (ASD) is a neurodevelopmental disease with repetitive behaviors and social interaction deficits. Epidemiological studies and an innate immune dysfunction in ASD suggest environmental risks like prenatal infections increase ASD pathology. Innate immune responses are evoked by toll-like receptor (TLR)-dependent signaling pathways. However, it is unknown whether diverse TLR responses result in unique pathological outcomes by differentially affecting NPCs during early development. We determined whether different infections cause distinct effects on fetal-maternal interaction in the mouse. Pregnant mice were challenged with TLR agonists during mid-gestation and we analyzed the pregnancy outcomes and NPCs. After bacterial (TLR4) and viral (TLR3) mimetics-mediated immune responses, changes in NPC division/fate determination in the developing fetuses and behavioral batteries in the postnatal offspring were tested to short-term and long-term effects, respectively. We assessed differences in NPC proliferation, cell cycle alterations, stem cell niche cryoarchitecture and neuronal subtype specification in the fetal brains from the TLR-challenged pregnancy compared with control. Cortical patterning was severely impaired by prenatal challenges accompanied with elevated immune cytokine/chemokine. Proliferation and divisions of NPCs were disturbed by TLR activation. ASD-like neuropsychiatric behavioral deficits were detected in the offspring from TLR agonist-challenged pregnancy. Together, this study indicates distinct NPC dysfunctions in early brain devel-

opment lead to the wide ranges of neuropsychiatric outcomes to increase ASD risks.

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F2041

NEUROLIGIN 3 AND ADAPTIVE MYELINATION

Mount, Christopher[1], Arnold, Evan[1], Sudaresh, Shree[1] and Monje, Michelle[2], [1]Stanford University, Stanford, CA, U.S., [2]Stanford University, Stanford, CA, U.S.

There is growing evidence that myelin undergoes dynamic changes throughout the course of adult life. These changes may be adaptive, and likely play an underappreciated role in normal processes of postnatal learning and memory. Currently, the molecular mechanisms underlying adaptive myelination are unclear. Intriguingly, the proliferation and differentiation of oligodendrocyte precursor cells (OPCs) in mice is modulated by neuronal activity, suggesting that communication between neurons and OPCs may modulate oligodendroglial lineage dynamics. A leading candidate for the mode of this communication is neuron-OPC synaptic communication, which has been well described in rodents but whose role remains unclear. Neuroligin 3 (Nlgn3) – a key regulator of neuronal synaptic function – is highly expressed in OPCs. Given the key role of neuroligins in establishing and maintaining synaptic function in neuronal synapses, we hypothesize that Nlgn3 may play a critical role in mediating activity-dependent communication between neurons and OPCs. To evaluate this hypothesis, we have utilized constitutive mouse models of Nlgn3 deletion, as well as conditional models in which Nlgn3 is deleted either across the entire oligodendrocyte lineage or specifically within OPCs. We have studied the impact of these Nlgn3 deletions upon myelin tract structure and oligodendrocyte lineage dynamics, as well as quantified neuron-OPC synapses. We have found structural alterations in white matter tract development in the absence of Nlgn3, as well as reductions in neuron-OPC glutamatergic synapses. Intriguingly, these differences do not appear during the course of juvenile development, but only in the young adult period, suggesting that adult myelin development and neuron-OPC synaptic communication may depend upon Nlgn3. Our data suggest a previously unknown role for a critical modulator of synaptic function in adult white matter development and shed light on the regulation of neuron-OPC synaptic communication.

F2043

CHARACTERIZATION OF HUMAN IPSC-DERIVED ALZHEIMER'S DISEASE ASTROCYTES

Oksanen, Minna Elina[1], Puttonen, Katja Annina[1], Ruponen, Marika[1], Hamalainen, Riikka[1], Viitanen, Matti[2], Rinne, Juha[3,4], Malm, Tarja[1], Kanninen, Katja[1] and Koistinaho, Jari[1], [1]University of Eastern Finland, Kuopio, Finland, [2]University of Turku and Turku City Hospital, Turku, Finland, [3]University of Turku, Finland, [4]Turku University Hospital, Turku, Finland

Astrocytes are the most abundant cell type in the central nervous system, and they have several essential functions including maintenance of synaptic plasticity and homeostasis, removal of excessive transmitters and energy metabolism. Astrocytes are considered equally important to neurons in neurodegenerative disorders, and they are known to play a role in Alzheimer's disease (AD). Current human models for studying the role of astrocytes in AD are inadequate and mainly limited to the availability of post-mortem samples. Although iPS cells have great potential for human disease modelling, all the published AD studies so far have focused on neurons instead of astrocytes. In the current study, iPS cells were generated from familial AD patients with deletion of exon 9 in presenilin-1 gene (n=3) and from healthy age-matched controls (n=3). iPS cells were further differentiated to astrocytes, and a variety of biochemical analyses were applied to characterize their phenotype. A significant reduction in the maximal respiratory capacity of AD astrocytes was detected through studying mitochondrial function by Seahorse XF technology, thereby indicating mitochondrial dysfunction in AD cells. The expression of autophagy proteins was altered concomitantly with an increased release of glutathione, thereby suggesting altered autophagic and antioxidant responses. In addition, inflammatory stimulation of AD astrocytes resulted in increased release of cytokines when compared to control cells. In conclusion, our data suggests that pathological findings commonly seen in AD patients are recapitulated in iPSC-derived astrocytes. Therefore, the iPS-derived patient-specific AD astrocytes offer a valuable tool for studying the disease mechanisms and for testing novel therapeutic approaches.

F2045

INTRACOCHLEAR TRANSPLANTATION OF NEURONAL PROGENITORS TO REPLENISH LOST SPIRAL GANGLION NEURONS AFTER EXPERIMENTAL BACTERIAL MENINGITIS

Perny, Michael[1,2], Roccio, Marta[2,3], Grandgirard, Denis[1], Zimmermann, Jeannine[1], Leib, Stephen[1] and Senn, Pascal[2,4], [1]Neuroinfectiology Laboratory, Institute for Infectious Diseases (IFIK), University of Bern, Switzerland, [2]Laboratory of Inner Ear Research, Department of Clinical Research, University of Bern, Switzerland, [3]Department of Otorhinolaryngology, Head & Neck Surgery, Inselspital Bern, Freiburgstrasse, Switzerland, [4]Department of Otorhinolaryngology, Head & Neck Surgery, Hôpitaux Universitaires de Genève, Geneva, Switzerland

Bacterial meningitis (BM) is the most common cause of acquired profound bilateral sensorineural hearing loss (SNHL) in childhood occurring in up to 30% of patients with *Streptococcus pneumoniae* infection. Hearing loss is irreversible because spiral ganglion neurons (SGNs) and hair cells have a limited regenerative capacity. Surviving SGNs are a prerequisite for the correct functioning of cochlear implants, which represents the only treatment option for severe SNHL. The aim of the project is to develop a stem cell-based therapy to replenish lost SGNs after experimental BM in infant rats. This will generate the foundation towards a regenerative therapy to improve the efficacy of cochlear implants in children with hearing deficits.In order to analyze the fate and functional effect of transplanted stem cells in the inner ear, we characterized sensorineural hearing loss in a well-established rat model of BM. We show in great detail how SGNs and hair cells are lost upon BM with the biggest damage occurring in the basal part of the cochlea. The initial bacterial inoculum accurately determines the severity of cochlear cell pathologies and thereby allows us study the fate of transplanted cells in different cochlear microenvironments. Preliminary stem cell transplantations were performed two weeks after the infection in rats with bacteriologically cleared meningitis. Thereby, green fluorescence protein (GFP) progenitor cells were isolated from neonatal rat spiral ganglions and grown for two passages. They were transplanted directly into the basal part of the modiolus via a retroauricular, transbullar approach. We show that grafted progenitor cells survive and morphologically integrate into the modiolus with the formation of axonal structures.In conclusion, our initial data demonstrates proof of concept for effective surgical cell transplantation into the cochlear nerve trunk and we have evidence that transplanted stem cells survive and differentiate into neurons. Optimization of the surgical access to minimize traumatic damage to inner ear structures and the molecular characterization of SGN progenitors is currently ongoing. The functional role of grafted cells on hearing function will be assessed by auditory brainstem recordings.

F2047

PURIFICATION OF CORTICAL PYRAMIDAL NEURON PROGENITORS USING DEVELOPMENT-RELATED SURFACE MARKERS

Sano, Noritaka[1,2], Ioroi, Yoshihiko[1], Morizane, Asuka[3], Miyamoto, Susumu[2] and Takahashi, Jun[1,2], [1]CiRA, Kyoto University, Kyoto, Japan, [2]Kyoto University Graduate School of Medicine, Kyoto, Japan, [3]Kyoto University, Kyoto, Japan

Neurological disorders including stroke, ALS and Parkinson's disease are major causes of severe physical disability, and pluripotent stem cells (PSCs) including iPS cells have attracted much attention as a source of many kinds of donor cells including cortical pyramidal neurons. Reconstructing a long neural circuit by neural cell transplantation, especially the corticospinal tract, is challenging, but might be the final target for the recovery stroke. Many kinds of protocol of inducing cortical neurons from PSCs have been invented in the last decade, but there has been no method of purifying cortical pyramidal neurons. Several reports indicated that grafted neurons from embryonic cortex of mice extended their axons to the spinal cord in the neonatal animals, and in a certain situation, constituted a part of corticospinal tract even in the adult mice. One of these articles proved that extending axons from the graft showed rather immature cortical neuron characteristics, for example, Doublecortin+/ Neu N/Fox3-. Other reports proved that pyramidal neuron-directed differentiation of PSCs facilitated elongation of axons along corticospinal tract. However, there is no report that indicated which type of cells in the grafted cerebral cortex are needed to reconstruct the corticospinal tract. Purification of certain type of neurons is necessary for clinical application, considering the stable efficiency and the risk of tumor formation after transplantation. In this study, we dissected and dissociated the frontal cortex of E14.5 eGFP knock-in mice. Then the dissected cells were applied to the cell sorter with several different antibodies against surface antigens expressed on developmental cortical neurons. The purified cells were then grafted to the cortex of neonatal mice. 35days after transplantation we estimated the number of neurites from the graft in the internal capsule, pons, and spinal cord. We identified an antibody that could recognize the "elite" neural progenitors that more efficiently would extend neurites along corticospinal tract after transplantation. Moreover, qPCR and immunocytochemical analysis reviled that the purified "elite" progenitors could attain mature characteristics of cortical pyramidal neuron earlier (in a few days) than the surrounding other cells.

F2049

HUMAN ADULT HIPPOCAMPAL NEUROGENESIS IN HEALTH AND DISEASE

Steiner, Embla[1], Bergmann, Olaf[1], Huttner, Hagen[1,2], Alkass, Kanar[1], Spalding, Kirsty L.[1], Bernard, Samuel[3], Salehpour, Mehran[4], Possnert, Göran[4], Druid, Henrik[1] and Frisén, Jonas[1], [1]Karolinska Institutet, Stockholm, Sweden, [2]University of Erlangen-Nuremberg, Erlangen, Germany, [3]University of Lyon, Villeurbanne, France, [4]Uppsala University, Uppsala, Sweden

Hippocampal neurogenesis appears to be altered in multiple neurological and psychiatric diseases. Depression is associated with reduced hippocampal neurogenesis and antidepressant drugs stimulate neuronal turnover in rodents. In Alzheimer's disease and Parkinson's disease the results are more contradicting. Some suggest an adaptive stimulating response to neuronal death and others suggest that neurogenesis is reduced already before clinical and neuropathological changes occur. After stroke, reactive neurogenesis in the cortex has been demonstrated in rodent, but not in human. Most studies on adult neurogenesis are performed in animal models, because studying adult neurogenesis in humans is technically difficult. To investigate the turnover of cell populations in human tissues, our lab has developed a retrospective birth dating method. We analyze the concentration of ^{14}C derived from nuclear bomb testing during the cold war in genomic DNA. We have used this strategy to study the turnover in neurogenic niches and the extent of neuronal turnover in human brains. We have found both differences and similarities between rodent and human adult neurogenesis in healthy conditions. In contrast to rodents, there seems to be very little, if any, neurogenesis in the human olfactory bulb. A subpopulation of interneurons in the striatum also seems to have a substantial turnover in humans, something that has not been shown in animals. Comparable to rodents, however, there is substantial neurogenesis in the human hippocampus. This supports the theory that adult neurogenesis may play an important role in diseases. In view of the differences between human and rodent neurogenesis, further human studies in pathological conditions are needed. In order to investigate the role of adult neurogenesis in human pathology, we have set out to investigate the neuronal turnover in the hippocampus of patients suffering from major depression, stroke, Parkinson's disease and Alzheimer's disease using retrospective birth dating.

F2051

EPIGENOMIC DYNAMICS DURING NEURAL TUBE FORMATION

Valensisi, Cristina[1], Andrus, Colin[2], Buckberry, Sam[3], Malonzo, Maia[4], Lund, Riikka[5], Lahdesmaki, Harri[4], Lister, Ryan[6] and Hawkins, David[2], [1]Division of Medical Genetics, Department of Medicine, Department of Genome Sciences, Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Seattle, WA, U.S., [2]University of Washington, Seattle, WA, U.S., [3]The University of Western Australia, Crawley, Australia, [4]Aalto University, Helsinki, Finland, [5]University of Turku and Åbo Akademi University, Turku, Finland, [6]University of Western Australia, Crawley, WA, Australia

hESCs-derived neural rosettes (NRs) represent a valuable tool to recapitulate the neural tube formation in humans, providing a powerful resource for in vitro modeling of neural tube formation-related disorders (NTDs). NTDs are the second most common congenital birth defects. NTDs have complex and multifactorial etiology with both genetic and environmental factors involved in the pathogenesis. The impact of environmental factors on NTDs is likely mediated through epigenetic mechanisms. To date, epigenomic studies are lacking in a 3D neural tube system, with limited data available from monolayer culture of neural progenitor cells. We constructed ChIP-seq maps for H3K4me1, H3K27ac, H3K27me3 and H3K4me3 in a highly pure population of hESCs-derived NRs to ascertain the chromatin landscape at regulatory elements. By comparing our data to those previously generated in monolayer cultured neural progenitor cells, such as radial glial cells, we identified over eight thousands NR-restricted putative active enhancers. TFBS motif enrichment analysis of these enhancers revealed that these regulatory elements are enrichment for canonical neural factors, such as LHX2 and RFX5. These results suggest that the same core master regulators are involved in governing different neural stem cells populations, but the regulatory landscapes are quite distinct. These distinctions likely result in different development potentials and recapitulate different developmental stages and cell types. We also conducted whole genome bisulfite sequencing and identified NR-specific genomic regions characterized by un-methylated and low-methylated regions (UMR/LMRs), which were used to predict active and cell type-specific regulatory elements. We have identified thousands of differentially methylated regions (DMRs) between NRs and monolayer neural progenitors. Finally we profiled coding and non-coding transcripts and identified approximately two thousand NR-restricted genes. A number of which are indicative of neural tube formation. Overall, our analysis showed that NRs have a distinct epigenomic landscape and transcriptome with respect to monolayer cultured neural progenitor cells, and that are likely to be important for neural tube

formation. Our study provides the first epigenomic map in a 3D human neural tube model.

F2053

A NOVEL CELLULAR HIERARCHY OF NEURAL PROGENITOR CELLS DOWNSTREAM OCT4-EXPRESSING PNSCS AND GFAP-EXPRESSING DNSCS IN MICE

Yammine, Samantha, Gosio, Jessica and van der Kooy, Derek J., University of Toronto, Toronto, ON, Canada

There are two distinct types of neural stem cells (NSCs) in the developing mouse embryo that generate the diversity of neural progenitor cells (NPCs) that build the brain. Primitive (p)NSCs form clonogenic neurospheres when grown in LIF and persist into the adult brain, where we have shown they express the pluripotency gene *Oct4* and give rise to the GFAP$^+$ definitive (d)NSCs that form neurospheres in EGF and FGF2. We used the neurosphere assay in vitro to enrich for progenitor cells downstream of either NSC type to characterize the functional differences between these two NSC populations. Here we show both NSC types derived from the E17.5 mouse brain give rise to NPCs that are bipotent in the neuronal and glial lineages, though pNSCs give rise to more neuron-only NPCs. Interestingly, progenitor cells from either NSC that gave rise to both lineages generated significantly more progeny than those committed to either glial or neuronal fates, suggesting that multipotent progenitors have more self renewal ability than unipotent progenitors. As pNSCs can give rise to dNSCs as well as neurons and glia, we asked whether pNSCs can directly produce neurons and glia without a dNSC intermediate step. To test this, we grew clonal spheres from early postnatal mice with herpes simplex virus thymidine kinase expression driven by the GFAP promoter, which causes GFAP-expressing cells (ie. definitive NSCs) to be killed upon division following administration of ganciclovir. There is a significant reduction in the number of neurospheres that form in EGF and FGF2 from definitive NSCs from the subependymal zone of early post-natal mice with this manipulation, as expected. Preliminary results show pNSCs from these mice could still form GFAP$^+$ astrocytes, however the number of ßIII$^+$ neurons were reduced. This suggests that astrocytes can be formed from pNSC-derived progenitor cells that do not upregulate GFAP expression until after they are post-mitotic, whereas neurons downstream pNSCs derive from a GFAP-expressing intermediate cell. By combining the retroviral lineage tracing and GFAP-TK data, we have constructed a novel hierarchy for progenitor cells downstream primitive and definitive NSCs that shows that these distinct NSC types give rise to unique sets of NPCs.

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F2055

MECHANISTIC STUDY OF CHEMICAL-INDUCED CELLULAR REPROGREAMMING

Zhang, Mingliang[1], Li, Ke[1], Cao, Nan[2] and Ding, Sheng[1], [1]Gladstone Institutes, San Francisco, CA, U.S., [2]Gladstone Institutes of Cardiovascular Disease, San Francisco, CA, U.S.

Chemical-induced cellular reprogramming provides a promising approach to generating clinically relevant cell types for regenerative medicine, and a unique process to study the mechanism underlying. To achieve a chemical-induced cell fate conversion, and explore the mechanism, we carried out step-wise chemical screening. We first transduced fibroblasts with episomal plasmids carrying OSKM, and identified lead chemicals that greatly improved the efficiency of OSKM-induced neural stem cells generation. Using this as a model system, a focused chemical library was screened again which led to identification of chemical cocktails that induce expression of endogenous neural master gene Sox2 in the absence of exogenous transgenes. The resulting cells exhibit uniformly cell morphology and molecular features resemble the primary neural progenitor cells. They are highly proliferative and could generate neurons in vitro. Gene expression analysis indicates that in the presence of chemical cocktail (1) the neural genes required for ectoderm fate determination, such as Shh, Ntf3, Hes5, and Nkx2.2, were greatly upregulated; (2) the neural fate determine genes, such as Ascl1, Pax6, and Sox1, were increased; and (3) the proneural genes, such as NeuonD1, NeuroG2, were induced and the repressive gene REST was downregulated. These results highlight a neural induction function of the cocktail we identified. The mechanistic study is currently on going. Our study provided a potential to obtaining lineage specific progenitor cells via chemicals, which holds the promise to drug discovery and cell replacement therapy for neurodegenerative disease.

F2057

HUMAN NEURAL STEM CELLS AND A SELF-ASSEMBLING PEPTIDE HYDROGEL IMPROVE FUNCTIONAL RECOVERY AND TISSUE REPAIR AFTER SPINAL CORD INJURY IN RATS

Sperling, Laura Elena[1], Reis, Karina[2], Pozzobon, Laura[2], Chagastelles, Pedro[2], Rodriguez, Alexandra[3], Nisbet, David[3], Nicola, Fabricio[4], Netto, Carlos A.[4] and Pranke, Patricia[2,5], [1]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Stem Cell Laboratory, Fundamental Health Science Institute, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, Porto Alegre, Brazil, [2]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Stem Cell Laboratory, Fundamental Health Science Institute, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [3]ANU College of Engineering and Computer Science, Canberra, Australia, [4]Department of Biochemistry, Fundamental Health Science Institute, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [5]Institute for Research with Stem Cells, Porto Alegre, Brazil

Cell transplantation therapy is a promising approach for spinal cord injury (SCI) and is extensively used to improve recovery. Neural stem cells are considered one of the best candidates for SCI transplants. However, this strategy is limited by the poor survival and uncontrolled differentiation of transplanted stem cells. In order to achieve greater survival and integration with the host tissue, the efficacy of tissue repair and motor recovery after transplantation of human embryonic stem cell derived neural stem (NSCs) and a self-assembling nanofibrous hydrogel into a subacute contusion model of rat SCI was evaluated. Male adult Wistar rats (n=28) were subjected to a spinal cord injury by contusion using the MASCIS impactor. The animals were divided into four groups: hydrogel, NSCs, hydrogel together with NSCs and lesion control. 10 μL of a 20 mg/mL Fmoc-DIKVAV hydrogel was injected into the lesion epicenter one hour after the SCI. All the animals were subjected to a second operative procedure 7 days after SCI when 2x 10^5NSCs were transplanted centrally into the lesion site. All the other animal groups received an injection with vehicle only. Functional recovery was assessed using the Basso, Beattie and Bresnahan (BBB) locomotor rating scale at different time points, before surgery, two days after and weekly up to six weeks after transplantation. The rats transplanted with NSC/Fmoc-DIKVAV and NSCs showed improved but not statistically significant BBB scores relative to the vehicle alone. The rats injected with Fmoc-DIKVAV showed significantly higher BBB scores when compared with the vehicle. By hematoxylin and eosin staining, visible cavities of varying sizes were observed in the injury site. The rats transplanted with NSCs showing smaller cavities and the presence of increased cellular material at the injury site. To quantify and examine the inflammatory response, the glial scar, the NSCs fate and neural degeneration, flow cytometry analysis was performed with various antibodies. Together, the flow cytometry data shows that the NSC-transplanted groups showed improved neural regeneration and the inflammation and amount of astrocytes was increased in the transplanted groups. The use of biomaterials for stem cell transplantation is promising for enhanced recovery following spinal cord injury.

Funding Source: CNPq, CAPES, FAPERGS, Stem Cell Research Institute

F2059

ADENOSINE SIGNALLING AS AN UNDERLYING PATHOLOGY IN LESCH-NYHAN DISEASE

Crapper, Liam[1], Bell, Scott[2], Peng, Huashan[2], Maussion, Gilles[2], Rosneberger, Thad[3], Jinnah, Hyder[4], Visser, Jasper[5], Turecki, Gustavo[2] and Ernst, Carl[2], [1]McGill/Douglas Research Hospital, Montreal, QC, Canada, [2]McGill University, Montreal, QC, Canada, [3]University of North Dakota, Grand Forks, ND, U.S., [4]Emory University, Atlanta, GA, U.S., [5]Radboud University, Nijmegen, Netherlands

Lesch-Nyhan Disease (LND) is a severe neurodevelopmental disorder caused by mutations in the gene HPRT1 and characterized by hyperuricemia, dystonia, intellectual disability, and compulsive self-injurious behaviour. Although the disease was first identified in 1963, there is still no treatment for the neurological symptoms, nor has a functional association between HPRT1 mutations and self-injury or dystonia been established. Patients with LND have very low levels of dopamine, and it is believed that this contributes to the neurological symptoms, but again the connection of HPRT1 to dopamine remains unknown. Using patient IPS derived cortical and dopaminergic neurons we have shown substantially reduced levels of key genes the adenosine pathway, and reduced adenosine receptor A1 signalling. We have also examined the effects of reduced adenosine signalling on dopamine production in patient cell lines and the effects of HPRT deficiency on adenosine signalling in HPRT KO mice. Adenosine provides a direct link between the metabolic activity of HPRT and the dopaminergic dysfunction that has been observed in LND.

REPROGRAMMING

F2061

INTEGRATION-FREE REPROGRAMMING OF PRIMARY HUMAN FIBROBLASTS INTO NEURAL STEM CELLS USING BRIEF EXPOSURE TO PLURIPOTENCY FACTORS.

Barta, Tomas[1,2] and Hampl, Ales[1,2], [1]St. Anne's University Hospital, International Clinical Research Center (ICRC), Brno, Czech Republic, [2]Masaryk University, Brno, Czech Republic

Cell reprogramming has recently attracted an increasing attention as a tool for a range of applications including autologous cell therapy, drug discovery, as well as disease modelling. In order to change cell fates, multiple approaches including induction of pluripotency and direct cell lineage conversion have been used. Induction of pluripotency have become a promising tool, however, concerns related to acquired pluripotency including higher probability of genomic instability, low efficiency, immune rejection, and high costs represent major hurdles towards practical applications of this approach. Recently, an alternative concept of cell fate switch has been introduced. Induction of cell plasticity involves the transient cell opening leading to "plastic state" that is permissive to lineage switch in response to specific stimulus. Induction of cell plasticity has the advantage of higher efficiency, elevated proliferation and erasure of epigenetic memory, when compared to direct cell lineage conversion. Furthermore, it also generates a rejuvenated multipotent progenitor cell population, which is able to differentiate into specific cell types, hence lacking all limitations typical for transdifferentiated cells. Here we describe a protocol for integration-free reprogramming of human fibroblasts (hFs) into neural stem cells (NSCs) by brief exposure to pluripotency factors. We used integration-free Sendai virus expressing Oct4, Sox2, Klf4, and c-Myc to infect hFs. Reprogrammed cells do not express viral pluripotency factors, express typical markers for neural stem cells, have typical morphology of NSCs, can be propagated in vitro (45 passages tested), and differentiate into astrocytes, neurons, oligodendrocytes. Importantly, recent evidence suggests that brief exposure to pluripotency factors may lead to induction of pluripotency, however our data indicates that using our approach does not lead to induction of pluripotency.

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F2063

DIRECTLY CONVERTED INDUCED NEURAL STEM CELLS (iNSC) AS A ROBUST AND SAFE SOURCE FOR AUTOLOGOUS CELL REPLACEMENT

Edenhofer, Frank[1], Wörsdörfer, Philipp[2], Meyer, Sandra[2] and Günther, Katharina[2], [1]University of Wuerzburg, Wuerzburg, Germany, [2]University of Würzburg, Institute of Anatomy and Cell Biology, Würzburg, Germany

Generation of iPSCs from adult skin fibroblasts and subsequent differentiation into somatic cells provides fascinating prospects for the derivation of autologous transplants that circumvent histocompatibility barriers. However, progression through a pluripotent state and subsequent complete differentiation into desired lineages remains a roadblock for the clinical translation of induced pluripotent stem cell technology because of the associated neoplastic potential and low cost efficacy. Recently, we showed that somatic cells can not only be converted into iPSCs but also into different types of multipotent somatic stem cells by using defined factors, thereby circumventing progression through the pluripotent state. In particular, the direct conversion of human fibroblasts into induced neural progenitor cells (iNPCs) heralds the possibility of a novel autologous cell source for various applications such as cell replacement, disease modeling and drug screening.Here, we describe the direct conversion of patient-specific fibroblasts into iNPCs by timely restricted expression of Oct4, Sox2, Klf4, as well as c-Myc within 2 months. Sox2-positive neuroepithelial colonies appear after 17 days of induction and iNPC lines can be established efficiently by monoclonal isolation and expansion. Precise adjustment of viral multiplicity of infection and supplementation of leukemia inhibitory factor during the induction phase represent critical factors to achieve conversion efficiencies of up to 0.2%. Thus far, Patient-specific iNPC lines could be expanded for more than 15 passages and uniformly display morphological and molecular features of neural stem/progenitor cells, such as the expression of Nestin and Sox2. The iNPC lines can be differentiated into neurons and astrocytes as judged by staining against TUJ1 and GFAP, respectively. In conclusion, we report a robust protocol for the direct conversion of human fibroblasts into stably expandable neural progenitor cells that might provide a cellular source for biomedical applications including disease modeling and autologous neural cell replacement.

F2065

DISSECTION OF EARLY PHASE OF REPROGRAMMING BY COMPARATIVE GENOMIC ANALYSIS ON DIRECT LINEAGE REPROGRAMMING

Ha, Jeongmin[1,2], Yoon, Byoung-Ha[1,2], Lee, Minhyung[1,2], Cho, Sang Mi[3], Nam, Ki-Hoan[3], Kang, Yong-Kook[1,2], Kim, Mirang[1,2] and Kim, Janghwan[1,2], [1]Korea University of Science & Technology, Daejeon, Korea, [2]Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea, [3]Laboratory Animal Resource Center, KRIBB, Cheongju, Korea

Recently, applications of reprogramming technologies have been broadened extensively to potential autologous regenerative therapies and disease modeling for drug discovery. However, mechanistic interpretation of the reprogramming process is still remained elusive. In addition, most of these studies are focusing on the process of generating induced pluripotent stem cells (iPSCs). Thus, we sought to investigate the direct lineage reprogramming (DR) process. Depending on the reprogramming factors used, the DR can be divided into two approaches, i.e. somatic cell-specific factor-mediated direct reprogramming and pluripotent cell-specific factor-mediated direct reprogramming (PDR). Interestingly, PDR uses the same reprogramming factors as iPSC reprogramming (iPSCR), but generates various lineage-specific cells instead of iPSCs. Thus, we compared these two reprogramming processes, PDR and iPSCR, to figure out how and when these processes are bifurcated from each other during the reprogramming. Dox-inducible reprogrammable cells were used to control the expression of the reprogramming factors. We analyzed the cells, which are sampled every other day by microarray. Until day 6, all samples (PDR and iPSCR) showed a similar pattern of gene expression, but the pattern has changed dramatically after day 6 regardless of dox-induction or specific cues for fate determination. These suggest that the process of PDR is also divided into two steps as known in iPSCR and the cells around day 6 in our experiment could be the intermediate and epigenetically flexible cells. When we focused on the early phase of reprogramming, i.e., from day 0 to day 6, we found the expression patterns could be divided into four distinct groups. Interestingly, one of the groups showed exclusively up-regulated pattern on day 6 but down-regulated in fibroblasts, iPSCs, and induced neural progenitors. Thus, we assumed that this group might represent the intermediate cells (ICs). Gene Ontology analysis shows that these genes are related to epithelial development and retinoic acid metabolism. We expect that by studying the roles of these genes, we could understand the early process of both PDR and iPSCR which is currently assumed as stochastic and characterize the IC which is still unidentified.

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F2067

HOMOGENEOUS GENERATION OF IDA NEURONS WITH HIGH SIMILARITY TO BONA FIDE DA NEURONS USING A DRUG INDUCIBLE SYSTEM.

Park, Hanseul and **KIM, Jongpil**, Dongguk University, Seoul, Korea, South

Recent work generating induced dopaminergic (iDA) neurons using direct lineage reprogramming potentially provides a novel platform for the study and treatment Parkinson's disease (PD). However, one of the most important issues for iDA-based applications is the degree to which iDA neurons resemble the molecular and functional properties of their endogenous DA neuron counterparts. Here we report that the homogeneity of the reprogramming gene expression system is critical for the generation of iDA neuron cultures that are highly similar to endogenous DA neurons. We employed an inducible system that carries iDA-inducing factors as defined transgenes for direct lineage reprogramming to iDA neurons. This system circumvents the need for viral transduction, enabling a more efficient and reproducible reprogramming process for the generation of genetically homogenous iDA neurons. We showed that this inducible system generates iDA neurons with high similarity to their bona fide in vivo counterparts in comparison to direct infection methods. Thus, our results suggest that homogenous expression of exogenous genes in direct lineage reprogramming is critical for the generation of high quality iDA neuron cultures, making such culture systems a valuable resource for iDA-based drug screening and, ultimately, potential therapeutic intervention in PD.

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F2069

LYSINE (K)-SPECIFIC DEMETHYLASE 4A (KDM4A) ENHANCE REPROGRAMMING OF SCNT EMBRYO USING FROZEN-THAWED MOUSE OOCYTES

Lee, Ah Reum[1], Bang, Jae Il[2] and Lee, Dong Ryul[2,3], [1]CHA University, Seongnam-si, Korea, South, [2]CHA University, Seongnam-si, Korea, [3]CHA University, Seoul, Korea

The efficiency of cellular reprogramming is very low in both somatic cell nuclear transfer (SCNT) and induced pluripotent stem cell (iPS) technologies. In fact, SCNT is a process associated with gradual epigenetic changes, implying that epigenetic manipulation in somatic nucleus is a key to increase the efficiency of reprogramming. Recently, it was shown that the microinjection of Kdm4a or Kdm4d mRNA significantly improve developmental efficiency of SCNT embryos in mouse and.).Moreover, Kdm4a mRNA also can improve the establishment of human SCNT-ES cells. Meanwhile, human in vitro fertilization–embryo transfer (IVF-ET) is routinely performed in the clinics for infertility treatment, some of the retrieved oocytes has cryopreserved for the next use.If the pregnancy has been achieved, preserved oocytes will be discarded or donated. If possible to find the way to use these cryopreserved oocytes after human IVF-ET, it would be a good resource for human therapeutic cloning.In the present study, we have examined whether injection of Kdm4a mRNA can improve the efficiency of SCNT using frozen-thawed oocytes.The rate of 2 cell block in the Kdm4a mRNA injected-SCNT embryo was significantly decreased than in the control and the sham injected embryos, respectively.Moreover, blastocyst formation of SCNT embryos derived from frozen-thawed oocytes was increased when injection of Kdm4a mRNA was applied. Also, the numbers of total and inner cell mass (ICM) were increased in Kdm4a mRNA-injected group. Taken together, the results indicate that the epigenetic regulation using Kdm4a mRNA injection can enhance the efficiency of SCNT in frozen-thawed oocytes as well as fresh one.It would be a good resource for human therapeutic cloning.

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F2071

REPROGRAMMING FIBROBLASTS INTO HAIR-FORMING CELLS IN VIVO WITH DEFINED ENVIRONMENTAL PROTEINS

Fan, Sabrina Mai-Yi[1], Tsai, Chia-Feng[2], Plikus, Maksim V[3], Chen, Yu-Ju[2] and **Lin, Sung-Jan**[1,4], [1]National Taiwan University, Taipei, Taiwan, [2]Academia Sinica, Taipei, Taiwan, [3]University of California, Irvine, Irvine, CA, U.S., [4]National Taiwan University Hospital, Taipei, Taiwan

Organ development is a complicated yet well-orchestrated process of self-organization involving different cell types. Despite the growing knowledge of the mechanisms for organ development, little is known about how they can be re-elicited in adults for regeneration. We found that cell-free extract from the embryonic skin of perifolliculogenetic stage conferred adult fibroblasts with the competence to form new hair follicles. IGF and Wnt signaling were activated and required for this process in adult fibroblasts. Transciptome and functional analysis showed that fibroblasts were broght to a state with hair inductive property after exposure to embryonic cell-free extract. The gained hair inductive competency was reversible and lost after a washout period. Through proteomics analysis, we identified three secreted proteins enriched in the embryonic skin that together were essential and sufficient to induce new hair follicles in adult mice. Therefore, extracellular proteins can confer the competency for regeneration on fibroblasts by partially reprogramming them toward embryo-like state in which they can re-engage in developmental interactions. Identification of factors to recreate tissue-specific embryonic extracellular context can be a novel strategy to promote regeneration in various adult organs.

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F2073

DATA-DRIVEN CELL CONVERSION WITH MOGRIFY

Rackham, Owen[1],., Firas, Jaber[2]Petretto, Enrico[3], Polo, Jose Maria[2], and Gough, Julian[1], [1]University of Bristol, Bristol, U.K, , [2]Monash University, Melbourne, VIC, Australia, and [3]Duke-NUS Medical School, Singapore, Singapore

It has been extensively described that the introduction of sets of carefully selected transcription factors can induce directed cell conversion between cell types. Given the number of human transcription factors and cell types the bottleneck has become correctly identifying

the set of transcription factors for any given cell conversion. To help alleviate this, we have developed Mogrify, a predictive system that combines gene expression data with regulatory network information to predict the reprogramming factors necessary to induce cell conversion. Mogrify correctly predicts the transcription factors used in known transdifferentiations and has been made available to the community at www.mogrify.net.Here I will present two examples of the application of Mogrify; Firstly, how we have used the predictions to perform and experimentally validate novel cell conversions without the need for trial-and-error selection of the transcription-factor sets. Secondly, how we have adapted the algorithm to facilitate the identification of alternatives to transcription factor over-expression for driving cell conversion. Both of these examples taken together will demonstrate how taking a data-driven approach (such as Mogrify) can accelerate the field of cell conversion.

F2075

SUBSTRATE STIFFNESS REGULATES FOCAL ADHESION KINASE IN THE DIRECT CONVERSION OF FIBROBLASTS INTO NEURONS

Soto, Jennifer, Wong, SzeYue, Chu, Julia, Park, Hyungju, Poo, Mu-Ming and Li, Song, University of California, Berkeley, CA, U.S.

Direct reprogramming is the process of converting from one cell type into a very distantly related cell type without proceeding through an intermediate proliferative stem-cell like stage. Previous studies have shown that somatic cells can be directly reprogrammed into specific neuronal subtypes using different combinations of transcription factors and microRNAs and in addition, biochemical factors can help reprogram these cells into induced neuronal (iN) cells. However, how biophysical factors regulate iN reprograming through mechanotransduction is not well understood. Here we show, for the first time, that substrate stiffness can enhance iN reprogramming efficiency in a biphasic manner that is dependent on the matrix stiffness. The involved mechanism relies on the mechanomodulation of focal adhesion kinase (FAK). We found that stiffness modulates the expression of FAK in a linear fashion, such that the phosphorylation of FAK decreases as stiffness decreases. In addition, we found that by disrupting focal adhesion kinase signaling through chemical inhibition we can mimic the reprogramming trend that is observed with substrate stiffness. The derived iN cells display a typical neuronal morphology, express neuronal markers and exhibit functional neuronal properties. Altogether, the results suggest that substrate stiffness regulates the expression of focal adhesion kinase and thus there is an optimal level of expression that allows for a more efficient conversion of fibroblasts into neurons. In essence, findings from this study will aid to broaden our understanding of how cell-matrix interactions can influence the fibroblast-to-neuron reprogramming process. These novel findings can provide insights into the mechanisms that determine cell fate with potential applications in neurological disease modeling and drug discovery.

F2077

IMPROVED REPROGRAMMING OF MOUSE GALLBLADDER CELLS TO GENERATE INSULIN-PRODUCING CELLS

Wang, Yuhan[1], Galivo, Feorillo[2], Pelz, Carl[2], Haft, Annelise[2] and Grompe, Markus[2], [1]OHSU, PORTLAND, OR, U.S., [2]Oregon Health & Science University, Portland, OR, U.S.

Type I diabetes is a disease caused by deficits of insulin-producing ß cells in the islets of the pancreas. Although islet transplantation is the only definitive treatment for the disease, this approach is largely limited by the availability of transplantable material. Therefore, finding alternative cell sources to generate insulin-producing cells has been a major research goal in the field. Cells originate from the endodermal lineage, such as acinar cells in the pancreas, the liver and the gallbladder cells, have been of particular interest because of their developmental proximity to the ß cells. Previously, our lab demonstrated that mouse gallbladder cells could be propagated and reprogrammed in vitro to generate insulin-producing cells. In this study, we further optimized the reprogramming protocol and were able to demonstrate improvement in both reprogramming efficiency and efficacy towards the pancreatic lineage. With the new protocol, more than 30% of insulin-producing cells were stably generated from in vitro cultures. The second generation reprogrammed cells (rGBC2.0) have a more than 50-fold increase in insulin expression and secrete insulin in response to glucose stimulation. Gene expression analysis indicate that the rGBC2.0 cluster closer with ß cells and have a similar metabolic profile as neonatal ß cells. Upon transplantation into immune-deficient animals, rGBC2.0 persist for at least 5 months and further mature in vivo.

F2079

A COCKTAIL OF SMALL MOLECULES DRIVES A DIRECT CONVERSION OF HUMAN FIBROBLASTS INTO NEURAL STEM CELLS

Choi, Soon Won[1], Shin, Ji-Hee[2], Uddin, Md. Hafiz[1], Shin, Tae-Hoon[1], Kang, Tae Wook[1], Kim, Hyung-Sik[1], Seo, Yoo Jin[1] and Kang, Kyung-Sun[1], [1]Adult Stem Cell Research Center, Seoul National University, Seoul, Korea, South, [2]Seoul National University, Seoul, Korea, South

A next generation of patient-specific cell therapies for neural degenerative disorders, such as Alzheimer's and

Parkinson's diseases, are based on direct conversion technologies to generate target neural cell types. As recently reported in many studies, human fibroblasts can be directly converted into functional neuronal cells, bypassing an induced pluripotent stem cell (iPSC) stage and also removing exogenous transgenes. However, sources of such direct conversions have been restricted and the converted neurons have a limited capacity to proliferate. Here, we report a direct conversion of human fibroblasts into expandable, induced neural stem cells by four chemicals (ciNSCs), without passage of a transient pluripotent stage. These ciNSCs resembled hiPSC- and human fibroblast-induced NSCs, with neurosphere formation and NSC-specific gene and protein expressions. After a further maturation, ciNSCs can be differentiated into three main neural lineages, namely neurons, astrocytes and oligodendrocytes in vitro/in vivo. Additionally, they can be terminally differentiated into specific neuronal subtypes, such as dopaminergic neurons, under defined culture conditions. Thus, our data demonstrate that a specific chemical cocktail can drive the neural lineage-specific direct conversion of human somatic cells into progenitors with self-renewal and multipotency. This chemical-only approach for iNSCs can, therefore, be suggested as an alternative technique to produce transgene-free and large-scale sources for patient-specific cell-based therapies.

IPS CELLS

F2083

AUTOLOGOUS HUMAN IPSC AND THEIR MYOGENIC DERIVATIVE ARE TOLERATED WHILE THEIR ALLOGENEIC COUNTERPARTS ARE REJECTED BY DISTINCT MECHANISMS IN HUMANIZED MOUSE MODELS

Benabdallah, Basma, CHU-Ste Justine, Laval, QC, Canada

Generation of induced pluripotent stem cells (iPSC) and their differentiation into various cell types represents a promising strategy for regenerative therapy. This technology is expected to yield unlimited amount of patient-specific iPSC without the concern of immune rejection. However, several studies using murine iPSC and iPSC-differentiated cells were shown to elicit an immune response in syngeneic mice. Similarly, immune responses to various autologous human iPSC derivatives were also observed in a humanized mouse model. These recent findings highlights the urgent need to better evaluate the immunogenicity of these cells. In that effect, we observed that undifferentiated iPSC are targeted by NK cells in vitro, probably due to their loss of MHC-I expression after reprogramming. In contrast, iPSC failed to activate both autologous and allogeneic T-cells. Conversely, iPSC-derived myogenic progenitor cells (iPSC-huMyoP) could induce allogeneic but not autologous T-cells activation in vitro. When injected in humanized mice, iPSC and iPSC-huMyoP were also evaluated for their capacity to form teratomas or to engraft in skeletal muscle respectively. To do so, we used two distinct humanized mouse models (hereafter named hu-BLT or hu-PBMC) generated following the injection of fetal liver and thymic tissues or peripheral blood mononuclear cells respectively. Our results showed that allogeneic but not autologous teratomas are rejected in humanized mouse models. Similarly, our results also showed that autologous myogenic progenitor cells derived from iPSC are well tolerated in both humanized models. In contrast, we found allogeneic fibers derived from iPSC-huMyoP to be rejected by CD8+ T-cells. Our results provide important information on the immunogenicity of iPSC and iPSC-huMyoP and provide essential pre-clinical data for the use of iPSC-based cell-replacement therapies in humans.

F2085

USING CARDIOMYOCYTES DERIVED FROM LVNC CARDIOMYOPATHY HIPSC FOR THE MECHANISTIC STUDY AND DRUG SCREENING

Yu, Yu-Xin, Institute of Pharmacology, National Taiwan University, Taipei, Taiwan and **Chen, Wen-Pin**, Institute of Pharmacology, National Taiwan University, Taipei/Taiwan, Taiwan

Dilated cardiomyopathy (DCM) causes left ventricular dilation and systolic failure. The heterogeneous etiologies underlying DCM limited our understanding of the long-term pathogenesis of DCM. A unique familial DCM family with significant left ventricular noncompaction (LVNC) was identified with two mutations in the sarcomeric genes (TNNT2:R151W and MYPN:S1296T). Furthermore, DCM progression in the daughter carrying the mutations was faster than that in the DCM father. The lack of available donors results in significant mortality for pediatric patients awaiting transplantation. Thus, it is appealing to establish the individualized gene-specific therapy. Using cardiomyocytes derived from DCM-hiPSC (hiPSC-CM), the present study aimed to investigate the underlying mechanism and to identify the therapeutic drugs. Our study found that hiPSC-CM derived from these DCM-LVNC families could successfully recapitulate the disease phenotype with big cell size, the decrease of cell shortening, and the abnormal intracellular calcium handling property around day 60 after differentiation. It was also found the nuclear translocation of TNNT2 in DCM-derived hiPSC-CM. Furthermore, a significant increase of the sensitivity to milrinone in increasing cell shortening was found in DCM-derived hiPSC-CM on day 60. The contractile dysfunction could be partly reversed with the treatment of statins since day 15 after differentiation, though the treatment did not change in the nuclear translocation of TNNT2. This study established a DCM-LVNC-hiPSC-CM platform to characterize

the pathogenesis for mechanic study and the development of therapeutic drugs.

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F2087

MODELLING ALZHEIMER'S DISEASE EMPLOYING HUMAN PATIENT-DERIVED NEURONS BASED ON INDUCED PLURIPOTENT STEM CELL (IPSC) TECHNOLOGY

De Filippis, Roberta, Janssen, Katharina, Vasileva, Maria, Liebel, Bettina, Spieler, Katharina, Gomm, Daniela, Kiefer, Claudia, Van Bergeijk, Jeroen, Nimmrich, Volker, Reinhardt, Peter, Lakics, Viktor, Bakker, Margot H.M. and Terstappen, Georg C., Abbvie, Ludwigshafen, Germany

Human induced pluripotent stem cell (hiPSC) technology enables a promising approach to model neurodegenerative disorders in vitro. This technology offers the possibility to establish a drug discovery platform for target identification/validation and generation of lead molecules for therapeutic intervention employing human neurons, directly derived from healthy controls or patients, promising better 'translatability' of results. In this study we developed and optimized two neuronal differentiation protocols to generate specific neuronal subtypes (i.e. cortical and midbrain) to setup a phenotypic in vitro models for Alzheimer´s disease (AD). These protocols were optimized for application with an automated cell culture system to increase reproducibility and to allow for a medium- to high-throughput scale. Molecular characterization studies carried out during several stages of the differentiation process employing RT-PCR and immunofluorescence staining, demonstrated an increase in expression of neuronal markers (e.g. vGLUT1/2, GAD65, Tuj1 and Map2), as well as the presence of synapses (e.g. synaptophysin and PSD95). The hiPSC-derived neurons regional identity was confirmed by using a customized RT-PCR-based gene expression array. Neurite outgrowth was investigated in the presence or absence of different growth-promoting or -inhibiting compounds employing live cell and high content imaging technologies. Employing whole cell patch clamp analyses 6 weeks after neuronal induction sodium and potassium like currents were detected, and action potentials and spontaneous synaptic activity were indicative of appropriate neuronal functioning. To establish disease-relevant phenotypic assays neuronally differentiated hiPSCs, derived from age-matched healthy controls and familial AD patients, were compared regarding the production of Aß40 and Aß42 in a time course experiment. Dysregulations in Calcium signaling were revealed employing calcium imaging by investigating spontaneous Calcium oscillations in healthy controls and familiar AD neurons. Taken together, our studies demonstrate the utility of patient-derived iPSC-based neurons for the implementation of human disease-relevant model systems for drug discovery, which will support the development of new effective therapies.

Funding Source:

F2089

HUMAN INDUCED PLURIPOTENT STEM (IPS) CELL-BASED APPROACHES TO MODEL AND TREAT ATAXIA-TELANGIECTASIA

Franco, Sonia, Bhatt, Niraj, Ghosh, Rajib, Gao, Yongxing, Armanios, Mary and Cheng, Linzhao, Johns Hopkins University School of Medicine, Baltimore, MD, U.S.

Ataxia Telangiectasia (A-T) is an autosomal recessive syndrome caused by mutations in ATM, a large serine-threonine kinase encoded by the ATM locus at 11q22. Among its pleiotropic clinical manifestations, ataxia develops early in childhood and typically results in severe disability in the second decade of life. Consistently, radiology of affected patients reveals progressive cerebellar atrophy and pathology of postmortem A-T cerebellums shows loss of Purkinje neurons. A-T has no cure and most patients die in early adulthood due to severe recurrent infections or cancer. The goal of our work is to develop stem cell-based approaches to: 1) understand the requirement for ATM in the maintenance of cerebellar neurons; and 2) develop cell-based regenerative therapies for the disease. Towards these goals, we have recently generated and characterized induced pluripotent stem (iPS) cells from an A-T patient. Previous studies had reprogrammed A-T fibroblasts using feeder layers and integrating vectors and found a marked decrease in reprogramming efficiency. In contrast, we find that A-T peripheral blood (PB) is a robust source of iPSCs even in xeno-free, feeder-free, episomal vector-based protocols. Moreover, we find that, in baseline conditions, A-T iPS cells maintain pluripotency with passage, have a normal karyotype and reset telomere length similar to ATM-proficient iPS cells. However, exposure of A-T iPS cells to ionizing radiation reveals defective activation of the G2/M cell cycle checkpoint and increased radiosensitivity, two main features of the disease. Finally, we find that A-T iPS cells differentiate to neural stem cells (nestin-positive, Sox1-positive cells) in vitro. Altogether, this work supports the use of blood-derived A-T iPS cells for disease modeling and, in the longer term, gene therapy and tissue regeneration.

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F2091

GENERATION OF CLINICALLY COMPATIBLE AND GENETICALLY STABLE IPS CELL LINES FROM HUMAN PERIPHERAL AND CORD BLOOD USING MICRORNA-FACILITATED SRRNA REPROGRAMMING

Eminli-Meissner, Sarah[1], Moon, Jung-Il[2], Yi, Kevin[2], Kiskin, Fedir[3], Kwieder, Baraa[3], Chang, C-Hong[3], Rana, Amer[3] and **Hamilton, Brad**[4], [1]Stemgent, a Reprocell Group Company, Lexington, MA, U.S., [2]Stemgent, Part of the ReproCELL Group, Lexington, MA, U.S., [3]University of Cambridge, Cambridge, U.K., [4]Stemgent, Part of the ReproCELL Group, Cambridge, MA, U.S.

Repeated transfection of fibroblasts with a cocktail of reprogramming mRNAs results in the generation of stable, integration-free human iPS cells. While much advancement has been made to refine this process on fibroblasts, little has been done to advance the application of RNA reprogramming to 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. Notably, blood-outgrowth endothelial progenitor cells (EPCs) can be clonally isolated from only 10 mL of fresh or cryopreserved mononuclear cell (MNC) preparations from both human peripheral and cord blood. In 2013, published results demonstrated the reprogramming of human neonatal fibroblasts into iPS cells using self-replicative RNA (srRNA), with as few as one transfection. Subsequently, we have extended the application of srRNA for cellular reprogramming to peripheral and cord blood derived EPCs. Optimization of mRNA delivery, culture media composition and transitions, as well as incorporation of reprogramming associated microRNAs allowed us to develop a singular EPC reprogramming protocol, whereby we were able to generate integration-free, wholly pluripotent human RNA-EPC-iPS cell lines from 42 out of 54 different primary patient samples (78% reprogramming efficiency) in the first pass. Subsequent improvements have resulted in a simple and robust two transfection, no-split protocol using only GMP-compatible substrates (vitronectin and laminin-511) and media, without the need for conditioned or FBS containing medium. These integration-free RNA-EPC-iPS cells exhibit superior genetic stability when compared to fibroblasts derived RNA-iPS cells, making them an exceptional choice for applications requiring clinical grade cells. Additionally, they demonstrate highly consistent cardiomyocyte and neural differentiation. The unique combined application of microRNA and srRNA, using GMP-compliant reagents, for the cellular reprogramming of human EPC lines derived from peripheral and cord blood results in genetically stable, clinically relevant iPS cells.

F2093

INDUCTION OF PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS (IPSCS) TO ADIPOCYTE LINEAGE AS A DISEASE MODEL TO STUDY ADIPOCYTE DYSFUNCTION IN POLYCYSTIC OVARY SYNDROME

Huang, Mei-Chi[1], Ho, Hong-Nerng[2,3] and Chen, Hsin-Fu[2,3], [1]NTU College of Medicine Graduate Institute of Medical Genomics and Proteomics, Taipei, Taiwan, [2]Graduate Institute of Medical Genomics and Proteomics, College of Medicine and the Hospital, National Taiwan University, Taipei, Taiwan, [3]Department of Obstetrics and Gynecology, College of Medicine and the Hospital, National Taiwan University, Taipei, Taiwan

Polycystic ovary syndrome (PCOS) is a common female endocrine disorder presenting with chronic anovulation, hyperandrogenism and polycytic ovaries. Dysfunctions of adipose tissue, such as abnormal enlargement of adipocytes, defects in insulin signaling pathway and dysregulated expression of adipokines, have been shown to be associated with the occurrence of metabolic syndrome in PCOS. Induced pluripotent stem cells (iPSCs) are derived by reprogramming somatic cells into embryonic stem-like cells that have the potential to differentiate to all cell types in the body. We thus aimed to derive PCOS patient-specific iPSCs and differentiate the cells to adipocyte lineage in order to study the potentially defective adipogenesis and dysregulated function in PCOS. Now we have established the iPSCs by reprogramming luteinized granulosa cells from PCOS and non-PCOS patients using Sendai virus reprogramming system. Adipocyte differentiation was done firstly through mesenchymal progenitor cells (MPCs) stage and then the MPCs were induced toward adipocyte lineage by treatment with adipogenesis cocktail. After differentiation for 30 days, we successfully derived putative adipocytes fulfilling the criteria of morphology, presence of lipid droplets, expression of adipocyte markers as ZNF423, CEBPb&d (early-stage), DLK1, CEBPa, PPARd (middle-stage), CEBPa, PPARg (late-stage) and FABP4, GLUT4 (mature-stage). We found out that the expression of most adipocyte markers were significantly higher in PCOS patients (though with the exception of two earlier markers, CEBPa and DLK1) and the number of cells with lipid droplets were higher in PCOS patients, both suggesting enhanced adipogenesis. Interestingly, we also identified lower expression level of brown adipocyte marker UCP1, a marker of enhanced energy expenditure, in PCOS patients. Now studies are actively going on for the global gene expression profilings, adipokines expression, lipolysis function and glucose uptake ability between PCOS and non-PCOS patients, which have shown some interesting results especially on the defective lipolysis and dysfunctional insulin signaling. These

results thus suggest a novel and likely efficient approach of using this iPSCs-adipocytes disease model for exploration of the PCOS mechanisms and future drug testing.

F2095

EVALUATION OF GENETIC FACTORS OF OPLL USING PATIENT-SPECIFIC iPSCs

Kawai, Shunsuke[1,2], Hada, Masataka[3], Koyama, Yuko[3], Ikeya, Makoto[4], Alev, Cantas[1], Hotta, Akitsu[5], Ikegawa, Shiro[6], Nakamura, Masaya[7], Yoshitomi, Hiroyuki[1,3], Matsuda, Shuichi[2] and Toguchida, Junya[1,3], [1]Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [2]Department of Orthopaedic Surgery, Gradate School of Medicine, Kyoto University, Kyoto, Japan, [3]Department of Tissue Regeneration, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan, [4]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [5]Department of Life Science Frontier, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [6]Laboratory for Bone and Joint Diseases, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan, [7]Department of Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Japan

Ossification of the posterior longitudinal ligament (OPLL) is one of spinal disorders among the elderly. The ossified ligament compresses the spinal cord and nerve roots, causing motor and sensory impairments, and may cause tetraplegia in the worst case. Ossification is supposed to develop via endochondral process, although the precise mechanism is unknown and no effective preventive therapies are available. OPLL is a multifactorial disease, and both genetic and acquired factors are involved. The involvement of genetic factors is suggested by the familial occurrence, and a genome-wide association study (GWAS) identified six disease-associated SNPs.To examine the effect of genetic factors precisely, we established iPS cells (iPSCs) from patients in families with more than two affected members. Six patients from two families (three from each) were enrolled by collaboration with the OPLL research group in Japan, and iPSCs were established from peripheral blood cells of each patient (OPLL-iPSCs). We checked the difference of osteogenic and chondrogenic differentiation potential between MSCs induced from OPLL-iPSCs and standard iPSCs. We found higher osteogenic differentiation potential in MSCs induced from OPLL-iPSCs than those from standard iPSCs, but no significant difference between them in chondrogenic differentiation potential. Genotyping of six disease-associated SNPs of these patients revealed that all three members of one family were homozygous for the risk allele at one of six SNPs. To evaluate the biological effect of this risk allele precisely, we are establishing SNP-modified OPLL-iPSCs in which the risk allele is converted to the non-risk allele by genome editing. These results suggest that the iPSC-based approach for OPLL is a useful tool to study underlying genetic factors of OPLL.

F2097

AN INTEGRATED APPROACH FOR GENERATION OF VALIDATED HUMAN IPSC CELL BANKS

Quintanilla, Rene H., Sridharan, Mahalakshmi, van Baarsel, Eric and **Lakshmipathy, Uma**, Thermo Fisher Scientific, Carlsbad, CA, U.S.

The reprogramming of somatic cells into induced Pluripotent Stem Cells (iPSC) has emerged as a powerful tool for dissecting basic biology with the added potential of applications in drug screening and cell therapy. With advances in reprogramming technologies, use of diverse media systems and somatic cell sources, there is a compelling need for comprehensive characterizations methods that facilitate the generation of high quality standardized iPSC cell banks. In this study, we utilize an integrated approach to track somatic cell reprogramming, qualify resulting iPSCs to generate validated cell banks, and employ routine quality control measures to confirm high quality of the cells prior to use in downstream applications. Progression in reprogramming is monitored using a combination of positive and negative self-renewal markers via real-time monitoring and flow cytometry methods. Selected iPSC colonies are expanded and comprehensively characterized utilizing antibody staining and hPSC ScoreCard qPCR assays to confirm self-renewal marker expression and tri-lineage differentiation potential. Functionally pluripotent iPSC with normal karyotype, confirmed cell line identity, and free of mycoplasma and pathogens are banked to generate master frozen cell stocks. Viability and post thaw recovery of the frozen cell stocks are confirmed and a routine 2 marker flow cytometry analysis is carried out as a quality control measure prior to downstream applications such as gene editing and directed differentiation to specific lineages. A streamlined method of iPSC qualification and banking is critical for traceability to ensure the identity and quality of cells chosen for drug screening. In addition it serves as a foundational tool for the generation of clinical-grade banks that have potential use in extensive trails.

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F2099

PREMATURE OVARIAN FAILURE PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM (IPS) CELLS SHOW COMPROMISED POTENTIAL TO DEVELOP TO GERM CELLS AND GRANULOSA CELLS

Lu, Chen-Yu[1], Chen, Yu-An[2], Wu, Fang-Chun[3], Huang, Mei-Chi[1], Ho, Hong-Nerng[1,3] and Chen, Hsin-Fu[1,3], [1]Graduate Institute of Medical Genomics and Proteomics, College of Medicine and the Hospital, National Taiwan University, Taipei, Taiwan, [2]Graduate Institute of Medical Genomics and Proteomics, College of Medicine, Nati, Taipei, Taiwan, [3]Department of Obstetrics and Gynecology, College of Medicine and the Hospital, National Taiwan University, Taipei, Taiwan

Premature ovarian failure (POF; also called primary ovarian insufficiency, POI), is a common endocrine disorder in females, accounting for 1-2% in reproductive-age women. Patients with the disease experience spontaneous menopause before the age of 40 years, and frequently encounter significant difficulties in overcoming the problems of infertility and menopausal syndrome. However, the mechanisms underlying POF are difficult to study due to the lack of a suitable disease model. Recently pluripotent stem cells, including human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, are becoming useful cell sources to study and dissect the molecular mechanisms underlying in vitro differentiation into specialized cell types. We and other researchers have previously shown that pluripotent stem cells including both ES cells and iPS cells are capable of developing into primordial germ cells (PGCs) and ovarian granulosa-like cells. In this study therefore, we aimed to derive human iPS cells from POF patients and further differentiate the stem cells to germ cell-related lineages. Now we have successfully derived and characterize patient-specific iPS cell lines from five POF patients and two control subjects by transduction of skin fibroblasts or peripheral blood mononuclear cells (PBMCs) with four defined factors (OCT3/4, SOX2, KLF4 and c-Myc) using Sendai virus reprogramming method. The resultant human iPS cells were then induced by using the cytokine cocktails towards PGCs, which expressed germ cell markers VASA, GDF9 and meiosis marker SCP3, and also towards functional granulosa-like cells, which expressed FOXL2, FSHR, AMHR2, AMH and CYP19A1. We also observed that the POF-patient iPS cells derivatives showed differential and reduced expression levels of the granulosa cell markers (AMH, AMHR2, CYP19A1 etc.) and germ cell marker VASA, when compared to control iPS and ES cells. The present data thus supported that we could produce POF-specific iPS cells and induce the stem cells in vitro to PGCs and granulosa cells. Considering the altered phenotypes of the stem cell derivatives, the POF patient-specific iPS cells derived in this study thus represent a potentially useful disease model for further studies into the disease mechanisms and to screen drugs that may be useful for treating and arresting POI.

F2101

FUNCTIONAL ASSESSMENT OF REPROGRAMMING-ASSOCIATED AND CULTURE-ASSOCIATED MUTATIONS IN HUMAN INDUCED PLURIPOTENT STEM CELLS

Miga, Karen Hayden, Salama, Sofie and Haussler, David H., University of California, Santa Cruz, Santa Cruz, CA, U.S.

Generation of induced pluripotent stem cells (iPSCs) from patient-derived somatic cells offers tremendous potential for precision medicine. However, reprogramming of iPSCs from cultured somatic cells has been shown to introduce a new source of undesired sequence variants in coding regions and large-scale genome rearrangements, thereby inhibiting their clinical use. As a result, rigorous genetic screening and attention to culturing procedures of patient iPSCs is essential to eliminate lines with an unacceptable mutational burden. Analyses based on short-read sequencing technologies are challenged by inconsistencies in complex structural variant predictions and typically omit mutagenic events in repetitive DNA. Furthermore, it is difficult to confidently link introduced sequence and epigenetic variants to potential cellular function that could influence downstream therapeutic efforts. Here, we directly address these gaps in the genome-wide analysis of iPSC reprogramming and culture-associated mutagenic events by developing bioinformatics and genomic resources from iPSC lines derived from the extensively characterized GM12878 cell line, which is credited as a genomic standard in variant calling, haplotype phasing, and epigenomic datasets. Here we present functional and genomic characterization of two, independent IPSC lines derived from GM12878. In doing so, we can provide more complete assessment of structural variants introduced during reprogramming and subsequent long-term cell culture. Additionally, by using a Tier 1 ENCODE cell line, we are able to couple variant discovery with extensive functional annotation, critical to understanding the impact of introduced mutagenic events in transcriptional regulation and epigenetic inheritance, providing analysis from single nucleotide variants to tracking mono-allelic regulation of the inactive X chromosome. Further, this project will critically contribute to the functional assessment of retrotransposon-mediated insertions and satellite DNA stability in regions of constitutive heterochromatin, resulting in an exhaustive characterization of repeat-rich regions commonly omitted from standard protocols.

F2103

CHARACTERIZATION OF MITOCHONDRIAL DYNAMICS IN IPSC DERIVED NERUONS FROM ALZHEIMER'S PATIENTS

Navara, Christopher[1,2], Chaudhari, Shital[1,2], Lee, Hyoung-Gon[3], Perry, George[1,3] and Grow, Douglas Alvin[1,2], [1]University of Texas at San Antonio, San Antonio, TX, U.S., [2]San Antonio Cellular Therapeutics Institute, San Antonio, TX, U.S., [3]Case Western Reserve University, Cleveland, OH, U.S.

Accumulating evidence implicates mitochondrial dysfunction in neurological degenerative disease etiology, including Alzheimer's disease (AD). These defects include imbalanced mitochondrial fusion and fission, aberrant mitochondrial trafficking, and defects in oxidative phosphorylation, resulting in increased production of reactive oxygen species (ROS), ultimately impairing neuronal functions. Studies of the causes of neuronal degeneration at the cellular level are hindered by the difficulty of obtaining human samples. Induced pluripotent stem cells (iPSCs) can be derived from many easily accessible cell types including fibroblasts and peripheral blood. These cells have the potential to become every cell type in the body including neurons, enabling researchers to perform in vitro investigations of human neurons in the context of a genetic background associated with neurodegenerative disease. Here, we describe the production of iPSCs from normal healthy humans and patients with familial or sporadic AD, and their subsequent differentiation into neurons. We characterized the neuronal phenotype of these cells using immunocytochemistry and RT-qPCR. Further, we imaged mitochondria in the neuronal cultures and compared the mitochondrial volume in AD neurons to that of control neurons. Results from this work demonstrate the potential to use human iPSC·derived neurons as a dlsease·in·a-dish approach to studying the mechanisms of mitochondrial dysfunction during neuronal degeneration.

F2105

PRETREATMENT WITH GSI PREVENTS TUMOR-LIKE OVERGROWTH IN HUMAN IPSC-DERIVED TRANSPLANT FOR SPINAL CORD INJURY

Okubo, Toshiki[1,2], Iwanami, Akio[1], Kohyama, Jun[2], Itakura, Go[2], Matsumoto, Morio[1], Nakamura, Masaya[1] and Okano, Hideyuki[2], [1]Department of Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Japan, [2]Department of Physiology, Keio University, School of Medicine, Tokyo, Japan

Neural stem/progenitor cells derived from human induced pluripotent stem cells (hiPSC-NS/PCs) are considered to be a promising source for cell-based interventions targeting central nervous system (CNS) disorders. Previously we reported that transplantation of hiPSC-NS/PCs promoted functional recovery in animal models of spinal cord injury (SCI). However, transplantation of certain hiPSC-NS/PCs into the injured spinal cord results in tumor-like overgrowth of hiPSC-NS/PCs and subsequent deterioration of motor function. To avoid adverse effects of hiPSC-NS/PC transplantation, remnant immature cells should be removed or induced into more matured cell-types. Since Notch signaling plays a role in maintaining NS/PCs, we evaluated the effect of a gamma-secretase inhibitor (GSI). The purpose of the present study was to elucidate the effects of GSI on tumorigenic hiPSC-NS/PCs in vitro and in vivo. hiPSC-NS/PCs, a potentially tumor-like overgrowth cell line (253G1), were cultured with GSI. Immunocytochemistry and microelectrode array (MEA) were performed to compare their characterizations. Cell cycle/apoptosis analyses were also performed using flow cytometry, and global analyses of the gene expression profiles were performed by DNA microarray and RT-PCRs. Next, we induced contusive SCI in mice and performed hiPSC-NS/PCs transplantation pretreating with or without GSI 9 days after SCI. The survival and growth of transplanted cells was measured by bioluminescence imaging and immunohistochemistry. Behavioral analyses of motor function were performed by BMS score, rota-rod test and DigiGait system. Pretreating hiPSC-NS/PCs with GSI promoted neuronal differentiation and maturation in vitro, and in vivo GSI pretreatment reduced the tumor-like overgrowth of transplanted hiPSC-NS/PCs, inhibited deterioration of motor function and long-lasting functional recovery. These results indicate that pretreatment of hiPSC-NS/PCs results in decreased proliferative capacity of transplanted hiPSC-NS/PCs, triggers neuronal commitment, and improves the safety of hiPSC-based approaches to regenerative medicine.

F2107

TRANSMISSION OF MITOCHONDRIAL DYSFUNCTION FROM DISEASED CELLS TO HEALTHY CELLS BY DIRECT SEEDING OF ALPHA-SYNUCLEIN

Ryan, Tammy, Bamm, Vladimir, Coackley, Carla, Humphries, Kayla, Harauz, George and Ryan, Scott, University of Guelph, Guelph, ON, Canada

Neuronal loss in Parkinson's Disease (PD) is associated with aberrant mitochondrial function and impaired proteostasis in dopaminergic (DA) neurons. Linking these two pathologies is a major hurdle in developing new therapies for PD. It has been proposed that the interaction of α-syn with anionic membranes is critical for its folding to an α-helical structure. We thus asked whether a pathophysiological connection between mitochondrial dysfunction and proteostasis exists in PD that centers on the ability of α-syn to be folded at the outer mitochondrial membrane (OMM). Using a patient-derived hiPSC model of PD that

allows for comparison of A53T-*SNCA* mutant cells against isogenic mutation-corrected controls, we generated DA neurons (hNs) by following a floor plate-derived differentiation paradigm. We determined that A53T hNs display fragmented mitochondria and accumulate insoluble α-syn deposits that cluster to mitochondrial membranes. This results in recruitment of LC3 to the mitochondria and increased mitochondrial clearance. Indeed, we determined that this was initiated by externalization of the anionic phospholipid cardiolipin, to the OMM in A53T hNs, which specifically bound to and facilitated re-folding of a-syn oligomers. To test whether mitochondria in healthy neurons respond to seeding of a-syn oligomers by attempting to buffer a-syn folding, we combined and co-cultured A53T-hNs with their genetically corrected controls. We found that a-syn was not only transmitted from A53T hNs to corrected hNs, but that a-syn rapidly clustered to the OMM of corrected hNs in co-culture, resulting in LC3-mediated mitophagy. Transmission of mitochondrial pathology was effectively blocked using an anti-a-syn mAb. These findings suggest that endogenous mutant a-syn can be propagated from cell to cell, inducing pathology in previously healthy hNs. In addition to informing on the mechanism of α-syn mediated mitochondrial damage, our data highlight the mitochondria as a therapeutic target that can help buffer synucleinopathy in PD and support a role for mAb therapy in this disease.

Funding Source: Parkinson's Society of Canada, Natural Science and Engineering Reseach Council of Canada, Ontario Institute of Regenerative Medicine

F2109

AN INDUCED PLURIPOTENT STEM CELL MODEL OF X-LINKED DYSTONIA-PARKINSONISM

Shin, David[1], Hendriks, William[1], Vaine, Christine[1], Ito, Naoto[1], Dhakal, Jyotsna[1], Breakefield, Xandra[1], Sharma, Nutan[1] and Bragg, Cristopher[2], [1]Massachusetts General Hospital, Charlestown, MA, U.S., [2]Harvard Medical School, Charlestown, MA, U.S.

X-linked Dystonia-Parkinsonism (XDP) is a hereditary neurodegenerative disorder involving a progressive loss of striatal medium spiny neurons. The mechanisms underlying neurodegeneration are not known, in part because there have been few cellular models available for studying the disease. The XDP haplotype consists of multiple sequence variations in a region of the X chromosome containing TAF1, a large gene with at least 38 exons, and a Multiple Transcript System, MTS, comprised of five unconventional exons. A previous study identified a neural-specific TAF1 isoform, N-TAF1, which showed decreased expression in post-mortem XDP brain, compared to control tissue. We generated XDP patient and control fibroblasts and induced pluripotent stem cells (iPSCs) in order to further probe cellular defects associated with this disease. N-TAF1, which incorporates an alternative exon 34', was not expressed in fibroblasts, but was detectable in iPSC-differentiated neural stem cells (NSCs) at levels that were approximately 3-fold lower in patient cells than controls. Transcriptional profiling of patient fibroblasts compared to controlsderived a 53-gene signature distinguishing XDP from control cells ($p < 0.05$), which mapped strongly to gene sets linked to tumor necrosis factor-α and NFκB signaling. Luciferase reporter assays and expression of endogenous NFκB target genes confirmed genotypic differences in TNFα/NFκB responses in XDP vs. control cells. Collectively these data confirm previous reports of aberrant TAF1 expression in XDP cells while suggesting the potential involvement of NFκB in the biology of XDP.

F2111

THE EBISC INFORMATION MANAGEMENT SYSTEM: A PLATFORM TO SUPPORT AN INTERNATIONAL IPS CELL BANK

Streeter, Ian, European Molecular Biology Laboratory - European Bioinformatics Institute, Hinxton, U.K.

In February 2016 the European Bank for Induced Pluripotent Stem Cells (EBiSC) launched its public catalogue of iPS cell lines (https://cells.ebisc.org). EBiSC aims to be the largest European catalogue of human iPS cell lines, and its first public release made available cell lines generated by multiple institutes from across the continent. We describe here the Information Management System (IMS) underlying the operations of the EBiSC bank. The IMS comprises several interacting services, which in combination facilitate the complete journey of a cell line, from depositor to purchaser via banking at EBiSC's core facility. Cell line information enters the EBiSC system via hPSCreg (http://hpscreg.eu), the registration system in which European generators of iPS cells are required to describe and annotate their cell lines. Sales and distribution of lines are managed by the European Collection of Authenticated Cell Cultures (ECACC) (http://www.phe-culturecollections.org.uk/products/celllines/ebisc/). The IMS also comprises a central database to store the bank's data, to coordinate interaction between the federated services, and to provide a user portal from where customers can browse the EBiSC catalogue. The IMS adheres to community standards in describing iPSC meta data using controlled vocabularies and ontologies. It makes available all iPSC-associated protocols (generation, characterization, expansion, differentiation) using ontology terms to standardize annotations and to enable structured-searches of cell lines. Genetic data associated with EBiSC cell lines are distributed via the European Genome-phenome Archive (EGA, https://www.ebi.ac.uk/ega/), a secure managed-access archive that specializes in sharing patient-linked data with bona fide researchers. The implementation of community standards in the IMS

complements EBiSC's wider commitment to driving forward standards in the quality control, characterization, and distribution of European cell lines.

F2113

NEOFUNCTION OF ACVR1 IN FIBRODYSPLASIA OSSIFICANS PROGRESSIVA

Toguchida, Junya[1,2], Hino, Kyosuke[2,3], Ikeya, Makoto[2], Horigome, Kazuhiko[2,3], Matsumoto, Yoshihisa[2,4], Ebise, Hayao[5], Nishio, Megumi[2], Sekiguchi, Kazuya[2] and Matsuda, Shuichi[6], [1]DeInstitute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan, [2]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [3]Innovative Drug Discovery Laboratories, Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan, [4]Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan, [5]Genomic Science Laboratories, Sumitomo Dainippon Pharma Co., Ltd., Osaka, Japan, [6]Graduate School of Medicine, Kyoto University, Kyoto, Japan

Fibrodysplasia ossificans progressiva (FOP) is a rare genetic disease characterized by heterotopic ossification (HO) through the endochondral process. FOP patients harbor point mutations in the *ACVR1* (also known as *ALK2*) gene encoding one of type I receptors for bone morphogenetic protein (BMP). Two mechanisms of mutated ACVR1 (FOP-ACVR1) have been proposed: ligand-independent constitutive activity and ligand-dependent hyper activity in BMP signaling. But these hypotheses failed to explain the unique feature of FOP: HO is exaggerated periodically after physical trauma and inflammatory responses. Therefore, we hypothesized that FOP-ACVR1 abnormally responds to undetermined ligands induced by trauma and/or inflammation, and such FOP-ACVR1 specific ligands were screened by utilizing FOP patient-derived iPS cells (FOP-iPSCs) and gene-corrected (rescued) FOP-iPSCs (resFOP-iPSCs).FOP-iPSCs and resFOP-iPSCs are differentiated into induced mesenchymal stromal cells (iMSCs), and used for FOP-ACVR1 specific ligand screening and chondrogenic differentiation assay. FOP-ACVR1 specific ligand screening was performed by a BMP-specific luciferase reporter construct (BRE-Luc). Chondrogenic differentiation assay was performed in vitro and in vivo. As a result, we identified Activin-A as an FOP-ACVR1 specific ligand, which transduced abnormal BMP signaling via FOP-ACVR1 but not wild type ACVR1. Although Activin-A is a molecule that normally transduces only TGF-beta signaling and contributes to inflammatory responses, it enhanced the chondrogenesis of FOP-iMSCs via aberrant activation of BMP signaling in vitro and induced endochondral ossification of FOP-iMSCs in vivo. These results uncover a novel mechanism of HO in FOP and suggest a possible application of anti-Activin-A reagents as a new therapeutic tool for FOP.

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F2115

THE SMALL SCALE AND MULCH CHANNEL BIOREACTOR FOR PLURIPOTENT STEM CELL STIRRED SUSPENSION CULTURE

Wada, Masanori[1], Matsuura, Katsuhisa[2], Ishikawa, Yoichi[1] and Shimizu, Tatsuya[2], [1]ABLE Corporation, Tokyo, Japan, [2]Tokyo Women's Medical University, Tokyo, Japan

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 designed the bioreactor which is capable of the stirred suspension culture of the inoculated single iPS cells in the aggregates condition. We showed expansion and scale up culture by using three types of single-use bioreactor in the last conference. We show the small scale and mulch channel bioreactor suitable for evaluation of cell line or cell expansion and differentiation process in this conference.

The ABLE Corporation published three types of bioreactor (working volume 30mL, 100mL, 500mL) last year. These bioreactor series including delta shape paddle impeller prevents a turbulent flow and agitates medium by laminar flow. We now developed the small scale (working volume 5mL) and mulch channel bioreactor for iPS cell suspension culture. This bioreactor has 6 independent small vessels which are equipped with delta paddle impeller individually in the SBS format (same as the dimension of 6 well plate). The single cell suspension were prepared from 2D feeder free culture and inoculated into as 0.5 to 2x10^5cells/mL. The cell aggregates were collected after the stirred suspension culture for 4 to 5 days, the size of aggregates measured. The collected aggregates were dissociated into the single cells by using enzymes and the number of viable cells was measured.The 5mL-6ch. bioreactor using the delta shape paddle impeller enabled to create a lot of aggregates for 4 to 5 days. Furthermore the number of cells increased 4 to 5 times to the number of inoculated cells. The similar cell proliferation was observed like as our larger scale bioreactor series. Collec-

tively, we established the scalable expansion system from small to large scale for pluripotent stem cell.

Funding Source: This research is supported by Projects for Technological Development in Research Center Network for Realization of Regenerative Medicine from Japan Agency for Medical Research and Development, AMED.

F2117

SKELETAL MUSCLE DIFFERENTIATION OF HUMAN IPS CELLS USING CRISPR/CAS9 MEDIATED KNOCK-IN REPORTERS

Wu, Jianbo, Hunt, Samuel D and Darabi, Radbod, University of Texas Health Science Center at Houston, Houston, TX, U.S.

Directed differentiation of ES/iPS cells toward various tissue progenitors for disease modeling or regenerative medicine has been the focus of recent research. Therefore, generation of tissue-specific reporter human iPS lines provide better understanding of developmental stages in iPS cells. Although a few approaches have been developed for derivation of skeletal myogenic precursors from human ES/iPS cells, most of them are based on gene over-expression or long-term mesenchymal differentiation and retrospective identification of myogenic cells. Here, we have generated knock-in human iPS reporter cell lines for the early myogenic lineage specification genes of PAX7 and MYF5. By introduction of site-specific double-stranded breaks (DSB) in the genomic locus of PAX7 and MYF5 using CRISPR/Cas9 nickase pairs, 2A-GFP/ tdTomato reporters have been incorporated before the stop codon of each gene.Single and double reporter iPS cells have been evaluated for proper in-frame inclusion of the reporter cassettes using southern blot and sequencing. Furthermore, the activities of the reporters have been validated using a nuclease-dead Cas9 activator (dCas9-VP160) system. The promoter region of each gene has been targeted by gRNA activators for transient gene induction and reporter activity validation. This was further confirmed by flow cytometry, WB and immunostaining for PAX7 and GFP. Finally using EB differentiation, the iPS cells have been differentiated into myogenic progenitors and the reporter activity has been evaluated by FACS and immunostaining.This report describes first successful generation of human iPSC reporter lines for skeletal muscle lineage-specific genes using CRISPR/Cas9 technology and provides further insights for application of human iPS cells for skeletal muscle differentiation.

F2119

PREMATURE NEURONAL DIFFERENTIATION OCCURS IN NEURAL PROGENITOR CELLS FROM ALZHEIMER'S DISEASE PATIENT- IPSCS

Zhao, Hanzhi[1], Yang, Juan[1] and Jin, Ying[1,2], [1]Institute of Health Sciences, Shanghai Institute of Biological Sciences, Chinese Academy of Sciences, Shanghai, China, [2]Shanghai Stem Cell Institute, Shanghai JiaoTong University School of Medicine, Shanghai, China

Alzheimer's disease (AD) is the most common age-related dementia associated with progressive neuronal loss. However, the molecular mechanism for the neuronal loss is still debated. We take advantage of induced pluripotent stem cells (iPSCs) derived from somatic cells of familial AD patients to study the early development of AD. We find that premature neuronal differentiation with decreased proliferation and increased apoptosis occurs in AD-iPSC-derived neural progenitor cells (AD-NPCs) once neuronal differentiation is initiated, accompanied by higher levels of Ab42 and phosphorylated tau. Premature neuronal differentiation in AD-NPCs is caused by PSEN1 mutations and might be correlated to multiple dysregulated processes including but not limited to NOTCH and WNT pathways. Moreover, NPCs isolated from AD transgenic mouse embryos display gene expression patterns of premature neuronal differentiation. Our study uncovers previously unappreciated early NPC dysfunction in FAD-NPCs and AD transgenic mice, providing new insights into the mechanisms underlying AD development.

F2121

DELETION OF SIRT2 HAMPERS REPROGRAMMING TO PLURIPOTENCY THROUGH P16INK4A ACTIVATION

Kim, Ah-Young[1], Lee, Eun-Mi[1], Lee, Eun-Joo[2], Elfadl, Ahmed[1], Kim, Hyeong-Mi[1] and **Jeong, Kyu-Shik**[1], [1]Kyungpook National University, Daegu, Korea, South, [2]Kyungpook National University, Daegu City, Korea, South

Primary mouse embryonic fibroblasts (MEFs) were isolated from SIRT2(+/+), SIRT2(+/-) and SIRT2(-/-) mice embryo at E13.5 day, respectively. When reprogramming was induced by doxicycline-inducible lentiviral delivery of Oct4, Sox2, Klf4 and c-Myc, alkaline phosphatase-positive colonies were significantly less formed from SIRT2 knockout (KO) MEFs. Similarly, SIRT2 knockout MEFs had quite less numbers of reprogrammed cells than wild type (WT) cells in FACS analyses for identifying SSSEA-1 or Oct4-positive/CD44-negative fraction. Because SIRT2 hetero (HT) group did not show significant differences to wild type, it was focused that what the most distinctive difference between WT and KO is. KO cells displayed

prominently slow proliferation than WT cells. Therefore several genes involved in Ink4/Arf pathway were selected as candidates for those differences. Indeed, SIRT2-KO-MEFs had significantly increased expression of p16^{Ink4a}. p15 ^{Ink4b}, RB and p21 were also increased in KO cells. Because Ink4/Arf pathway inhibits aberrant cell proliferation for tumor suppression, it also works as a barrier for reprogramming to pluripotency, which requires robust proliferation as a prerequisite. Collectively, SIRT2-KO-MEFs seemed to have elevated levels of Ink4/Arf gene expression as a basal status. Since when the reprogramming factors are introduced, Ink4/Arf pathways are more activated, knockout cells may not overcome the increased stress and go to the way of cell cycle inhibition. In conclusion, SIRT2 is absolutely essential factor for efficient reprogramming to pluripotency even though both iPS cells can be generated without it.

Funding Source: This work was supported by the Bio-industry Technology Development Program (312062-5) of iPET (Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries), Ministry for Food, Agriculture, Forestry and Fishe

F2123

Pp18 INHIBITS REPROGRAMMING THROUGH INACTIVATION OF CDK4/6

Zhu, Shaohua, Cao, Jiani, Sun, Hongyan and **Zhao, Tongbiao**, Institute of Zoology Chinese Academy of Sciences, Beijing, China

Pluripotent stem cells (PSCs) show atypical cell cycle regulation characterized by a high proliferation rate and a shorter G1 phase compared with somatic cells. The mechanisms by which somatic cells remodel their cell cycle to achieve the high proliferation rate of PSCs during reprogramming are unclear. Here we identify that the Ink4 protein p18, which is expressed at high levels in somatic cells but at low levels in PSCs, is a roadblock to successful reprogramming. Mild inhibition of p18 expression enhances reprogramming efficiency, while ectopic expression of p18 completely blocks reprogramming. Mechanistic studies show that expression of wild-type p18, but not a p18^{D68N} mutant which cannot inhibit Cdk4/6, down-regulates expression of Cdk4/6 target genes involved in DNA synthesis (TK, TS, DHFR, PCNA) and cell cycle regulation (CDK1 and CCNA2) and thus inhibits reprogramming. These results indicate that p18 blocks reprogramming by targeting Cdk4/6-mediated cell cycle regulation. Taken together, our results define a novel pathway that inhibits somatic cell reprogramming, and provide a new target to enhance reprogramming efficiency.

Funding Source: China National Basic Research Program-Strategic Priority Research Program of the Chinese Academy of SciencesNational Natural Science Foundation of China

F2125

NOVEL iPSC-DERIVED CELLULAR SYSTEMS FOR IN VITRO DISEASE MODELING

Carlson, Coby B, Anson, Blake, DeLaura, Susan, Mann, David and Jones, Eugenia, Cellular Dynamics, Madison, WI, USA

A major challenge in drug discovery research is modeling human biology in an in vitro system that is both physiologically relevant and predictive of the disease state. Human induced pluripotent stem cell (iPSC) technology allows for the generation of unlimited quantities of virtually any cell type in the human body from numerous donors. This technology also enables access to human disease models which have been shown to recapitulate the native phenotype in vitro. The functional relevance of human iPSC-derived cell types in research and drug discovery programs is being demonstrated by a rapidly growing body of literature. Here, we present case study examples of induced, engineered, and innate disease models generated by the production of iPSC-derived cell types environmentally stimulated to elicit a disease phenotype, genetically modified to introduce a disease mutation, or from patient-derived material, respectively.In particular, we describe the application of iPSC-derived hepatocytes in hepatitis C virus (HCV) infectivity. We present an induced model of Alzheimer's disease (AD) where beta-amyloid-dependent toxicity is induced in iPSC-derived cortical neurons. This assay was developed for a pilot screen to identify compounds protective against AD. We also provide data from iPSC lines genetically modified to carry point mutations in the amyloid precursor protein yielding another neuron-based Alzheimer's model. Finally, we showcase an iPSC-derived diabetic cardiomyopathy model, in which culture conditions were optimized to induce the disease state in apparently normal iPSC-derived cardiomyocytes. This model was used in a phenotypic screen for rescue from the pathological phenotype during diabetic stress and identified candidate molecules that were subsequently shown to be protective in cardiomyocytes derived from diabetic patient-specific iPS cell lines. Overall, these illustrate how iPSC technology offers reliable and predictive model systems not otherwise attainable using currently available primary and immortalized cells, thus creating new tools and opportunities in drug discovery.

IPS CELLS: DIRECTED DIFFERENTIATION

F2127

EFFICIENT DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS TO HEMATOPOIETIC PROGENITOR CELLS IN SERUM-FREE CULTURE CONDITIONS

Kardel, Melanie Dawn[1], Noort, Rebecca[1], Chen, Jenny[1], Hadley, Erik[1], Wognum, Bert[1], Thomas, Terry E.[1], Eaves, Allen C.[1,2] and Louis, Sharon A.[1], [1]STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Reproducible protocols for differentiation of human pluripotent stem cells (hPSCs) to hematopoietic progenitor cells have been difficult to develop. Most protocols for hematopoietic differentiation use hPSCs maintained on feeder cells or rely on undefined serum-containing media or co-culture with stromal cells: all contributing factors to inconsistent results obtained across multiple hPSC lines. Here, we demonstrate that hPSCs maintained using TeSR™ media can be efficiently differentiated to hematopoietic progenitor cells in serum- and feeder-free conditions. Differentiation was reproducible on multiple human embryonic stem cell (H1, H9) and induced pluripotent stem cell (WLS-1C, STiPS-F016) lines. Briefly, small aggregates (100 - 200 µm) of hPSCs were plated in TeSR™ medium on Corning® Matrigel® at 15 - 20 aggregates/cm^2 and allowed to attach overnight. The next day differentiation was initiated by changing the medium to STEMdiff™ Hematopoietic Medium A. After 3 days, medium was changed to STEMdiff™ Hematopoietic Medium B; additional half medium changes were performed every 2 - 3 days during the protocol. At day 12, differentiated cells were harvested from the supernatant and adherent layer, and characterized separately for their phenotype and colony forming unit (CFU) frequency. Cells in the supernatant contained a high proportion of CD34$^+$CD45$^+$ hematopoietic progenitor cells: H9, 43 ± 4% (mean ± SEM, n =7); H1, 47 ± 4% (n = 7); WLS-1C, 58 ± 6% (n = 8); STiPS-F016, 59 ± 5% (n = 4). Adherent cells contained both CD31$^+$CD144$^+$ endothelial and CD34$^+$CD45$^+$ hematopoietic cells; however, typically 80 - 95% of the hematopoietic cells were in the supernatant. On average, 150,000 ± 18,000 (mean ± SEM, n = 26) CD34$^+$CD45$^+$ cells were harvested from the supernatant per cm^2 of culture, indicating the generation of about 100 hematopoietic progenitors per hPSC. CFU frequency was assessed using MethoCult™ methylcellulose medium (H4435) and was high in all cell lines (expressed per 10^4 supernatant cells): H9, 70 ± 15 (mean ± SEM, n =7); H1, 96 ± 10 (n = 7); WLS-1C, 200 ± 17 (n = 8); STiPS-F016, 137 ± 29 (n = 4) with both myeloid (range 24 - 238) and erythroid (0 - 77) colony types produced. In summary, the STEMdiff™ Hematopoietic Kit can be used to reproducibly differentiate hPSCs into hematopoietic progenitor cells with high efficiency.

F2129

MODELLING FAMILIAL HYPERCHOLESTEROLEMIA USING HUMAN ISOGENIC INDUCED PLURIPOTENT STEM CELLS

Santos, Rodrigo[1], Soares, Filipa[2], **Armesilla-Diaz, Alejandro**[1], Jhaveri, Kalpesh[3], Schofield, Christine L[1], Lowe, Christopher E[1] and Yeo, Marcus[3], [1]Horizon Discovery Group Plc, Cambridge Research Park, Waterbeach, Cambridge, U.K., [2]DefiniGEN Ltd, Ltd, Babraham Research Campus, Cambridge, U.K., [3]DefiniGEN Ltd, Ltd, Babraham Research Campus, Babraham, Cambridge, U.K.

Familial Hypercholesterolemia (FH) is an autosomal dominant disorder, characterized by plasma accumulation of cholesterol, transported in Low Density Lipoproteins (LDLs). Worldwide, about one in 200 people has FH, and if untreated, around one in two men and one in three women will develop premature coronary heart disease. The majority of FH patients are carriers of mutations in the LDL Receptor (LDLR), although some may also carry mutations in Apolipoprotein B, an LDL constituent. Phenotypes appear to be correlated with the level of expression of LDLR: heterozygotes have twice the normal plasma levels of LDL-cholesterol, and homozygous individuals four times the normal amount. A common LDLR mutation is c.301G>A, which results in substitution of a glutamic acid with a lysine at amino acid 101 (p.E101K). Here we report the generation of isogenic human induced pluripotent stem cells (hiPSCs) carrying the E101K mutation, followed by in vitro differentiation into hepatocyte-like cells to model FH. Using the CRISPR-Cas9 genome–editing technology in healthy donor iPSC cell lines, the following genotypes were achieved: LDLR (E101K/E101K), LDLR (E101K/+), LDLR (E101K/-), LDLR (-/-) and LDLR (+/-). A defined four-stage protocol was used to differentiate these isogenic hiPSCs into liver cells. In the first stage, iPSCs are differentiated into definitive endoderm (DE) cells. The second stage differentiates the DE cells into anterior definitive endoderm (ADE) cells, which represent a common progenitor between the liver, pancreas, lung and thyroid. During the third stage, ADE cells differentiate into hepatic progenitors. In the fourth stage of the protocol, the resulting hepatocyte-like cells undergo functional maturation, fetal characteristics diminish, and functions associated with adulthood increase to reach a maximum level at day 35. Generated isogenic hepatocyte-like cells have been validated at both the genotypic and phenotypic levels using an array of biochemical methodologies. This renewable and scalable source of patient relevant isogenic hepatocytes provides a biologically relevant

platform to expedite in vitro disease modelling and novel drug discovery.

F2131

DEVELOPMENT AND FUNCTIONAL APPLICATIONS OF HUMAN IPSC-DERIVED SPINAL MOTOR NEURONS

Chavez, Carrie[1], Meline, Benjamin[1], Liu, Jing[1], McLachlan, Michael[1], Burke, Thomas J.[1], Jones, Eugenia[2], McMahon, Christopher[1] and Wang, Wen Bo[1], [1]Cellular Dynamics International, Madison, WI, U.S., [2]Cellular Dynamics, Madison, WI, U.S.

The aim of this study was to produce spinal motor neurons from human induced pluripotent stem cells (iPSCs) with sufficient purity for use in a multitude of downstream assays including electrophysiological recordings using a multi-electrode array (MEA) system. In particular we wanted to produce motor neurons that could be cultured in defined conditions over long periods of time, without being hampered by outgrowth from proliferative cell types. Using an optimized 3D differentiation protocol that improves upon published methods, we were able to produce motor neurons from iPSCs at greater than 60% purity as measured by Isl 1/2 and Tuj1 positive staining. These cells can be stored frozen, thawed, and cultured in media without glia for extended periods, simplifying experimental design and data interpretation. We collected ICC, qPCR and MEA data to characterize the motor neuron cells and used iPSC lines from multiple donors to demonstrate a robust protocol that produces motor neurons independent of donor iPSC line. In addition, genetically modified iPSC lines were generated to create an isogenic disease model for Amyotrophic Lateral Sclerosis. These data show the characteristics and utilization of motor neurons produced from iPSC.

F2133

A NOVEL DIFFERENTIATION SYSTEM TO GENERATE HPS CELL-DERIVED HEPATOCYTES IN A ROBUST MANNER WITH POTENTIAL APPLICATION TO DRUG DISCOVERY, DRUG METABOLISM RESEARCH, AND HEPATOTOXICITY STUDIES

Ellerstrom, Catharina, Takara Bio Europe AB, Gothenburg, Sweden
Clontech Laboratories, Inc., a Takara Bio Company, Mountain View, CA, U.S.

Human pluripotent stem (hPS) cell-derived hepatocytes have the potential to serve as predictive human in vitro model systems for drug discovery, drug metabolism research, and hepatotoxicity studies provided that they possess relevant hepatic function. Until recently, however, the functionality of hPS cell-derived hepatocytes has been insufficient for applications that demand high expression of multiple drug metabolizing enzymes. We have recently developed a novel, robust differentiation system to generate hepatocytes from human induced pluripotent stem cells. These resulting hPS cell-derived hepatocytes have substantial CYP1A, 2C9, 2C19, 2D6, and 3A4 enzyme activities and important adult hepatic features, such as low expression of fetal genes (e.g., CYP3A7 and alpha-fetoprotein) and high expression of adult genes (e.g., CYP2C9, 2C19, and 3A4). We here illustrate the system's differentiation protocol that begins with culturing any hPS cell line, continues with directed differentiation into definitive endoderm (DE) cells, and ends with further differentiation into hepatocytes, thereby mimicking normal embryonic development. Morphological images and immunostaining data demonstrate the robustness and reproducibility of the system to generate hepatocytes from a panel of several different cell lines. More importantly, these hepatocytes generated from multiple human embryonic or iPSC lines show diverse CYP activity profiles, indicative of the inter-individual variation present in the human population. The hPSC-derived hepatocytes respond to test compounds in a similar manner as human primary hepatocytes and correctly identify known hepatotoxins; suggesting their potential use in toxicity testing assays. The new hepatocyte differentiation system can reliably generate an almost inexhaustible source of human hepatocytes from different genetic backgrounds for use in *in vitro* drug discovery, drug metabolism research, and toxicology-related studies.

F2135

IN VIVO FORCED EXPRESSION OF SOX2 TRANSCRIPTION FACTOR IN AN ANIMAL MODEL OF DEMYELINATION TRANS-DIFFERENTIATES REACTIVE ASTROCYTES INTO OLIGODENDROCYTES PROGENITOR CELLS

Javan, Mohammad[1,2], Farhangi, Sahar[3], Dehghan, Samaneh[3] and Totonchi, Mehdi[4], [1]Department of Physiology, Faculty of Medical Sciences, Tarbiat Modarres University,Tehran, Iran, Tehran, Iran, [2]Royan Institute, Tehran, Iran, [3]Tarbiat Modares University, Tehran, Iran, [4]Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran, Tehran, Iran

Direct trans-differentiation of brain somatic cells into neuroblasts or neurons as a new avenue in neural tissue repair has been in research focus in recent years. In this study we hypothesized the possibility of direct reprograming of reactive astrocytes to oligodendrocyte progenitor cells (OPCs) using forced expression of Sox2 and providing a demyelinating niche by 12 weeks feeding of mice with

cuprizone including chow. Lentiviral particles expressing SOX2 were injected into striatum of control and cuprizone treated mice just below the corpus callosum. Astrocytes were the main population of transduced cells when a GFP control vector was used. Seven and 21 days post transfection, the main population of cells transfected with Sox2-GFP expressing vector expressed OPC markers. At day 21, the number of transfected cells which expressed oligodendrocyte marker was increased. The conversion of astrocytes into oligodendrocyte lineage was confirmed by in vitro transfection of astrocytes and their consequent transplantation into the corpus callosum of demyelinated mice and additionally by in vivo expression of Sox2 under the control of GFAP promoter. Our data suggest that application of Sox2 transcription factors could convert the transduced astrocytes into OPCs and myelinating oligodendrocytes. Inducing a demyelinated niche significantly enhanced the conversion of astrocytes into oligodendrocyte lineage cells.

Funding Source: This study was supported by Tarbiat Modares University and Royan Institute, Tehran, Iran.

F2137

IDENTIFICATION OF HUMAN ASTROCYTE SUBTYPE-DERIVED SIGNALS ESSENTIAL FOR NEURONAL MATURATION

Krencik, Robert[1], Seo, Kyounghee[1], Rowitch, David[2] and Ullian, Erik[1], [1]University of California, San Francisco, CA, U.S., [2]Department of Pediatrics, University of California, San Francisco, CA, U.S.

One major roadblock in utilizing neuronal subtypes from human pluripotent stem cells to study development, disease, regeneration and response to drug screening is their inability to mature without the presence of astrocyte support. This phenomenon recapitulates normal brain development when considering synaptogenesis and synaptic refinement do not occur until astrocyte-derived signals are present post neurogenesis. To overcome this barrier in stem cell research, neurons are typically co-cultured in the presence of rodent glia even though the contributing factors involved are undefined. What are these factors and are human astrocyte subtypes intrinsically attuned to specific neuronal subtypes? Answering this question is crucial for proper maturation of stem cell derived neurons and for understanding mechanisms of human brain development. In order to investigate this relationship, we have examined the maturation of neuronal subtypes, including ventral midbrain dopaminergic neurons and spinal motor neurons, in a three dimensional organoid system containing pre-matured human pluripotent stem cell-derived astrocytes with specific regional identities. Furthermore, we have profiled the astrocyte subtypes with RNA-seq and identified unique signatures of astrocyte subtypes that are being functionally examined in the coculture system. Together, our studies will identify the critical signals that human astrocytes contribute in order for proper neuron circuit maturation.

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F2139

AN IN VITRO MODEL OF PRADER-WILLI SYNDROME BY GENERATION OF HYPOTHALAMIC NEURONS AND GENOME EDITING OF HUMAN INDUCED PLURIPOTENT STEM CELLS

Mathieux, Elodie[1], Banda, Erin[2], Stoddard, Christopher[2], Glatt-Deeley, Heather[2], Langouet, Maeva[2], Crandall, Leann[2] and Lalande, Marc[2], [1]UCHC, Farmington, CT, U.S., [2]UConn Health, Farmington, CT, U.S.

Prader-Willi syndrome (PWS) is characterized by neonatal hypotonia and failure to thrive followed by hyperphagia, obesity, short stature, dysregulated sleep, infertility and distinctive behavioral problems. These clinical manifestations are consistent with dysfunction of the hypothalamus, a ventral diencephalon structure implicated in the control of the endocrine system. PWS is a disorder of genomic imprinting that is caused by the absence of a normal paternal contribution to chromosome 15q11-q13. Most other cases of PWS result from uniparental disomy of the maternal chromosome 15. Recently the PWS critical region (PWSCR) has been narrowed to an ~91kb region encompassing several non-coding RNAs including a cluster of box C/D snoRNAs (SNORD116). In order to gain an understanding of how the loss of the SNORD116 cluster contributes to PWS phenotypic abnormalities, we used CRISPR/Cas9 to engineer isogenic pairs of human hESCs that differ exclusively at the SNORD116 cluster. We confirmed the loss of expression of the SNORD116 cluster in the isogenic deletion line by qRT-PCR and RNA fluorescence hybridization. In order to identify novel cellular and molecular targets of the SNORD116 regulation, we are differentiating normal and SNORD116 deletion lines into hypothalamic neurons. For this, we modified existing protocols involving activation of the Sonic Hedgehog pathway followed by inhibition of the Notch signaling pathway by the addition of DAPT, for both monolayer and 3-dimensional spheroid neuronal differentiation. We compared the ability of normal control and several PWS-specific induced pluripotent stem cells (iPSC) derived neurons to adopt hypothalamic cell fates by assaying for gene and neuropeptide expression. We observed a marked increase in the gene expression of NKX2.1, OTP, RAX and POMC for the hypothalamic enrichment relative to our standard protocol. These findings suggest that our

normal and PWS-specific iPSC lines can be differentiated into hypothalamic neurons, particularly, those of the arcuate nucleus (ARC) region. In conclusion we have generated new stem cell models of PWS to elucidate a cellular phenotype of PWS and to define the critical role of the SNORD116 cluster.

Funding Source: This project is supported by the Connecticut Stem Cell Research Fund (12SCB-UCHC-09).

F2141

HUMAN INDUCED PLURIPOTENT STEM CELL MODEL TO STUDY CHEMOTHERAPY INDUCED PERIPHERAL NEUROPATHY

Ramesh, Soneela, Schmidt, Kiley and Staff, Nathan P, Mayo Clinic, Rochester, MN, U.S.

About 30% of patients undergoing chemotherapy are susceptible to chemotherapy induced peripheral neuropathy (CIPN) but the mechanism or reasons why certain patients are more susceptible is not clearly understood. Peripheral neuropathy develops due to axonal/cellular damage in the peripheral nerves. Symptoms typically include tingling sensation, burning or pain that progresses in an ascending manner. Chemotherapy induced peripheral neuropathy (CIPN) is a dose-limiting condition and affects the quality of life. Rodent and fly models of CIPN are available, but with the advent of induced pluripotent stem cell (iPSC) technology, we have the potential to explore individualized therapeutic solutions using human patient samples. We have been able to differentiate patient derived iPSCs to sensory neurons (iSN) by modifying a previously established small molecule protocol. Traditionally, drug dosing in *i*n vitro disease models are done on a dish limiting us from understanding the mechanism of drug action specifically on axons. Hence, our goal was to develop a system that would enable us to conduct drug testing specifically in the axons of sensory neurons. Using a microfluidic platform, we were able to guide the iSN through channels using topographical cues and gradients of extracellular matrix, laminin. This device has two chambers - axonal and somatic, allowing us to treat either the somatic side or axonal side alone with chemotherapy drugs. We have demonstrated the integrity of iSN within the chambers by immunostaining for βtubulin III and peripherin. Morphometric analysis software, Axon Quant was used to evaluate the extent of axonal damage quantitatively in response to varying doses of Bortezomib, a chemotherapeutic drug. In conclusion, we have devised an elegant human patient specific peripheral neuropathy model that could potentially be used for drug testing; possibly predicting an individual patient's susceptibility to CIPN.

Funding Source: Funding provided by Mayo Clinic Center for Regenerative Medicine.

F2143

MODELLING NEUROLOGICAL DISEASE: IN-VITRO GENE EDITING AND IPSC DIFFERENTIATION COMBINE TO CREATE POWERFUL NEW TOOLS

Santos, Rodrigo[1], Sharma, Ruchi[2], Armesilla-Diaz, Rafael Alejandro[3], Newman, Benjamin[2], Schofield, Christine L[1], Lowe, Christopher E[1] and Shi, Yichen[2], [1]Horizon Discovery Group Plc, Cambridge Research Park, Waterbeach, Cambridge, U.K., [2]Axol Bioscience ltd. Chesterford Research Park, Little Chesterford, Cambridge, U.K., [3]U.S.

Neurodegenerative diseases, such as Parkinson's disease, Huntington's disease, Alzheimer's disease and other age-related dementias are incurable and debilitating conditions, with Alzheimer's disease alone accounting for ~60-70% of cases. With an increasingly ageing global population, the economic as well as human impact of these conditions is expected to increase unless novel therapeutics and care strategies can be developed. Induced pluripotent stem cells (iPSCs) and gene-editing technology, offers unprecedented biomedical potential for disease modelling, high-throughput drug screening and development of therapeutic strategies for such diseases. We have generated stable human iPSC lines from normal human dermal fibroblasts and patient derived fibroblasts (e.g. Huntingdon's and Alzheimer's diseases). The fibroblasts were reprogrammed using a non-integrating episomal method coding for Yamanaka factors (license agreement with iPS Academia Japan) and then differentiated into neuronal stem cells (NSCs) and cortical neurons to provide a complete modelling solution in a dish. The iPSC lines derived from normal human dermal fibroblasts were stable with all the hallmarks of pluripotency and a normal karyotype for over 13 passages. These could be cultured as single cells, an essential prerequisite for efficient genome editing. Using the CRISPR-Cas9 genome–editing technology, we generated patient relevant disease models carrying microtubule-associated protein Tau (MAPT) mutations. Tau protein is normally associated with microtubules and is involved in their assembly and stabilization. In turn, microtubules are critical for cellular function, especially for neurons to facilitate the growth and integrity of axons and dendrites and transport between the cell body and distant dendrites. Clinically identified missense mutations reduce the ability of Tau to promote microtubule assembly, resulting in neuronal cell death and subsequent disease phenotype. These renewable and biologically relevant resources will further enable investigation of the mechanisms of disease progression, with additional models relevant to Alzheimer's disease, Parkinson's disease, Huntingdon's disease and epilepsy being generated to aid in the identification of novel drug discovery targets.

F2145

MEGAKARYOPOIESIS FROM INDUCED PLURIPOTENT STEM CELLS: GRAY PLATELET SYNDROME IPS CELL DIFFERENTIATION UNCOVERS A ROLE FOR GFI1B IN MEGAKARYOPOIESIS AND ENDOTHELIAL SPECIFICATION.

Hansen, Marten[1], Marneth, Anne[2], van der Zwaan, Carmen[3], van Oorschot, Rinske[2], Laros-van Gorkom, Britta[2], Wust, Tatjana[1], von Lindern, Marieke[1], Meijer, Sander[3], van der Reijden, Bert[2] and **van den Akker, Emile**[4], [1]Sanquin Research, Department of Hematopoiesis, Amsterdam, Netherlands, and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands., Amsterdam, Netherlands, [2]Department of Laboratory Medicine, Laboratory of Hematology, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen,Netherlands., Nijmegen, Netherlands, [3]Sanquin Research, Department of Plasma Proteins, Amsterdam, Netherlands, and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, Amsterdam, Netherlands, Amsterdam, Netherlands, [4]Sanquin, Asmterdam, Netherlands

Limited platelet shelf-life, risk of infections and allo-immunization are problems in platelet transfusions. These problems could potentially be solved by using Induced pluripotent stem (IPS) cells, a promising (autologous) renewable source to initiate ex-vivo production. However, megakaryoid differentiation and yield from IPS is ill-defined and in need of optimization. We and others have shown that megakaryopoiesis in gray platelet syndrome (GPS) caused by GFI1B mutations is characterized by increased megakaryoblast frequency in bone marrow, inhibition of differentiation, reduced alpha granule content and maintained CD34 expression on platelets. Unravelling the molecular mechanism fundament to the increased megakaryopoiesis, may potentially be exploited to increase IPS-derived megakaryoid yield. Megakaryoblasts from healthy donors and GPS patients were reprogrammed using a polycistronic OCT4, SOX2, c-MYC and KLF4 lentiviral vector. IPS differentiation to megakaryoid cells was induced through colony differentiation. Megakaryocyte commitment and differentiation was assessed by morphology, flow cytometry, confocal microscopy, CFU-MK potential and Mass spectrometry. Differentiation resulted in a wave of megakaryopoiesis between days 14-17. Multi-nucleated CD41, CD42a/b and CD61 megakaryocytes, pro-platelet formation and CFU-MK were observed. Yields were 3 logs increased compared to CD34+ cell megakaryopoiesis. Interestingly, GPS-IPS differentiation showed maintained megakaryoblast specification leading to 20 times increased megakaryoid yield compared to control IPS. Mass spectrometry confirmed specific molecular hallmarks of GPS. In addition, terminal megakaryoblast differentiation was inhibited. Surprisingly, endothelial precursor yield was elevated compared to controls, suggesting a role for GFI1B in endothelial specification. In conclusion, IPS lines were generated from megakaryoblasts and re-differentiated to megakaryocytes. GFI1B mutations result in a selective growth advantage of megakaryoblasts and an endothelial population. These data confirm the usefulness of patient specific IPS-lines to study pathologies. The pathways resulting in this growth advantage may be exploited to increase hematopoietic output and specifically platelet yield.

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F2147

FUNCTIONAL CARDIAC FIBROBLASTS DIFFERENTIATED FROM HUMAN PLURIPOTENT STEM CELLS

Zhang, Jianhua[1], Carvalho, Juliana L.[1,2], Tao, Ran[1], Ruiz, Edward[1], Schmuck, Eric[1], Squirrell, Jayne[1], Eliceiri, Kevin[1], Swanson, Scott[3], Thomson, James A.[3] and Kamp, Timothy J.[1], [1]University of Wisconsin - Madison, Madison, WI, U.S., [2]Federal University of Minas Gerais, Belvedere, Brazil, [3]Morgridge Institute for Research, Madison, WI, U.S.

Cardiac fibroblasts (CFs) comprise two-thirds of the total cells in the heart and play critical roles in cardiac development, homeostasis, and response to injury. Access to primary human CFs is limited by the scarcity of viable cardiac tissue. The GiWi (GSK3β inhibition/Wnt inhibition) protocol was optimized for differentiation of human pluripotent stem cells (hPSCs) to cardiomyocytes (CMs), and we hypothesized that the fate of progenitor cells emerging during the GiWi protocol can be directed to the CF lineage by optimizing FGF signaling. We initiated the GiWi protocol and changed to a defined fibroblast medium supplemented with bFGF (0-125 ng/ml) at day 2-3 or 4, and assessed the cells present at day 20 by flow cytometry for CMs (MF20) and fibroblasts (TE-7). Adding bFGF (75-125 ng/ml) at day 2-3 gave rise to ~80% fibroblasts but <1% CMs. However, adding the same concentrations of bFGF at day 3-4 resulted in significant cell death before day 20. The hPSC differentiated CFs can undergo 10-13 passages before senescence. Gene expression analysis by RNA-seq demonstrated high similarity in the abundance of overall transcripts between hPSC-CFs and primary CFs calculated by Euclidean distance. Some highly expressed genes in hPSC-CFs include COL (type 1, 3, 5), TMSB4X, ACTG1, FN1, DPARC, TGFB1, TMSB10, ITGB1, F3, VIM, TIMP2, S100A6, POSTN. Flow cytometry and immunolabeling revealed that both hPSC-CFs and primary CFs expressed the fibroblast marker and GATA4 with a low per-

centage of cells expressing CD90 in contrast to dermal fibroblasts (DFs) which expressed fibroblast marker and CD90 ubiquitously but not GATA4. There was no expression of CD31 (endothelial), MF20 (CM), SM-MHC (smooth muscle) in the hPSC-CFs. Evaluation of extracellular matrix production by hPSC-CFs and primary CFs demonstrated a high content of collagen and fibronectin with distinct 3D organization revealed by second harmonic imaging as compared to DFs. The hPSC-CFs can undergo myofibroblast differentiation induced by TGFβ1 identified by significant increase of SMA expression. In conclusion, CFs can be efficiently differentiated from hPSCs by modulation of Wnt/FGF signaling in defined conditions, are comparable to primary human CFs and provide an unlimited cell source for research and therapeutic applications.

IPS CELLS: EPIGENETICS

F2151

COMPARISON OF ISOGENIC HUMAN ES AND IPS CELL LINES REVEALS NEITHER SPECIFIC TRACES OF THE REPROGRAMMING PROCESS NOR TISSUE-SPECIFIC SOMATIC MEMORY.

Lagarkova, Maria[1,2], Shutova, Maria[1], Surdina, Anastasia[1], Bogomazova, Alexandra[1], Naumov, Vladimir[2], Ischenko, Dmitry[2], Alexeev, Dmitry[2], Vassina, Ekaterina[1] and Kiselev, Sergey[1,3], [1]Vavilov Institute of General Genetics, Moscow, Russia, [2]Research Center for Physical-Chemical Medicine, Moscow, Russian Federation, [3]Vavilov Institute of General Genetics, Moscow, Russia

The pluripotency of newly developed human induced pluripotent stem cells (iPSCs) is usually characterized by physiological parameters; i.e., by their ability to maintain the undifferentiated state and to differentiate into derivatives of the three germ layers. Nevertheless, a molecular comparison of physiologically normal iPSCs to the “gold standard” of pluripotency, embryonic stem cells (ESCs), often reveals a set of genes with different expression and/or methylation patterns in iPSCs and ESCs. To evaluate the contribution of the reprogramming process, parental cell type, and fortuity in the signature of human iPSCs, we developed a complete isogenic reprogramming system. We performed a genome-wide comparison of the transcriptome and the methylome of human isogenic ESCs, three types of ESC-derived somatic cells (fibroblasts, retinal pigment epithelium and neural cells), and three pairs of iPSC lines derived from these somatic cells. Our analysis revealed a high input of stochasticity in the iPSC signature that does not retain specific traces of the parental cell type and reprogramming process. We showed that five iPSC clones are sufficient to find with 95% confidence at least one iPSC clone indistinguishable from their hypothetical isogenic ESC line. Additionally, on the basis of a small set of genes that are characteristic of all iPSC lines and isogenic ESCs, we formulated an approach of “the best iPSC line” selection and confirmed it on an independent dataset.

F2153

COMPARATIVE EPIGENOMIC PROFILING OF REGULATORY ELEMENTS IN HUMAN AND CHIMPANZEE STEM CELLS REVEALS SPECIES-SPECIFIC ENDOGENOUS RETROVIRUS ACTIVITY

Narvaiza, Iñigo[1], Benner, Christopher[1], Wang, Meiyan[1], Marchetto, Maria Carolina[1], Ku, Manching[1], Japelli, Roberto[1], Swigut, Tomasz[2], Wysocka, Joanna[2] and Gage, Fred H.[1], [1]Salk Institute for Biological Studies, La Jolla, CA, U.S., [2]Stanford University, Stanford, CA, U.S.

We have previously shown that the comparison of gene expression in induced pluripotent stem (iPS) cells derived for human and non-human primates revealed differences in the control of mobile elements, which contribute to explain the higher levels of genome diversity in great apes. Here, we have further investigated the differences between humans and our closest living relatives by carrying out a comparative epigenomic study in human and chimpanzee iPS cells. For epigenomic profiling we analyzed genome-wide chromatin accessibility, transcription factors and histone modifications associated with active and repressed regulatory elements by chromatin immunoprecipitation and sequencing (ChIP-seq). We identified a number of divergent enhancers driven by differences in the sequence of transcription factor binding motifs. We also found a direct association between enhancer divergence and differences in target gene expression between human and chimpanzees. Many regulatory elements overlap with transposable elements, however we observed that divergent regulatory elements are enriched for LTR-retrotransposons, more specifically HERV-Hs. Divergence in transcriptionally active HERV-Hs is partially driven by species-specific mutations at LTR7s and correlates with differentially expressed neighboring genes. These findings reveal that epigenomic differences at retroelements that were mobile before the human-chimpanzee split leads to species-specific phenotypes, uncovering a previously underscored mechanism for evolution and diversity in primates, and demonstrate the value of primate iPS cells for comparative and primate evolution studies.

CHROMATIN IN STEM CELLS

F2155

TRANSPOSABLE ELEMENTS AND THEIR KRAB-ZFP CONTROLLERS REGULATE GENE EXPRESSION FROM EMBRYONIC STEM CELLS TO ADULT TISSUES

Ecco, Gabriela[1], Cassano, Marco[1], Kauzlaric, Annamaria[1], Duc, Julien[1], Coluccio, Andrea[1], Offner, Sandra[1], Imbeault, Michaël[1], Rowe, Helen[1,2], Turelli, Priscilla[1] and Trono, Didier[1], [1]EPFL, Lausanne, Switzerland, [2]University College London, U.K.

KRAB-containing zinc finger proteins (KRAB-ZFPs) and their cofactor KAP1 are important early embryonic sequence-specific controllers of transposable elements (TEs), which they repress though histone-based chromatin modifications and DNA methylation, a process thought to result in irreversible silencing. Using a target-centered functional screen, we identified two closely related murine KRAB-ZFP paralogs. We found these proteins to bind partly overlapping but distinguishable subsets of ERVK (endogenous retrovirus K), to repress these elements in embryonic stem cells, and to regulate secondarily the expression of neighboring genes. In addition, we uncovered that these KRAB-ZFPs were individually expressed in some differentiated cells as well, where they and KAP1 regulated their ERV targets and some genes situated nearby. This process stemmed from KRAB/KAP1-induced histone modifications, and appeared independent from the DNA methylation status of these loci. Therefore, TEs and KRAB-ZFPs establish transcription networks that regulate not only development but also differentiation and probably many other physiological events. Given the high degree of species-specificity of TEs and KRAB-ZFPs, these results have important implications for understanding the biology of higher vertebrates, including humans.

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GERMLINE CELLS

F2159

PRIMED HUMAN PLURIPOTENT STEM CELLS CONTRIBUTE TO THE GERMLINE MORE EFFICIENTLY THAN NAÏVE PLURIPOTENT STEM CELLS

Chen, Di[1], Lukianchikov, Anastasia[1], Galic, Zoran[1] and Clark, Amander T.[2], [1]UCLA, Los Angeles, CA, U.S., [2]University of California, Los Angeles, CA, U.S.

Generating germline cells from human embryonic stem cells is an important new model for understanding causes of human infertility, and the factors that lead to transmission of disease alleles from parent to child. Lessons from somatic cell differentiation in vitro have shown that independent hESC lines exhibit bias in their differentiation potential, and we hypothesized that this bias could also extend to the differentiation of germline cells. To address this, we derived eighteen hESC lines under primed conditions and differentiated all eighteen through an induced mesoderm like cell (iMELC) intermediate to create primordial germ cell like cells (PGCLCs). We discovered tremendous variability in the efficiency of germline formation between independently derived hESC lines. When all eighteen hESC lines were differentiated in parallel we discovered that male hESC lines were more efficient at generating PGCLCs than female hESC lines. To determine whether the efficiency of PGCLC differentiation could be improved by reverting primed hESCs to the naïve state, we converted the female hESC line called UCLA1 to the naïve state in a media called 5iLAF. We confirmed naïve pluripotency based upon a highly enriched human pre-implantation epiblast transcriptome, global loss of DNA methylation from the genome as quantified using whole genome bisulfite sequencing, and the loss of the primed cell surface antigen Stage Specific Embryonic Antigen 4 (SSEA4). Using the naïve cells as a starting point, we differentiated PGCLCs from naïve and primed hESCs in parallel through iMELC intermediate and discovered that PGCLC differentiation from naïve cells is highly inefficient and the resulting cells have a very low germline identity index. Taken together, our data reveals that primed hESCs exhibit differentiation bias towards the human germline in vitro, and that naïve hESCs cannot be used to correct differentiation bias.

F2161

DIFFERENTIAL EXPRESSION OF SELF-RENEWAL FACTORS FOR SPERMATOGONIAL STEM CELLS IN THE MAMMALIAN TESTICULAR NICHE

Takashima, Seiji[1,2], Yagi, Mizuki[3], Masaki, Kaito[3], Kuroki, Shunsuke[4], Tachibana, Makoto[4], Fujimori, Yuki[5], Hoshina, Kazuo[5], Oka, Kenji[6], Amano, Toshiyasu[7], Shiozawa, Tanri[6], Ishizuka, Osamu[6] and Hochi, Shinichi[1,3], [1]Graduate School of Science and Technology, Shinshu University, Ueda, Japan, [2]Shinshu University, Ueda, Japan, [3]Faculty of Textile Science and Technology, Shinshu University, Ueda, Japan, [4]Institute for Enzyme Research, Tokushima University, Tokushima, Japan, [5]Nagano Animal Industry Experiment Station, Shiojiri, Japan, [6]Shinshu University School of Medicine, Matsumoto, Japan, [7]Nagano Red Cross Hospital, Nagano, Japan

Spermatogonial stem cells (SSCs) are foundation for male reproductive system and possess self-renewal activity to maintain spermatogenesis throughout life. Previous studies demonstrated that glial cell line-derived neurotrophic factor (GDNF) is indispensable for SSC self-renewal. Although a recent study revealed that fibroblast growth factor (FGF)s could maintain SSCs in vitro even under GDNF-free condition, the function of these molecules in the testicular niche remains unknown. In this study, we investigated the role of FGFs in the postnatal mouse testis by comparing the expression patterns with GDNF under various testicular conditions. Quantitative reverse transcription polymerase chain reaction demonstrated that, among Fgf genes, Fgf2 is predominantly expressed in the mouse testis. Western blot analysis revealed that FGF2 expression is conserved in mammalian testes including mouse, rat, pig, bovine and human. We also found that Fgf2 was not expressed in Sertoli cells, but in germ cells and testicular interstitial cells. In addition, our study demonstrated that Fgf2 expression was constant during aging and downregulated under the conditions of spermatogenic failure, whereas Gdnf expression was downregulated by aging and upregulated under spermatogenic defects. Moreover, hypophysectomy induced upregulation of Gdnf and donwregulation of Fgf2, arguing against previous reports showing that pituitary-derived follicle stimulating hormone induces Gdnf expression in Sertoli cells. Although FGF2 and GDNF have shown to be bona fide self-renewal factors for SSCs in vitro, present study suggested that the roles of these factors in the mammalian testicular niche are different from each other.

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TOTIPOTENT/EARLY EMBRYO CELLS

F2163

GLOBAL GENE EXPRESSION PROFILING OF HESC H2O2-OXIDATIVE STRESS MODEL AND ANALYSIS OF THEIR SENESCENCE BIOMARKERS

Barandalla, Maria[1], Shi, Hui[2], Colleoni, Silvia[3], Trotter, Matthew[4] and Lazzari, Giovanna[3], [1]Avantea, Cremona, Italy, [2]University of Cambridge, Cambridge, U.K., [3]Avantea Srl, Cremona, Italy, [4]Celgene Institute for Translational Research Europe (CITRE), Seville, Spain

Human embryonic stem cells (hESCs) potentially offer new routes to study, on the basis of the Developmental Origins of Health and Disease (DOHaD) concept, how the maternal environment during pregnancy influences the offspring health and can predispose to chronic disease risk in later life. Under certain maternal pathological conditions Reactive Oxygen Species (ROS) production exceeds cellular defences and can damage lipids, nucleic acids and proteins. It has been demonstrated that ROS, antioxidant defences, and cellular redox status state play an important role in the regulation of gene expression and should now be regarded as central players in diabetes and metabolic syndromes as in aging. Therefore we have designed an in vitro cell model of oxidative stress by exposing hESCs to hydrogen peroxide (H_2O_2) during 72 h to mimic the preimplantation period. We have analysed the global gene expression profiles of hESCs (HUES3) exposed to 4, 8 and 16 μM H_2O_2 concentrations, using Illumina microarray HT-12 v4 and quantitative real time RT-PCR to validate the differential expressed genes. Comparative analysis of the gene expression profiles of H_2O_2 conditions versus control indicated the differential expression of 552 up-regulated genes and 477 down-regulated genes. According to gene ontology analysis the most affected categories were those related with RNA processing and splicing, oxidation-reduction, sterol biosynthesis and metabolic processes. Further we observed that one of the down-regulated genes for the 4 and 8 μM overlapping treatments is involved in centrosome structure and function. Therefore we assessed the presence of supernumerary centrosomes in our oxidative stress model at different timing treatments (2 h, 24 h and 4 days). Our data show that the percentage of cells with more than two centrosomes increased sharply with H_2O_2 treatment in hESC (HUES3 and 7) and in a control somatic cell line (Hs27), inducing a premature entry into senescence; by contrast, this effect was not observed in HUVEC treated cells, confirming their higher resistance to oxidative stress induced by H_2O_2 exposure.

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F2165

THE AUTOIMMUNE REGULATOR (AIRE) CONTRIBUTES THE INDUCTION OF EMBRYOINIC DEVELOPMENTAL PLURIPOTENCY

Krupalnik, Vladislav[1], Novershtern, Noa and Hanna, Jacob, Weizmann Institute of Science, Rehovot, Israel

The autoimmune regulator (Aire) protein plays an essential role in thymic education of immune system and preventing the appearance of T cell mediated autoimmune diseases. Aire contributes to autoimmune tolerance by inducing global low levels of expression of peripheral tissue antigens in thymic cells, and thus leading to representation of most self-antigens that are typically not expressed in the thymus. Remarkably however, Aire expression is not restricted only to the thymus, but is also expressed during early pre-implantation development, where its function remains to be explored. Further, Aire is conserved throughout vertebrate evolution and is found in all distant vertebrate species including Zebrafish. We have systematically studies the role of Aire in assembling pluripotency in vitro and in vivo. While Aire is dispensable for murine ESC maintenance, induction of Aire during somatic cell reprogramming significantly increases iPSC formation, and can substitute for exogenous Sox2 induction. In vivo experiments in both mice and zebrafish early development, show a significant decrease in zygote toward blastocyst maturation, 15% and 40%, respectively, upon Aire depletion. Our mechanistic studies implicate Aire in co-opting its role as a global transcriptional activator, for boosting transcriptional activation of the genome, zygotic or during iPSC formation, which is a critical event leading to assembling the ground state of pluripotency in vitro and in vivo. Collectively, we elucidate a previously unidentified role for Aire in safeguarding robust early embryonic development and somatic cell reprogramming.

EMBRYONIC STEM CELL DIFFERENTIATION

F2169

DERIVATION OF ENGRAFTABLE SCHWANN CELL PRECURSORS FROM HUMAN PLURIPOTENT STEM CELLS FOR DRUG DISCOVERY IN DIABETIC PERIPHERAL NEUROPATHY

Fattahi, Faranak[1,2], Arroyo, Edgardo[3], Ghazizadeh, Zaniar[4], Lankford, Karen[3], Amin, Sadaf[4,5], Calder, Elizabeth[2], Tchieu, Jason[1], Kocsis, Jeffery[3], Chen, Shuibing[2] and Studer, Lorenz[1], [1]Memorial Sloan-Kettering Cancer Center Developmental Biology, New York, NY, U.S., [2]Weill Cornell medicine, Weill Graduate School of Medical Sciences, New York, NY, U.S., [3]Yale University School of Medicine, New Haven, CT, U.S., [4]Weill Cornell Medicine, New York, NY, U.S., [5]Weill Cornell Medical College, New York, NY, U.S.

Schwann cells are glia of the Peripheral Nervous System (PNS). They arise from neural crest during embryonic development and play crucial roles in functional regulation, maintenance and repair of the PNS. Schwann cell defects are involved in a broad range of human disorders including Diabetic Peripheral Neuropathy (DPN). In DPN, hyperglycemia, hypoxia and oxidative stress lead to dysfunction and degeneration of Schwann cells particularly in sensory nerves. Here we establish an efficient strategy for derivation and prospective isolation of Schwann cell precursors from human Pluripotent Stem Cells (hPSCs). The hPSC-Schwann cells are capable of myelinating hPSC-derived sensory neurons in vitro. Transplanted hPSCs-Schwann cells in injured sciatic nerves of rats contribute to myelination of regenerating host axons and promote appropriate ion channel localization in newly myelinated fibers. The hPSC-Schwann cells enable the in vitro modeling of hyperglycemia induced cytotoxicity in DPN. We further use this model to perform high throughput drug screening and identify candidate therapeutic targets for treatment of DPN.

F2171

DIFFERENTIATION AND FUNCTION OF HUMAN EMBRYONIC STEM CELL DERIVED GABAERGIC INTERNEURON PROGENITORS IN VITRO AND IN THE EPILEPTIC MOUSE BRAIN

Anderson, Nickesha C., Van Zandt, Meghan, Shrestha, Swechhya, Gupta, Jyoti, Moakley, Daniel Finnell, Boyi, Trinithas, Harrsch, Felicia, Chen, Christopher Y, Bobbitt, Toria, Aaron, Gloster, Naegele, Janice and Grabel, Laura, Wesleyan University, Middletown, CT, U.S.

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 human ESCs using sonic hedgehog treatment. To test the differentiation potential of the Nkx2.1+ cells in vitro, we utilized co-culture and tri-culture systems with mouse cortical astrocytes and mature hippocampal cells; deriving an enriched population of interneurons in which 75% of the MAP2-positive cells are also GABA-positive after 8 weeks. Studies examining the fate of human ESC-derived ventralized neural progenitors transplanted into the mouse hippocampus of severe compromised immune deficient (SCID) epileptic mice demonstrate increased expression of the mature neuronal markers, Hu, NeuN, and the inhibitory neurotransmitter GABA between 6 and 12 weeks post transplantation. Preliminary studies suggest the transplanted cells are able to suppress recurring seizures in a mouse model of temporal lobe epilepsy. In addition, mice with transplanted cells exhibited significant improvement in the Morris Water Maze spatial memory task by six weeks post transplant. Patch clamp analysis indicates that hESC derived neurons are capable of firing mature action potentials following long-term in vitro culture and post transplantation into mouse host.

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F2173

EVALUATION OF THREE-DIMENSIONAL CELL CULTURE SYSTEMS FOR IMPROVED HEPATIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

Sgodda, Malte[1,2], Papp, Oliver[1,2], Alfken, Susanne[1,2], Zweigerdt, Robert[2,3] and **Cantz, Tobias**[1,2], [1]Hannover Medical School, Hannover, Germany, [2]REBIRTH Cluster of Excellence, Hannover Medical School, Hannover, Germany, [3]Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover, Germany

Human pluripotent stem cell (hPSC) -derived hepatic cells are broadly used as cell source for disease modeling and pharmacotoxicology studies. So far, hepatic differentiation of hPSCs applying conventional monolayer cell culture systems is well studied, but efficiencies and enrichment of functional active hepatic derivatives might be supported by three-dimensional cell culture systems. In this study, we evaluated gene expression levels and characteristics of hPSCs grown in three-dimensional aggregates in direct comparison to an established monolayer differentiation protocol. In our first set of experiments, we analyzed the capability of fragments, obtained from the hepatoblast stage of the monolayer protocol to form hepatic aggregates in 6-well plates applying suspension culture conditions. Here, we detected a strong increase of hepatic marker gene expression in the hepatic aggregates, which provided clear evidence for an enhanced hepatic maturation in the three-dimensional culture system. Subsequently, we switched to a scalable Erlenmeyer flask-based suspension culture system to investigate critical parameters (rotation speed, inoculation density, fragment size) for a standardized generation of hepatic aggregates. Paraffin-embedded aggregates depicted solid-tissue like structures surrounding some cysts and support the notion of spontaneous formation of liver organoids. Interestingly, the size of the inoculated monolayer fragments interfered with the hepatic lineage commitment and larger fragments yielded more cholangiocyte features but small fragments provided more homogenous hepatocyte features with significant activity of Cytochrome P450 enzymes. Finally, we tested different dissociation enzymes to digest the organoids for subsequent re-plating on a collagen-matrix based monolayer culture. Gene expression profiles of these cells revealed that the acquired hepatic features was maintained in this culture setting with only minor changes in the hepatic marker gene expression levels. In conclusion, we evaluated three-dimensional cultivation conditions for growing hPSC-derived hepatic cells and establish an organoid culture system, which enhanced the maturation of hepatic hPSC-derivatives for down-stream use in disease-modeling or pharmacotoxicology applications.

F2175

UNRAVELING THE ROLE OF TREM2 MUTATIONS IN ALZHEIMER'S DISEASE USING HUMAN PLURIPOTENT STEM CELLS

Claes, Christel[1], Boon, Ruben[2], Sleegers, Kristel[3], Van Broeckhoven, Christine[3], De Strooper, Bart[4] and Verfaillie, Catherine[2], [1]KU Leuven, Leuven, Belgium, [2]SCIL, Leuven, Belgium, [3]VIB, Antwerp, Belgium, [4]VIB, Leuven, Belgium

Currently, no good therapies exist to treat Alzheimer's disease (AD). In addition, diagnosis only takes place at a later stage, at a time that extensive brain damage has already occurred. It should however remain possible to tackle neuroinflammation to slow down the further progression of AD. We here focused on the role of monocytes, known to contribute to neuroinflammation. In addition, these cells express the receptor TREM2. Heterozygous rare variants of TREM2 are associated with a significant increased risk for AD, with TREM2-R47H showing a four-fold increased association. To enable the study of human monocytes, we first established a monocyte differentiation model for human pluripotent stem cells (hPSCs). Monocytes can be efficiently generated from different hPSC lines, including genetically modified lines containing either GFP or tdT, and can be collected from day 15 until day 30. Monocytes are enriched from the mixed progeny using CD14 MACS beads. FACS analysis showed that 97.6% of the MACS selected cells express CD14 and CX3CR1. In addition qPCR data demonstrated high transcript levels for monocyte specific markers including CD45, CD14, CX3CR1, CCR2 as well as TREM2 and DAP12. After collection, monocytes were cryobanked. To address the effect of the R47H variant and KO on monocytic function, construction of TREM2 R47H mutated and TREM2 KO human embryonic stem cell lines is in its final steps, using CRISPR/Cas9 nickases. In addition, the recent CRISPRi-KRAB system is being used to cause a > 90% decrease in TREM2 expression in hPSCs, and lymphocytes from a TREM2 R47H AD patient are being reprogrammed to induced (i)PSCs. To unravel the contribution of human monocytes and TREM2 to AD, we are optimizing co-cultures of hPSC-derived neural stem cells (NSC) with hPSC-derived monocytes in 2D as well as 3D. NSC are successfully differentiated from different hPSC lines and cryobanked. Cells stained positive for Nestin, FoxG1, Sox2 as well as Pax6, confirmed by qPCR data. In addition, the effect of wild-type and TREM2 aberrant monocytes will be assessed in vivo. We hypothesize that these in vitro and in vivo setups will result in better humanized models for drug screening.

F2177

PARASYMPATHETIC NERVES CONTROL ACINAR CELL GENERATION AND SPECIFICATION DURING SALIVARY GLAND DEVELOPMENT AND REGENERATION VIA SOX2+ PROGENITOR CELLS

Emmerson, Elaine[1], May, Alison[1], Cruz-Pacheco, Noel[1], Nathan, Sara[1], Lizama, Carlos O.[2], Zovein, Ann C[2], Tward, Aaron D[3], Ryan, William R[3], Muench, Marcus O[4] and Knox, Sarah M[1], [1]Program in Craniofacial Biology, University of California San Francisco, San Francisco, CA, U.S., [2]Cardiovascular Research Institute, University of California San Francisco, San Francisco, CA, U.S., [3]Otolaryngology, University of California San Francisco, San Francisco, CA, U.S., [4]Blood Systems Research Institute, San Francisco, CA, U.S.

Acinar cells in salivary glands (SGs) are irreversibly lost following therapeutic radiation for head and neck cancer resulting in xerostomia. Despite the fact that ~500,000 patients a year are treated for head and neck cancer, at present there are no therapies available to restore salivary gland function Stem/progenitor cell therapy has the potential to repair and regenerate injured tissue but the identity of salivary progenitor cells and their niches and the cues that regulate them is poorly understood. Using genetic lineage tracing we found that removal of parasympathetic innervation or inhibition of acetylcholine signalling reduced acinar cell formation and the expression of the progenitor marker SOX2. Ablation of *Sox2* in the epithelium increased epithelial cell death, reduced proliferation and inhibited the acinar but not ductal cell lineage. Using human fetal salivary gland ex vivo explant and cell culture systems, we show that SOX2+ cell proliferation and *SOX2*expression is also dependent on muscarinic receptor activation in the human SG. In the adult organ, muscarinic activation promotes acinar cell repopulation during homeostasis and after radiation-induced injury through expansion and differentiation of SOX2+ progenitors. Folowing radiation therapy adult human SGs undergo atrophy and a concurrent loss of parasympathetic nerves and *SOX2*. Crucially, SOX2 increases in response to the acetylcholine analog Carbachol in our novel adult explant culture. Together, these data demonstrate SOX2 to be a crucial regulator of salivary gland development and regeneration whose expression is dependent on cholinergic signalling in mice and humans.

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F2179

AN INVESTIGATION OF THE ROLE OF TRPC3 IN THE NEURAL DIFFERENTIATION OF MOUSE EMBRYONIC STEM CELLS (ESCS).

Hao, Helen Baixia[1], Webb, Sarah E.[1], Moreau, Marc[2], Leclerc, Catherine[2], Yue, Jianbo[3] and Miller, Andrew L.[1,4], [1]The Hong Kong University of Science and Technology, Hong Kong, Hong Kong, [2]University Paul Sabatier, Toulouse, France, [3]City University of Hong Kong, Hong Kong, [4]Marine Biological Laboratory, Woods Hole, MA, U.S.

The neural differentiation of embryonic stem cells (ESCs) is a dynamic process, which is regulated by several different signaling pathways. Among these signals, Ca^{2+}, as a highly versatile intracellular second messenger, plays an important role in regulating neural differentiation of ESCs. The goal of our research is to investigate the role of transient receptor potential canonical 3 (TRPC3), a cation channel that is permeable to both Ca^{2+} and Na^{+}, in the regulation of the neural differentiation of mouse ESCs. We showed that TRPC3 was expressed in mouse ESCs and it contributed to the thapsigargin-induced store-operated Ca^{2+} entry (SOCE) in these cells. The level of TRPC3 expression gradually increased as the ESCs differentiated into cells of the neural lineage. In addition, suppressing the expression of TRPC3 (by ~50%) via shRNA knockdown inhibited the expression of the neural differentiation markers: nestin, sox1 and tuj1. Furthermore, treatment of mouse ESCs with pyr3, a specific TRPC3 inhibitor, inhibited the expression of these neural differentiation markers and reduced the SOCE. Interestingly, TRPC3 knock out with CRISPR/Cas9 or inhibition with pyr3 caused dramatic cell death during the early stages of mouse ESC-derived neural differentiation. Taken together, TRPC3 appears to positively regulate the neural differentiation of mouse ESCs and it is also required for cell survival during this process. We are now studying how the suppression of TRPC3 affects the cellular and molecular mechanisms involved during the inhibition of neural differentiation and cell survival.

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F2181

ROLE OF MIR-1305 IN REGULATING PLURIPOTENCY, CELL CYCLE, AND APOPTOSIS IN HUMAN EMBRYONIC STEM CELLS

Jin, Shibo[1], Zhu, Lili[1], Neganova, Irina[2], Collin, Joseph[3], Armstrong, Lyle[3] and Lako, Majlinda[3], [1]Institute of Genetic Medicine, Newcastle, U.K., [2]International Centre for Life, Newcastle upon Tyne, U.K., [3]Newcastle University, Newcastle upon Tyne, U.K..

Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are defined as pluripotent in view of their ability to maintain self-renewal and differentiation to cells of all three germ layers. So far the mechanism underlying the regulation of self-renewal, pluripotency of human pluripotent stem cells are not fully understood. In this study we screened for candidate miRNAs which might play important roles in regulating pluripotency and cell cycle by using a microarray based approach. miR-1305's expression was significantly downregulated during hESC differentiation and upregulated during G1 to S transition and for this reason was chosen for further investigations. Our results provide evidence that overexpression of miR-1305 leads to hESC differentiation, increases cell apoptosis, and speeds up the G1/S transition, while its downregulation facilitates maintain of hESC pluripotency, increases cell survival and slows down the G1/S transition slower. Using target prediction softwares and luciferase based reporter assays we identified POLR3G as a downstream target by which miR-1305 regulates hESC differentiation. Overexpression of POLR3G rescues hESC differentiation induced by miR-1305 overexpression, and knock-down of POLR3G expression abolishes the miR-1305-knockdown mediated hESC pluripotency. Together our data corroborate previous findings on existence of intrinsic link between cell cycle regulation and maintenance of pluripotency in hESC and identifies new candidate genes and miRNA regulating these interlinked processes.

F2183

SEPARATION OF HUMAN MESENCHYMAL STEM CELLS FROM DIFFERENTIATED HUMAN EMBRYONIC STEM CELLS USING INTEGRIN BINDING RECEPTORS

Kim, Jin-Su, and Lee, Soo-Hong, CHA University, Gyeonggi-do, Korea, South

The successful differentiation of human pluripotent stem cells into cells that similar adult mesenchymal stem cell (MSC) is one of the most promising approaches to obtain a readily available source of progenitor cells for tissue engineering and cell therapy. The extracellular matrix (ECM) consists of a complex mixture of structural and functional

macromolecules. The various ECM components are expressed during the tissue development and play a key role as a modulator during the cell growth and tissue development. Cell-ECM interactions largely depend on integrin cell-surface receptors. Integrin α5β1 is highly expressed during the differentiation of the mesenchymal stem cells from embryonic stem cells. In the present study, we describe a new isolation method to rapidly derive MSC-like cells from hESCs in one step using a fibronectin receptor (integrin α5β1) that allows maintaining stable survival and attachment of MSC-like cells. The ratio of 18.1 % CD90+CD105+ cells was obtained in the total differentiated hESCs. The separated cells through fibronectin (Fn) were highly expressed mesenchymal stem cell surface markers compared to the other groups. Furthermore, fibronectin separation method was significantly enhanced initial cell attachment than other groups. In addition, the initial cell attachment on fibronectin was mediated by α5β1 integrin. Fibronectin-separated hESC-MSCs exhibited a typical MSC phenotype such as fibroblastic morphology, MSC surface marker expression. Moreover, these cells also have the multi-differentiation potential into osteocyte, chondrocyte and adipocyte in vitro. These data demonstrate that fibronectin coating system can be used to guide a rapid, efficient derivation of ES-derived MSC.

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F2185

TARGETED DISRUPTION OF HEB IN HUMAN EMBRYONIC STEM CELLS

Li, Yang[1], Zuniga-Pflucker, Juan Carlos[2], Brauer, Patrick M.[1] and Xhiku, Sintia[3], [1]Sunnybrook Research Institute, Toronto, ON, Canada, [2]Department of Immunology University of Toronto, Toronto, ON, Canada, [3]University of Toronto, Toronto, ON, Canada

HEB, the product of TCF12 gene, is a member of helix-loop-helix (HLH) E-protein family. Most studies associated with HEB mainly focused on lymphopoiesis by studies involving HEB mutant mice. However, the role of HEB in human development remains unknown. In this study, we disrupted TCF12 gene using CRISPR-Cas9 genome editing in human embryonic stem cells. Subsequently, HEB knockout in human embryonic stem cells was confirmed on protein and mRNA level by western-blot and RT-PCR. Surprisingly, the expression of NANOG and OCT4 down-regulate in HEB KO cells compared to wild type cells. Furthermore, KO cells have higher proliferation rate than wild type cells. We also investigated the role of HEB in hematopoietic and T-cell developmental potential. The data show that knockout of HEB in human embryonic stem cells leads to a reduction in early hematopoietic and T-cell differentiation ablility. This work illustrates how human embryonic stem cells can provide unique insights into human development.

F2187

SYNCHRONIZED DIFFERENTIATION IN MOUSE EMBRYONIC STEM CELLS

Minakawa, Tomohiro, Yamamizu, Kohei and Yamashita, Jun, Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto City, Japan

Embryonic development proceeds through many cellular events (lineage segregation, division, migration etc.) in a highly orchestrated manner. However, how cells communicate each other and control their behavior during the dynamic change is still largely unknown. Embryonic stem cells (ESCs), which are able to differentiate from a pluripotent state to three germ layers, are useful tools for investigating cell differentiation and cell-cell communication processes. Previously, we reported that protein kinase A (PKA) regulates differentiation timing of ESCs. We generated a mouse ESC line that carries Dox-inducible (Dox-OFF) constitutive active form of PKA (PKA-ESCs), and demonstrated that PKA activation in the early phase of differentiation could induce Flk1+ mesoderm differentiation approximately 2 times faster than normal differentiation condition. With the use of the ESC differentiation system, here we show that differentiation timing of cells is synchronized through cell-cell communication. We established a cell-chimeric culture system using PKA-ESCs and another ESC line that carries ubiquitous promoter-driven GFP gene alone (GFP-ESCs). We generated chimeric cell aggregates with PKA-ESCs and GFP-ESCs and induced differentiation with the chimeric aggregates. Pure PKA-ESC aggregates and GFP-ESC aggregates are used as positive and negative control, respectively. In normal differentiation condition, both Pure PKA-ESC and GFP-ESC aggregates gave rise to Flk1+ cells at differentiation day 4.5 (approx. 10% of total cells). When PKA was activated (Dox-), pure PKA-ESC aggregates showed earlier appearance of Flk1+ cells, that is approx. 43% at day 3.5, but pure GFP-ESC aggregates did not (approx. 4%). On the other hand, in the chimeric aggregates, GFP-ESCs efficiently differentiated into Flk1+ cells at day 3.5 under PKA activation (approx. 40%). This result indicates that when co-existing with the faster differentiating PKA-ESCs, GFP-ESCs also showed faster differentiation even though PKA was not directly activated in GFP-ESCs, and synchronized their differentiation timing with the faster differentiating cells. Thus, we would like to call this phenomenon “synchronized differentiation”. Currently, we are investigating cellular and molecular mechanisms of this new type cell-cell communication.

F2189

UNDERSTANDING PATTERNING ONE STEM CELL AT A TIME

Warmflash, Aryeh and **Nemashkalo, Anastasiia**, Rice University, Houston, TX, U.S.

Embryonic stem cells (ESCs) represent a promising system to dissect spatial patterning in vitro. During stem cell differentiation, cell fates are specified by a combination of exogenously supplied ligands and paracrine signals between the cells – effects that are difficult to parse. Here we disentangle these effects using a bottom-up approach: by growing stem cells confined to very small colonies (microcolonies, 1-8 cells/colony) using micropatterning techniques. This approach allows us to study the effects of exogenously supplied ligands in isolation and precisely modulate the level of paracrine signaling. We differentiated microcolonies of human embryonic stem cells with BMP4, a ligand critical for primitive-streak formation and gastrulation in vivo. Our results indicate that BMP4 acts as a simple switch rather than as a morphogen: pluripotent (Sox2+) stem cells completely transition to extra-embryonic (Cdx2+) above a threshold BMP4 ligand concentration. To study underlying cell-cell interactions, we analyzed the results of signaling and differentiation as a function of the number of cells in the colony. Interestingly, under pluripotent conditions, a fraction of isolated cells spontaneously downregulate Sox2 and express the trophectodermal marker Cdx2, but as the colony size grows to 4 cells/colony or larger, spontaneous differentiation is not observed. We argue that this is the manifestation of the community effect, the enforcement of a common fate in groups of cells, in our microcolonies. We show that in pluripotent conditions, the community effect is mediated by paracrine FGF signaling that prevents spontaneous differentiation. When cells are differentiated with BMP4, the community effect is enforced in the signaling response itself. Live-cell imaging analysis shows more coherent and sustained responses in larger colonies that correlates with more uniform fate conversion. To explain these observations, we exploit an analogy with magnetic systems to create an Ising-like model of the influence of exogenous and paracrine signals on cell fate. This simple two-parameter model explains a wealth of quantitative data from the microcolony system. Our results provide proof of principle for dissecting signaling interactions underlying embryonic patterning using a bottom-up approach.

F2191

LARGE-SCALE SINGLE CELL RNA-SEQ RESOLVES CELLULAR HETEROGENEITY IN LONG-TERM CULTURES OF HUMAN CEREBRAL ORGANOIDS

Quadrato, Giorgia[1,2], Nguyen, Tuan[1], Macosko, Evan[2,3], Sherwood, John[1,2], Maria, Natalie[1], Zhang, Helen[1], McCaroll, Steven[2,3] and Arlotta, Paola[1,2], [1]Harvard University, Cambridge, MA, U.S., [2]Broad Institute of Harvard and MIT, Cambridge, MA, U.S., [3]Harvard Medical School, Boston, MA, U.S.

Genomic sequencing has rapidly expanded our understanding of the polymorphisms underlying neurodevelopmental disorders. However, we still have a limited understanding of the cellular phenotype associated with these risk-associated alleles. Cerebral organoids represent an emerging model that meets many of the criteria for an optimal in vitro system to phenotype specific mutations. However, current organoids appear to only represent early time points in brain development, and whether they reproducibly generate endogenous cellular diversity has not been fully explored. There is a clear need to use high-throughput screening methods on organoids to fully reveal their cellular composition. We have implemented a modified version of the culturing protocol developed by Lancaster et al.,[1] to enable efficient long term cultures, and have achieved healthy growth of cerebral organoids for over nine months. We have characterized a time course for generation of different brain structures and cell types within one to nine month old organoids by immunohistochemistry. This data show that organoids develop by first producing neural progenitors followed by production of distinct cell-types expressing class-specific markers of cortical inhibitory and excitatory neurons. At later stages of differentiation, following a developmentally-correct temporal sequence, we observed astrogliogenesis and synaptogenesis. To understand the exact cellular composition of each organoid and determine the reproducibility with which distinct brain regions and cell classes are generated, we used high-throughput single cell RNA sequencing via droplet sequencing[2] to profile a large number of cells (4000-6000 per organoid), across separate culture flasks. Initial analysis shows that while organoids are highly heterogeneous, there are specific cell populations that are reproducibly generated within every organoid. These data provide insight into the range of cellular heterogeneity that results when generating cerebral organoids from hPSC. Building on these results, we are investigating neurodevelopmental diseases by phenotyping the cellular composition of organoids carrying risk-associate alleles. We aim to examine changes in single-cell transcriptomes in response to stimulated microcircuit activity in mutant organoids.

F2193

DLG2 IS EXPRESSED IN NEURAL PROGENITOR CELLS AND HAS A ROLE IN NEURAL DEVELOPMENT

Shin, Eunju Jenny, Li, Meng and Harwood, Adrian J, NMHRI, Cardiff University, Cardiff, U.K.

Various genes expressed in postsynaptic density have been found to be associated with increased risk of neurodevelopmental disorders such as schizophrenia. Implicated genes include members of the DLG (disks large) family of membrane associated guanylate kinases (MAGUKs) and related synaptic proteins. In particular, recent genome-wide association studies found de novo mutations in DLG2 are associated with increased risk of schizophrenia. Unlike the general concept that DLG2, a postsynaptic protein, has a role mainly in synapses of mature neurons, it was found to be expressed throughout the neural differentiation process including neural progenitor stage where there is no synapse formation. The aim of the study is to reveal the function of DLG2 not only on mature neurons but also during neural development. The knockout human embryonic stem cell (hESC) lines were derived using the new genome editing technology called clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system. DLG2$^{-/-}$ hESCs differentiated into nestin^{+} neural precursors and gave rise to cortical projection neurons. However, compared to wildtype hESCs, differentiation kinetics seemed to be altered. DLG2$^{-/-}$ hESCs differentiated in more speedy way resulting in more neurons at D40 and D50 of cortical differentiation. At later time points, the proportion of Tbr1^{+} layer 6 cortical neurons was less and the percentage of Ctip2^{+} layer 5 cortical neurons was higher compared to wildtype counterparts. In order to pinpoint from when the two lines are behaving differently, flow cytometry analysis is ongoing with samples from various differentiation time points. Unveiling the role of DLG2 during and after neural differentiation will let us understand more about neural development itself and give us a clue to uncover underlying mechanisms of neurodevelopmental diseases.

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F2195

THE EXPRESSION AND ROLE OF ARYL HYDROCARBON RECEPTOR IN HUMAN EMBRYONIC STEM CELLS AND ITS DIFFERENTIATING COUNTERPARTS

Teino, Indrek, Pook, Martin, Tiido, Tarmo and Maimets, Toivo, University of Tartu, Tartu, Estonia

The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor initially known as a major mediator of toxicity of various environmental contaminants. In the following studies, the role of AHR in normophysiology was established. This includes its importance in reproductive system, liver homeostasis, cell cycle regulation etc. In addition, recent data has elucidated the important role of AHR in cells with high developmental potential (e.g. neural, hematopoietic, breast cancer stem cell/progenitors). However data, regarding the expression and role of AHR in human embryonic stem (hES) cells and during the first steps of differentiation, is scarce. Thus our aim is to investigate the regulation and role of AHR in hES cells and its differentiating counterparts. Our experiments show that AHR is expressed in pluripotent hES cells. Moreover, a known carcinogen and the most potent ligand of AHR – TCDD – had no effect on the pluripotency factor expression as well as on the cell cycle of hESC, indicating that AHR is constitutively activated. This was corroborated by immunofluorescence analysis, which showed that AHR is mainly located in the nucleus. Subsequent analysis of AHR expression in embryoid bodies (EB) showed varying results, suggesting that AHR might be differentially expressed depending on the linage. In our ongoing experiments, we aim to elucidate the expression pattern of AHR during differentiation into distinct lineages and describe the mechanism by which the AHR is modulated. In addition, we seek to clarify the role of AHR in association with pluripotency and differentiation of human embryonic stem cells.

F2197

GENERATION OF FUNCTIONAL SOMITE-LIKE CELLS FROM HUMAN PLURIPOTENT STEM CELLS

XI, Haibin[1], Fujiwara, Wakana[1], Jan, Majib[1], Liebscher, Simone[2], Van Handel, Ben[3], Schenke-Layland, Katja[4] and Pyle, April[1], [1]UCLA, Los Angeles, CA, U.S., [2]Eberhard Karls University Tübingen, Tübingen, Germany, [3]CarthroniX Inc., Los Angeles, CA, U.S., [4]Fraunhofer IGB Stuttgart, Germany

Human pluripotent stem cells (hPSCs) hold great promise in advancing medicine. To fully harness their power, it is imperative to design efficient protocols to differentiate hPSCs toward desired lineages. Somites are transient mesodermal structures formed during early development that are common ancestors to a plethora of cell types, including skeletal muscles as well as bones and cartilages of ribs and vertebrae. Somitogenesis involves formation of primitive streak (PS), migration of PS cells to form posterior presomitic mesoderm (pPSM), progression of posterior to anterior PSM (aPSM), and segregation of aPSM cells to generate somites. There are no well-established protocols to generate somites from hPSCs. We set out to devise a protocol to differentiate hPSCs to somite-like cells in vitro by mimicking the environmental cues instrumental to somitogenesis in vivo. We have found that transiently activating canonical WNT-β-catenin signal-

ing drives hPSCs toward a PS followed by a pPSM fate. Subsequently, inhibiting BMP signaling specifies pPSM-like cells toward aPSM- and somite-like cells. Moreover, inhibition of TGFβ signaling further enhances the somite specification efficiency. To validate the functionality of our somite-like cells, we assayed their myogenic, osteogenic and chondrogenic potential. Cells under myogenic differentiation conditions show a progressive increase of myogenic marker expression and form myosin heavy chain positive skeletal muscle cells. Similarly, under osteogenic and chondrogenic conditions, cells upregulate expression of the respective lineage markers and are positively stained by Alizarin Red (osteogenesis) and Alcian Blue (chondrogenesis). To gain more insights on human somitogenesis, we micro-dissected nascent somites from early human embryos and compare their transcriptional profile to that of hPSC-derived somite-like cells via RNA sequencing. This approach has uncovered similarities and differences between the in vivo and in vitro human somite cells and has enabled further improvements to the somite specification protocol. In summary, we have developed a protocol to derive functional somite-like cells from hPSCs, which could serve as a useful platform for optimally generating somite-derived lineages for disease modeling and regenerative medicine.

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F2199

DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS INTO CFTR-EXPRESSING (LUNG) EPITHELIAL CELLS

Ulrich, Saskia[1,2], Engels, Lena[2,3], Merkert, Sylvia[2,3], Olmer, Ruth[2,3] and **Martin, Ulrich**[2,3], [1]Hanover Medical School, Hannover, Germany, [2]Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), Hannover, Germany, [3]Hannover Medical School, Hannover, Germany

Pluripotent stem cells (PSCs), like embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), offer promising therapeutic options to treat lung diseases, such as cystic fibrosis, based on cellular/tissue replacement therapies, disease modelling and drug screening. These regenerative approaches crucially rely on the efficient differentiation of PSCs into lung epithelial cells, which is the aim of our present study. We made use of the human (h) ESC reporter cell line hES3 NKX2.1-eGFP (kindly provided by A. Elefanty) expressing eGFP under the endogenous NKX2.1 promoter. Furthermore, we took advantage of a lab-internal generated double transgenic cell line based on the hES3 NKX2.1-eGFP cells, additionally expressing dTomato controlled by the endogenous CFTR promotor. Immunofluorescence stainings, qRT PCR analysis and flow cytometry were used to analyze the generated definitive endoderm (DE), anterior foregut endoderm (AFE) and NKX2.1-eGFP^{pos} cell populations. Using the commercially available STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™ Optimized) resulted in the robust and efficient generation of a highly enriched DE population of > 93 % CXCR4^{pos}/c-Kit^{pos} cells and CXCR4^{pos}/EpCAM^{pos} cells from two different hESC lines and one iPSC line. DE generation was additionally verified by co-expression of the transcription factors FOXA2 and SOX17. Further differentiation resulted in a distinct FOXA2^{pos}/SOX2^{pos} AFE population, which finally gave rise to up to 46 % NKX2.1-eGFP^{pos} cells. Subsequent maturation of purified NKX2.1-eGFP^{pos} cells demonstrated the formation of CFTR-dTomato^{pos}/NKX2.1-eGFP^{pos} co-expressing cells around day 40 of differentiation, whose detailed phenotypic analysis is currently ongoing.We were able to generate almost pure DE followed by the induction of a distinct AFE population, resulting in a decent percentage of NKX2.1-eGFP^{pos} cells. Further maturation demonstrated the generation of CFTR-dTomato^{pos}/NKX2.1-eGFP^{pos} co-expressing cells, most likely associated with the lung lineage. Future work will focus on additional optimization of the differentiation strategy and phenotypic and functional analysis of the cells.

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EMBRYONIC STEM CELL PLURIPOTENCY

F2203

MIRNAS: POSSIBLE MECHANISM OF HUMAN AMNIOTIC EPITHELIAL CELLS TO MAINTAIN THE PLURIPOTENCY IN HUMAN EMBRYONIC STEM CELLS

Avila, Daniela[1], Garcia-Castro, Irma[1], Flores-Herrera, Hector[1], Molina-Hernandez, Anayansi[1], Portillo, Wendy[2], Garcia-Lopez, Guadalupe[1] and Diaz, Nestor Fabian[1], [1]Instituto Nacional de Perinatologia, Mexico City, Mexico, [2]Instituto de Neurobiologia UNAM, Queretaro, Mexico

Most existing human embryonic stem cells (hESC) lines have been derived and maintained in mouse embryonic fibroblasts (MEFs). However, the use of non-human feeder layers had several drawbacks, since they have the potential to transmit xenic-contaminants to the cell culture and, therefore, induce an immune response upon transplantation that would present a barrier for future medical application of hESC. In addition, the use of synthetic extracellular matrices could induce genetic and epigenetic alterations, compromising the pluripotency of hESC. For this reason, studies have searched for alternatives to culture hESC in a feeder-free environment. Using human feeder layers in xeno-free conditions is still the safest

method to derive and maintain hESC. Our laboratory previously demonstrated that human Amniotic epithelial cells (hAEC) support the derivation and maintenance of hESC from poor quality embryos; however, the mechanisms involved in the interaction between both cell types to promote the pluripotency still remain unknown. On the other hand, it has been reported that specific microRNAs families (miR-92, miR-302, miR-200) can modulate the pluripotency at the posttranscriptional level. Our aim was to determine if hAEC can maintain the pluripotency of hESC through their miRNAs. When the hESC were cultured on feeder-hAEC, after several passages (15), the colonies were positive to alkaline phosphatase, expressed pluripotency markers and the analysis by Epi-Pluri-Score (based on DNA methylation at specific genes) classified these lines in hAEC as pluripotent. On other hand, we detected the expression of miR-92a, miR-92b and miR-200c by RT-PCR in the conditioned media (CM) from hAECs (hAEC-CM). Afterwards, H1, H9 and Amicqui-1 lines were cultured with hAEC-CM on feeder-free conditions (Matrigel). Interestingly, the cells still expressed pluripotent markers, which suggested the maintenance of this characteristic. Analysis by qPCR showed that hESC maintained on feeder-hAEC or Matrigel with hAEC-CM, expressed high levels of microRNAs (e.g. miR-200c) as compared with feeder-MEF or MEF-CM. The above mentioned evidence suggests that hAEC can maintain the pluripotency of hESC through specific miRNAs.

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F2205

GENERATION OF NONHUMAN PRIMATE EMBRYONIC STEM CELL CHIMERIC EMBRYOS AND RECOVERY OF A CHIMERIC FETUS

Curnow, Eliza C.[1], Morris, David R.[2], Fernandez, Mark C.[1] and Hayes, Eric S.[1], [1]University of Washington, WaNPRC, Seattle, WA, U.S., [2]University of Washington, Seattle, WA, U.S.

The gold standard test for determining the pluripotency of embryonic and induced pluripotent stem cells is the embryonic chimera assay. The degree to which pluripotent stem cells (PSCs) contribute to the chimeric embryo is dependent on their pluripotent potential. With no definitive in vivo tests with which to characterize the pluripotency of human PSCs, the nonhuman primate (NHP) represents the most ideal model in which to test the questions surrounding the significance of naïve and primed pluripotency. Here we describe the use of 'primed' macaque (M. fascicularis) embryonic stem cell lines (MfESCs) for the generation of MfESC-embryo chimeras and the generation of an MfESC-chimeric fetus. Preliminary studies involved aggregation of zona free morula stage embryos with MfESCs labelled with a cell tracker dye (Mitotracker). Culture of the ESC-embryo aggregates to the blastocyst stage in a PDMS microwell culture system resulted in high rates of blastocyst development in both control and ESC-chimeras (34 vs. 53% respectively, n=23-38). Confocal imaging of blastocysts shows that MfESC-embryo chimeras have a significantly greater total cell number compared to control blastocysts (237.0±18.2 vs. 141±14.5 respectively, n=7-10) with MfESCs contributing to 32±5.8% of the cells in the blastocyst. For in vivo studies, eGFP labeled MfESCs (15-20 cells) were injected into morula and cultured to the blastocyst stage. Fresh or vitrified/warmed MfESC-embryo chimeras were transferred to naturally cycling recipient females (n=5) with one pregnancy established. A fetus was recovered following spontaneous labor at GD103 and evaluation and sampling of key organs was performed. Using nested PCR eGFP was detected in fetal blood, spleen, muscle, intestine, bladder and brain. Our preliminary data shows that MfESCs cultured under conditions that support a 'primed' state have the ability to integrate and contribute to the pre-implantation embryo and the developing fetus. Further studies are aimed at understanding the functional significance of naïve and primed pluripotency in the generation of ESC-embryo chimeras. The efficient production of liveborn MfESC-chimeras will advance the application of the NHP in the area of stem-cell based transplant therapies and model development for human therapeutics discovery.

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F2207

THE ANTI-APOPTOTIC PROTEIN MCL-1 AS A REGULATOR OF PLURIPOTENCY

Park, Kyungho[1], Dumitru, Raluca[2], Deshmukh, Mohanish[2] and **Gama, Vivian**[1], [1]Vanderbilt University, Nashville, TN, U.S., [2]University of North Carolina at Chapel Hill, NC, U.S.

Mcl-1 is an anti-apoptotic protein that promotes the survival of different cell lineages and it is frequently upregulated in several types of cancer. Mcl-1 anti-apoptotic function relies on its ability to inhibit Bax activation, the main mediator of cell death in mammalian cells. We previously reported that human embryonic stem (hES) cells differentially regulate the apoptotic machinery by maintaining Bax in its active conformation at the Golgi rather than at the mitochondria. This allows hES cells to effectively minimize the risks associated with having pre-activated Bax and makes them acutely sensitive to DNA damage. While investigating the role of Mcl-1 in inhibiting active Bax in hES cells, we found that Mcl-1 has an additional role regulating pluripotency of hES cells. Mcl-1 localizes to the outer mitochondrial matrix where it antagonizes Bax, but is also localized to the matrix, where it is believed to regulated cellular metabolism. Our data demonstrate that inhibiting Mcl-1 induces the dramatic loss of Nanog and Oct-4,

promoting the rapid differentiation of hES cells. We also show that overexpressing a truncated form of Mcl-1 which localizes exclusively at the mitochondrial matrix, partially rescues the loss of pluripotency markers. This data supports the idea that Mcl-1 may be required to support the metabolic requirements of pluripotency. Our findings suggest that Mcl-1 is an important mediator of pluripotency in hES cells, and suggests an unexpected non-apoptotic function for Mcl-1 in the maintenance of pluripotency.

F2209

MOUSE EMBRYONIC STEM CELLS CULTURED IN STIRRED-SUSPENSION ENVIRONMENT EXHIBIT SPATIALLY DISTINCT DIFFERENCES IN PLURIPOTENCY MAINTENANCE AT THE AGGREGATE LEVEL

Hsu, Charlie Yu-Ming[1,2] and Rancourt, Derrick E.[1,2], [1]University of Calgary, AB, Canada, [2]McCaig Institute for Bone and Joint Health, Calgary, AB, Canada

Pluripotent stem cells (PSC) have the capacity for long-term self-renewal and multi-lineage differentiation, making them the ideal platform for producing a wide range of tissue-specific cells for clinical applications. The demand for PSC is expected to increase and we have developed methods for the large-scale derivation and expansion of PSCs in a stirred suspension bioreactor (SSB). However, controlling the fate of PSC has been challenging as we have found that, after directed differentiation into tissue-specific lineage, a sub-population of cells remain undifferentiated. Modulating stem cell response in the bioreactor is complicated by the spatial and temporal heterogeneity in the hydrodynamic environment of the stirred-suspension culture and the microenvironment of the multi-cellular spheroid. When cultures are first inoculated with single-cell suspension at the time of seeding, cells are primarily subjected to fluid shear stress created by internal agitation of the impeller. However, as cells grow and aggregate into multi-cellular spheroid, shear stress becomes localized to the aggregate surface, while transport of nutrient to the inner region of the aggregate become limited, which would result in a transport gradient that would ultimately lead to differential cellular responses between the inner and outer regions of the aggregate. To test this, we developed a modified approach to dissociate aggregates into single cell suspension. Because cells on the outer layers of the aggregate get dissociated into suspension first, by performing repeated rounds of partial dissociation and separating the supernatant from each successive round into different fractions, aggregate can be gradually peeled away, with each fraction representing a spatially distinct section of the aggregate. We applied this approach to probe for pluripotency expression in mouse ESC aggregates cultured in a stirring flask by FACS. Unexpectedly, we found that Oct4/Sox2 expression were significantly higher in the core of the aggregate when compared to the surface, and that this differential in pluripotency expression widens with increasing stirring speeds. These data suggest that the hydrodynamic environment and the aggregate microenvironment may play a dichotomous role in modulating stem cell pluripotency and differentiation.

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F2211

THE EMBRYOLOGICAL STATUS OF HUMAN PLURIPOTENT STEM CELLS

Lau, Kevin X.[1], Mason, Elizabeth Anne[2,3] and Pera, Martin F.[3], [1]Stem Cells Australia, Parkville, Australia, [2]University of Melbourne, Australia, [3]University of Melbourne, Australia

It is widely held that human pluripotent stem cells (hPSC) propagated under conventional culture conditions closely resemble mouse primed pluripotent stem cells derived from the post-implantation epiblast. However, cellular heterogeneity, particularly evident under non-defined culture conditions (serum, serum replacements, and feeder cells), has obscured the hPSC phenotype. The self-renewing subpopulation of hPSC represents a minority of cells in conventional cultures, and it is endowed with a unique set of key molecular and cell biological characteristics. These properties include high level and uniform expression of key pluripotency markers, a lack of lineage priming, a very brief G1 cell cycle phase, use of oxidative phosphorylation in energy metabolism, and a high capacity for self-renewal. Expression of genes found in the human pre-implantation epiblast is highest in the self-renewing subpopulation, including genes involved in nodal signalling, and markers of the mouse and human naïve pluripotent states including KLF4, ZFP42, NR5A2, PRDM14, ZSCAN10, TFCP2L1, DNMT3B, and others. Defined culture conditions reduce the heterogeneity of hPSC, and increase the proportion of cells in the self-renewing subpopulation, which like the human pre-implantation epiblast, is highly dependent upon activin/nodal signalling, Addition of the protein kinase C inhibitor Go 6983 to defined mTeSR medium containing activin and FGF2 further promotes the maintenance of the self-renewing subpopulation. The existence of a distinct subpopulation of cells in conventional hPSC cultures that co-expresses markers of primitive endoderm and pluripotency is not easily reconciled with later post-implantation epiblast identity. Thus, the self-renewing subpopulation of conventional hPSC bears considerable similarity to the human pre-implantation epiblast; attempts to define a human state of naïve pluripotency should take into consid-

eration the heterogeneity in stem cell cultures, and should be performed with reference to the human embryo.

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F2213

WHOLE GENOME CRISPR SCREEN DURING NAIVE TO PRIMED HESC TRANSITION REVEALS KEY REGULATORS OF IMPLANTATION STAGE EMBRYO

Mathieu, Julie[1], Kuppers, Daniel[2], Cavanaugh, Christopher Charles[1], WANG, Yuliang[3], Detraux, Damien[1], Fischer, Karin A.[1], Sidhu, Sonia[1], Ware, Carol B.[1], Paddison, Patrick[2] and Ruohola-Baker, Hannele[1], [1]University of Washington, Seattle, WA, U.S., [2]Fred Hutchinson Cancer Research Center, Seattle, WA, U.S., [3]The Chinese University of Hong Kong, Shatin, Hong Kong

High percentage of pregnancy loss occurs during the early steps of embryonic development, however, very little is known about these stages. Early human development is poorly understood due to the unavailability of primary tissue, requiring the use of human cell cultures that represent this window. Two stable pluripotent stages have been derived in mouse and human; preimplantation naïve and postimplantation primed embryonic stem cell (ESC) stages. The naïve and primed hESC are different in terms of signaling dependence, gene expression, epigenetic features, metabolic requirement and developmental potential. To determine the genes required for the naïve to primed transition, we performed a whole genome CRISPR-KO screen, using lentiviral delivery of a genome-scale CRISPR-Cas9 knockout (GeCKO) library in naïve hESC. Cells were allowed to develop to the primed hESC state and the primed hESC were selectively killed due to their metabolic requirements. The enriched guide-RNAs in the surviving cells correspond to the genes required for the transition. Using this functional screen we uncovered novel regulators of naïve-to-primed transition involved in metabolic switching, signaling and chromatin remodeling. In a secondary screen, we validated genes from the three classes, including CREB1, PKLR, GPR161, NELL2, MAP2K7, FLCN, SMARCD2 and SUV4-20H2 as genes required for the transition. To dissect the mechanisms of GPR161 and SUV4-20H2 action, we tested the differential gene expression signatures between the mutant and control lines using RNA seq and bioinformatics platforms. Principal component analysis revealed that while after 7 days of culture in TeSR, naïve cells have moved toward the primed state, GPR161 KO and SUV4-20H2 KO cells were not able to transition, still clustering with the naive hESC. GPR161 is a repressor of Hedgehog (Hh) pathway that localizes in primary cilium. In mouse embryo the first primary cilia arise in the post-implantation epiblast. Similarly, we observed that primed, but not naïve hESC possess primary cilia. Genome wide RNA seq revealed that Hh pathway targets are significantly upregulated in GPR161 KO mutant compared to control naïve hESC. These data suggest that regulation of Hh pathway plays an important role in the naïve to primed hESC transition, and thereby human embryonic implantation.

F2215

UNDERSTANDING MECHANISMS OF PLURIPOTENCY THROUGH MULTI-OMIC NETWORK ANALYSIS OF 68 STEM CELL LINES DIFFERENTIATED TO THREE GERM LAYERS

Perumal, Thanneer Malai[1], Daily, Kenneth[1], Pantano, Lorena[2], Dexheimer, Phillip[3], Huo, Jeffrey[4], Lutzko, Carolyn[5], Zambidis, Elias[6], Mayhew, Christopher N.[7], Wells, James M.[8], Hide, Winston[9], Cancelas, Jose[5], Hatzopoulos, Antonis[10], Malik, Punam[11], Ho Sui, Shannan J.[2], Aronow, Bruce[12], Salomonis, Nathan[3] and Omberg, Larsson[1], [1]Sage Bionetworks, Seattle, WA, U.S., [2]Harvard School of Public Health, Boston, MA, U.S., [3]Cincinnati Children's Hospital Medical Center, Cincinnati, OH, U.S., [4]Johns Hopkins School of Medicine, Baltimore, MD, U.S., [5]Cincinnati Children's Hospital, Cincinnati, OH, U.S., [6]Johns Hopkins School of Medicine, Baltimore, MD, U.S., [7]Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, U.S., [8]Cincinnati Childrens Hospital, Cincinnati, OH, U.S., [9]Harvard Stem Cell Institute, Cambridge, MA, U.S., [10]Vanderbilt University Medical Center, Nashville, TN, U.S., [11]Cincinnati Children's Hospical Medical Center, Cincinnati, OH, U.S., [12]Cincinnati Children's Hospital Research Foundation, Cincinnati, OH, U.S.

Pluripotency, the ability of cells to give rise to any cell of the embryo proper, is a complex regulatory event that is carefully orchestrated by transcriptional and epigenetic changes. Understanding the mechanism of pluripotency helps gain deeper insights in human development and disease modeling, and has applications in regenerative medicine. Although substantial progress has been made in developing distinct experimental techniques for re-establishing pluripotency in somatic cells, these methods have neither been systematically benchmarked nor the determinants of pluripotency rigorously characterised. To address these challenges, the Progenitor Cell Biology Consortium (PCBC) has systematically characterised 68 pluripotent stem cell lines derived from 18 different reprogramming methodologies to 3 germ layers and embryoid bodies. These pluripotent stem cell lines have undergone cellular phenotyping and molecular characterization with RNA-seq, miRNA-seq, and array-based DNA methylation assays. After controlling for technical variables using mixed effects modeling, differential expression and differential co-expression network analyses were performed. An integrated network analysis between multiple types

of genomic assays identify functional modules of transcriptional and epigenetic changes that are orchestrated together to establish lineage-specific differentiation. Specifically, we identify miRNA and DNA methylation regulations of mRNA that describe putative regulatory mechanisms of pluripotency in stem cells and differentiated cells. Furthermore, these analyses identified sub-modules that corresponds to experimental design choices including culture conditions and method/origin of reprogramming. In addition, we observed gender differences in stem cells and embryonic bodies, and differences between endoderm derived from hESC and iPSCs. In summary, we present our observations of the pluripotent specific transcriptional and epigenetic machinery including differentially (co)-expressed genes, miRNAs, and methylated DNA probes.

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F2217

A DPPA4-INTERACTING PROTEIN NETWORK IN PLURIPOTENT CELLS AND CANCER

Somanath, Priyanka, University of California, Davis, Sacramento, CA, U.S. and Knoepfler, Paul, UC Davis, Sacramento, CA, U.S.

Stem cells have strong potential in regenerative medicine; however, one of the main roadblocks remaining in applying stem cell-mediated therapies in the clinical setting is the link between pluripotency factors and their contribution to tumorigenesis. In order to understand these oncogenic events in pluripotent cells, elucidation of the molecular machinery that governs pluripotency and its relationship to cancer is essential. Developmental Pluripotency-Associated (DPPA4) is a component of the pluripotency machinery that functions in both pluripotent stem and cancer cells. While the expression of DPPA4 is normally restricted to embryonic stem cells (ESCs), DPPA4 is expressed in a variety of embryonic and adult cancers and our lab has found that it can act as a strong oncogene. In development, DPPA4 is essential for early embryogenesis. However, apart from this, the cellular and molecular functions of DPPA4 protein are mostly unknown. Using a proteomics-based approach, we have identified novel DPPA4 cofactors in human ESCs. Interestingly, these cofactors have been of interest in cancer research due to their contributing role in tumorigenesis, but our work points to a novel role for these key signaling molecules in pluripotency via DPPA4. Furthermore, both DPPA4 and these cofactors have elevated expression in embryonic carcinoma cells compared to ESCs, indicating that their interaction could have a substantial role in tumorigenesis of pluripotent cells. Our studies have determined that human and mouse DPPA4 proteins can preferentially interact with these cofactors in both ESCs and pluripotent cancer cells, but this interaction is absent in normal fibroblasts, indicating a strong potential function in promoting the shared molecular characteristics between pluripotency and oncogenesis. Overall, our data suggest that this DPPA4 protein complex functions through integrated signaling and epigenetic pathways known to play a prominent role in cancer.

F2219

NORMAL TELOMERE LENGTHS RESTORED IN NTESCS DERIVED FROM TELOMERASE HAPLO-INSUFFICIENT MOUSE CELLS BY SERIAL CLONING

Xu, Jie[1], Chang, Wei-Fang[2], Wang, Shen-Wen[2], Ou-Yang, Huan[2] and Sung, Li-Ying[2], [1]University of Michigan Medical Center, Ann Arbor, MI, U.S., [2]National Taiwan University, Taipei, Taiwan

Mutations of genes that encode telomerase components cause dysfunctional telomeres in the individual's cells which lead to premature aging and age related diseases, including aplastic anemia, dyskeratosis congenital, and idiopathic pulmonary fibrosis, collectively referred to as telomere syndromes. Heterozygous mutations of TERC represents a major mutation in genetically defined telomere syndromes. It has been a challenge to establish patient-specific induced pluripotent stem (iPS) cells with proper restoration of telomere defects using telomerase haplo-insufficient patient cells. Somatic cell nuclear transfer (SCNT) is another major means to derive patient-specific stem cells (i.e. ntESCs). We previous reported that telomeres are robustly elongated in mouse Terc$^{+/-}$ ntESCs as compared to their donor cells. In this study, we investigated whether telomeres of Terc$^{+/-}$ ntESCs can be further elongated by additional rounds of SCNT (i.e. serial cloning). Relative telomere content/length (RTL) was measured as relative telomere to single copy gene (T/S) ratio by qPCR. After one round SCNT, we established the first generation Terc$^{+/-}$ ntESCs (G1 ntESCs), whose RTLs are significantly elongated (0.92±0.20) from those in the donor Terc+/- TTFs (0.61). Using G1 ntESCs as donor cells, we conducted a second round SCNT and achieved satisfactory cloned embryo development, with morula and blastocyst rates of 62.4% (53/85) and 50.6% (43/85), respectively, and established multiple lines of second generation Terc$^{+/-}$ ntESCs (G2 ntESCs), all of which expressed genes and antigens characteristic of pluripotent stem cells. Remarkably, telomeres in G2 ntESCs were further elongated to 1.64±0.37, not only significantly longer than those in the TTF donor cells (0.61) and G1 ntESCs (0.92), but reached a level similar to those of WT ntESCs (1.46). The present work indicates that serial cloning, for as few as two rounds, is capable to restore short telomeres in telomerase haplo-insufficient cells to a length of the WT cells. Further work will determine if this serial cloning

strategy has the capacity to fully restore telomeres of telomerase null (e.g. $Terc^{-/-}$) cells.

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F2221

A DYNAMIC GENE EXPRESSION PROFILING OF MOUSE EMBRYONIC STEM CELL DERIVATION FROM BLASTOCYST

Totonchi, Mehdi[1,2], Hassani, Seyedeh-Nafiseh[1], Sharifi-Zarchi, Ali[1], Tapia, Natalie[3], Adachi, Kenjiro[4], Araúzo-Bravo, Marcos J.[3], Greber, Boris[3], Sabour, Davood[3], Gourabi, Hamid[5], Schöler, Hans R.[3] and **Baharvand, Hossein**[1,2], [1]Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran, Tehran, Iran, [2]Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran., Tehran, Iran, [3]Department of Cell and Developmental Biology, Max Planck Institute for Molecular Biomedicine, Münster, Germany, Muenster, Germany, [4]Max Planck Institute for Molecular Biomedicine, Muenster, Germany, [5]Department of Genetics, Cell Science Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Mouse embryonic stem (ES) cells are derived from inner cell mass of 3.5-day blastocysts and thought to exist in a naïve state of pluripotency. During ICM-ES cell course, a normal developmental program is perpetuated as an infinite self-renewal and pluripotency in vitro. Here, we have examined a dynamic expression profiling within the intermediate stages of ES cell derivation from the ICM. The results revealed two major changes during this cell transition, which suggested that genes expressed on day three ICM-outgrowths played a prominent role in the establishment of the ES cells. Gene clustering and ontology (GO) analyses showed a significant change in the expression of epigenetic modifiers and DNA methylation-related genes in the intermediate stages. This study provides a time-course transcriptome profiling resource for the dissection of gene regulatory networks that underlie the transition from ICM to ES cells.

EMBRYONIC STEM CELL CLINICAL APPLICATION

F2225

CULTURE CONDITIONS TO PRODUCE HEPATOCYTE LIKE CELLS FOR TRANSPLANTATION

Geti, Imbisaat[1], de Brito, Miguel[1], Vitilo, Loriana[1], Saeb-Parsy, Kourosh[2] and Vallier, Ludovic[1,3], [1]Wellcome Trust - Medical Research Council Stem Cell Institute, Anne McLaren Laboratory, Department of Surgery, University of Cambridge, UK, Cambridge, U.K., [2]Department of Surgery, University of Cambridge and NIHR Cambridge Biomedical Research Centre, Cambridge, U.K., [3]Wellcome Trust Sanger Institute, Hinxton, UK, Cambridge, U.K.

Liver transplantation is the only treatment for end-stage liver diseases and the lack of suitable organ limits the number of patient who could benefit from this life saving treatment. Cell-based therapy using primary hepatocytes could address this growing health care challenge. However, primary hepatocytes are difficult to obtain in sufficient numbers. Indeed, they can only be obtained from organ donation while they cannot be expanded *in vitro*. Consequently, alternative source of hepatocyte are urgently needed. Human pluripotent stem cells (hPSCs) can self renew almost indefinitely *in vitro* while maintaining their capacity to differentiate into diverse cell type including hepatocytes like cells (HLCs). Thus, hPSCs could be used to produce large quantity of cells necessary for cell based therapy approaches in the context of liver diseases. However, the current protocols are not compatible with Good Manufacturing Practice (GMP) and thus, the resulting HLCs can not be transplanted. Furthermore, the safety and efficacy of these cells need to be demonstrated by systematic studies *in vivo*. Here, we describe a method to produce HLCs in conditions compatible with clinical applications. For that, we have established a protocol to differentiate hPSCs into homogenous population of HLCs compatible with clinical applications. Each reagent included in this protocol has been successfully translated into GMP grade materials. Furthermore, we showed that the GMP compliant protocol was equally robust as research grade protocol to produce HLCs displaying key characteristics of their in vivo counterparts including Albumin secretion, expression of a range of hepatic markers and also Cyp activity. Importantly, the efficacy of this new GMP protocol was validated on five hPSC lines, including three GMP grade hESC lines. Finally, we have also characterized the resulting cells in vivo and demonstrated their safety after transplantation in immune deficient mice. Considered together, these results show that production of HLCs in GMP like condition is feasible and that the resulting cells could be useful for cell based therapy in the context of

liver diseases. This study provides a new platform for production of hPSCs derived HLCs under safe GMP conditions and represent an essential step toward fist in man clinical trial.

F2227

COMPARISON OF DEFINED FEEDER-FREE CULTURE SYSTEMS FOR THE MAINTENANCE OF EUTCD-COMPLIANT HUMAN EMBRYONIC STEM CELL LINES

Man, Jennifer Sui-Sum, Nowell, Craig, Hunt, Charles and Stacey, Glyn, UK Stem Cell Bank, National Institute for Biological Standards and Control, Hertfordshire, U.K.

Validated protocols for the expansion of human embryonic stem cells (hESCs) within the UK Stem Cell Bank (UKSCB) include the use of inactivated feeders as well as the feeder-free alternative Matrigel-mTeSR™2 combination. These approaches, however, include the use of animal-derived products, undefined serum and other components that may pose a risk of pathogen transmission as well as carrying inherent batch-to-batch variability, which may ultimately result in non-desirable changes in cellular function. These issues become even more pertinent when hESCs with potential clinical applications are being expanded, and therefore the development of a more standardised and well-defined approach to culturing hESCs with product safety and reliability in mind becomes crucial. Since the UKSCB is currently preparing to derive seed stocks of clinical-grade hESCs, a pilot study to generate feeder-free protocols for the cultivation of EUTCD-compliant hESCs has been performed. A meta-analysis of published methods was initially conducted to identify candidate media and matrices and 14 different culture systems have been selected. Using the well-characterised cell line H9, these commercially available media and matrices combinations were assessed for their ability to maintain viable and functional hESCs, which will retain their stem cell characteristics over multiple passages. In this pilot study, cells were morphologically examined for 10 serial passages and were assessed for differentiation potential by embryoid body formation, proliferative capacity, karyotypic stability and expression of known pluripotency markers by qPCR and multi-colour flow cytometry. Of the 14 different culture systems analysed, three have been identified to consistently outperform all other combinations in the maintenance of undifferentiated hESCs without loss of functional characteristics. Furthermore, hESCs maintained on these systems were able to quickly transition from feeder-dependent to feeder-free conditions as well as maintaining a shorter doubling time without altering chromosomal stability. These final three culture systems will now be introduced into Phase II of in-house testing, which will consist of testing these combinations against several clinically relevant hESC lines.

CANCER CELLS

F2229

EXPANDED POPULATION OF BREAST CANCER STEM CELLS(BCSC) WITHIN TREATMENT NAIVE HUMAN BREAST TUMORS ELUCIDATES AGGRESSIVE BEHAVIOR OF HISTOPATHOLOGICALLY AND MOLECULARLY DEFINED HIGH GRADE TUMORS

Dhanota, Ninjit, Arora, Sunil Kumar, Singh, Gurpreet and Bal, Amanjit, , Postgraduate Institute of Medical Education and Research, Chandigarh, India

There is certain degree of similarity in terms of molecules & underlying machinery used by normal stem cells for homing or mobilization and BCSCs for invasion, migration & metastasis. Defining aggressiveness of tumors based on proliferation, invasion, tumorigenic & metastatic potential of BCSCs helps in defining clinical outcome of disease. In present study we are primarily trying to correlate aggressiveness of breast cancer to enrichment of BCSCs in tumor microenvironment using clinical, molecular & histopathological parameters, with an aim to use this as one of the parameters for better management of these tumors. Primary tumor samples from 37 chemotherapy naïve breast carcinoma female patients were included in study. BCSCs were identified with help of cell surface markers $CD44^{+}$ & $CD24^{-/low}$ through flowcytometery. Sorted BCSCs were characterized with help of functional assays like mammosphere formation, side population assay & Immunohistochemistry staining for Aldehyde Dehydrogenase 1 A1(AlDH1A1). Percentage of BCSCs was compared with histopathological grade & molecular stage of tumor in each patient. Higher percentage of BCSCs was observed in histopathologically defined aggressive tumors (Grade II{n=13} &Grade III{n=19}) as compared to Grade I{n=5}tumors. A statistically significant median value (0.027) was obtained when we compared BCSCs in grade I and grade II tumors. No. of BCSCs didn't correlate well with metastatic lymph node, tumor size, presence or absence of lymphovascular emboli & proliferative index. Percentage of BCSCs was found to be higher in Triple Negative(TNBC) and HER 2+ cases(molecularly aggressive tumors). High immunohistochemistry score for AlDH1A1 was reported in higher grade tumors (Grade III&II{IHC score2=8cases,IHC score3=2cases}) than grade I tumors(IHC Score ≤ 1). AlDH1A1 scoring showed significant (0.0522) difference in low grade (Grade I) & high grade(II & III) tumors. Our study indicates that higher no. of BCSCs in tumor tends to associate positively with aggressive behavior of tumor. Higher no. of BCSCs in TNBC & HER2+ cases gives us cellular evidence of aggressive behavior of these molecular subtypes. However, a larger study group may prove to be helpful in predicting behaviour of a tumor in a particular

patient by providing relevant information based on the no. of BCSCs.

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F2231

ACTIVATION OF AURORA A KINASE THROUGH THE FGF1/FGFR SIGNALING AXIS SUSTAINS THE STEM CELL CHARACTERISTICS OF GLIOBLASTOMA CELLS

Hsu, Yi-Chao, Mackay Medical College, New Taipei City, Taiwan

Fibroblast growth factor 1 (FGF1) binds and activates FGF receptors, thereby regulating cell proliferation and neurogenesis. Human FGF1 gene 1B promoter (-540 to +31)-driven SV40 T antigen has been shown to result in tumorigenesis in the brains of transgenic mice. FGF1B promoter (-540 to +31)-driven green fluorescent protein (F1BGFP) has also been used in isolating neural stem cells (NSCs) with self-renewal and multipotency from developing and adult mouse brains. In this study, we provide six lines of evidence to demonstrate that FGF1/FGFR signaling is implicated in the expression of Aurora A (AurA) and the activation of its kinase domain (Thr288 phosphorylation) in the maintenance of glioblastoma (GBM) cells and NSCs. First, treatment of FGF1 increases AurA expression in human GBM cell lines. Second, using fluorescence-activated cell sorting, we observed that F1BGFP reporter facilitates the isolation of F1BGFP(+) GBM cells with higher expression levels of FGFR and AurA. Third, both FGFR inhibitor (SU5402) and AurA inhibitor (VX680) could down-regulate F1BGFP-dependent AurA activity. Fourth, inhibition of AurA activity by two different AurA inhibitors (VX680 and valproic acid) not only reduced neurosphere formation but also induced neuronal differentiation of F1BGFP(+) GBM cells. Fifth, flow cytometric analyses demonstrated that F1BGFP(+) GBM cells possessed different NSC cell surface markers. Finally, inhibition of AurA by VX680 reduced the neurosphere formation of different types of NSCs. Our results show that activation of AurA kinase through FGF1/FGFR signaling axis sustains the stem cell characteristics of GBM cells. This study identified a novel mechanism for the malignancy of GBM, which could be a potential therapeutic target for GBM.

F2233

ACQUISITION OF TUMORIGENIC POTENTIAL AND ENHANCEMENT OF ANGIOGENESIS IN PULMONARY STEM/PROGENITOR CELLS THROUGH OCT-4 HYPEREXPRESSION

Gu, Sing-Yi, and **Ling, Thai-Yen**, Institute of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan

Cancer stem cells, also known as cancer initiating cells (CICs), are considered to be responsible for tumor growth and chemoresistance. Different hypotheses have been proposed to explain the origin of CICs, including mutations in adult stem/ progenitor cells or the acquisition of stem-like characteristics in differentiated cells; however, studies have yielded conflicting identification for CICs and have little information for the origin to generate CICs. Part of the difficulty in identifying CICs may stem from the fact that the CICs studied have been largely derived from cancer cell lines or well-developed tumors. In previous studies, we have reported the enrichment of mouse pulmonary stem/progenitor cells (mPSCs) by using serum-free primary selection culture followed by FACS isolation using the coxsackievirus/ adenovirus receptor (CAR) as the positive selection marker. Here, we demonstrated that overexpression of the pluripotent transcription factor Oct-4 is sufficient to induce CAR+/ mPSCs transformation, which we name CAR+/mPSCsOct-4_hi. These transformed cells possess cancer initiating and chemoresistance potential, as well as exhibiting remarkable expression of certain proangiogenic factors, including angiopoietins (ANGs) and VEGF, and enhanced angiogenic potential. Moreover, CAR+/mPSCsOct-4_hi actively participated in tumor blood vessel formation and triggered a novel angiogenic mechanism, the angiopoietins/Tie2 signaling pathway. These study provide critical evidence supporting the possible origin to generate CICs, and help elucidate the pathways responsible for CICs-mediated blood vessel formation.

F2235

REPURPOSING A CLASSIC CHEMOTHERAPEUTIC DRUG TO A TARGETED THERAPY ELIMINATES CHEMORESISTANT LEUKEMIA-INITIATING CELLS FROM DIVERSE HUMAN LEUKEMIAS

Perry, John[1], He, Xi. C.[1], Tao, Fang[2], Roy, Anuradha[3], Lu, Xiuling[4], Zhao, Meng[1], Venkatraman, Aparna[1], Qian, Pengxu[1], Weir, Scott[3], Sittampalam, G. Sitta[5], Lin, Tara[6], Ryan, Robin[7], Guest, Erin[7], August, Keith[7], Gamis, Alan[7], Perez, Raymond[6] and Li, Linheng[2], [1]Stowers Institute for Medical Research, Kansas City, MO, U.S., [2]Stowers Institute for Medical Research, Kansas City, MO, U.S., [3]University of Kansas, Lawrence, KS, U.S., [4]University of Connecticut, Storrs, CT, U.S., [5]National Institutes of Health, Rockville, MD, U.S., [6]University of Kansas Medical Center, Kansas City, KS, U.S., [7]Children's Mercy Hospital, Kansas City, MO, U.S.

Cancer treatment strategies are shifting from non-specific, cytotoxic drugs to mechanism-based, targeted therapies, but traditional chemotherapy remains the mainstay of treatment despite a deficiency in understanding how these treatments exert their effects. Doxorubicin (DXR) has long served as a backbone for chemotherapy and has the broadest spectrum of anti-cancer activity known. However, its use is limited by severe side-effects and its mechanism of action is controversial. By studying oncogenic self-renewal, our research has illuminated this mechanism and a path to use this drug in a targeted, more effective way. By activating the Wnt/β-catenin and PI3K/Akt signaling pathways, we have characterized a mouse model with aberrant self-renewal activity that progresses to leukemia via leukemic stem cell (LSC) development. Since Akt activates β-catenin by phosphorylation at serine 552 and thus represents a molecular link mediating the synergistic, self-renewal promoting activity of these pathways, we sought to specifically target pS^{552}-β-catenin. High-throughput screening (HTS) and validation assays found that DXR inhibits pS^{552}-β-catenin with minimal effects on total β-catenin. Standard chemotherapeutic treatment reduced the bulk leukemic blast cell population as expected but also induced pS^{552}-β-catenin uniquely in LSCs and stimulated LSC expansion. Using long-circulating nanoparticles to deliver very low, sustained doses of DXR (NanoDXR), we repurposed DXR as a targeted therapy for pS^{552}-β-catenin inhibition rather than a broadly cytotoxic agent. In vivo, this treatment reduced pS^{552}-β-catenin levels in LSCs, prevented LSC expansion, essentially eliminated tumorigenic activity in transplant recipients, and substantially increased survival in the animal model. Using patient samples, we found that more than half of human acute myeloid, T and B-lymphoid leukemias exhibit rare pS^{552}-β-catenin+ cells. Chemoresistant cells from human B and T-ALLs are enriched in pS^{552}-β-catenin+ cells and low-dose NanoDXR treatment can eliminate or significantly reduce tumorigenic cells in xenograft recipients. Our data reveals how understanding the mechanism of action for long-used chemotherapies can reduce or eliminate off-target effects while enhancing anti-cancer activity.

F2237

CYCLIN D1-SMAD2/3-SMAD4 SIGNALING PROMOTES LIVER CSC SELF-RENEWAL AND CSC DIFFERENTIATION BY SMAD INHIBITOR IS A KEY STAGE FOR CHEMOSENSITIZATION

Wang, Xiao Qi and Xia, Wei, University of Hong Kong Department of Surgery, Hong Kong, China

Targeting cancer stemness and induction of differentiation are the new directions for cancer therapy. We investigated the role of cyclin D1-SMAD2/3-SMAD4 signaling pathway in liver cancer stem cells (CSCs) and its potential as therapeutic target. Cyclin D1 dependent phosphorylation of SMAD2/3 and enhanced expression of SMAD4 were detected in HCC spherical cells as well as in over 50% of primary HCC tumor tissues. HCC patients with high levels of cyclin D1, pSMAD2/3, and SMAD4 together displayed a poor overall survival. Overexpression of cyclin D1 conferred liver cancer cells CSC features such as capacity of single sphere formation, increased CD90+ and EpCAM+ liver CSC population, enhanced pluripotency-associated gene and metabolic glycolysis gene expression, which was via activation of SMAD2/3 and SMAD4. Cyclin D1-SMAD2/3-SMAD4 signaling-regulated cancer spheres also showed enhanced epithelial-mesenchymal transition (EMT) phenotype and highly resistant to chemotherapy. Application of TGF-b/SMAD inhibitor (SB431542) inhibited CSC sphere growth moderately although the inhibition was specifically in cyclin D1-expressing but not in parental spheres (a further proof of cyclin D1-mediated activation of SMADs). However, pre-treated with low dose of SMAD inhibitor induced cancer spheres differentiation leading to significant chemosensitization. The same strategy (pre-treatment with low dose of SB431542 followed by cisplatin regularly) significantly decreased tumorigenicity in cyclin D1 sphere-derived xenograft tumor model, with 8 out of 14 (57%) cell injections were tumor free at endpoint, and the formed tumor was only 24% of tumor size of vehicle or cisplatin groups. Interestingly, xenograft tumor was unaffected when applying SMAD inhibitor and cisplatin simultaneously. Mechanistically, SMAD inhibitor reduced CD90+, EpCAN+, and CD133+ liver CSC populations; reduced stemness gene and glycolytic gene expression; reversed EMT phenotype; all these resulting in impaired self-renewal and induced differentiation, which is an effective step for reversing chemoresistance. Together, cyclin D1-SMAD2/3-SMAD4 signaling pathway promotes liver cancer CSC self-renewal. The induction of

CSC differentiation by TGF-b/SMAD inhibitor followed by chemotherapy provides a new path for combined therapy.

F2239

SINGLE CELL MRNA QUANTIFICATION FROM 1000S OF CELLS IN HEALTHY AND MALIGNANT TUMOR SAMPLES USING A HIGH-THROUGHPUT DROPLET-BASED SYSTEM

Zheng, Grace, 10X Genomics Inc, Pleasanton, CA, U.S.

Understanding the transcriptional landscape of individual cells, rather than bulk tissue, is fundamental to understanding complex biological systems such as the immune system and heterogeneous tumors. Current single cell RNA-sequencing methods are limited by their reliance on costly infrastructure and laborious experimental protocols. We developed a platform that combines microfluidics with molecular barcoding and custom bioinformatics software to enable 3' mRNA counting of up to 48,000 single cells in each 10-minute run. We demonstrated the superior performance of our platform by profiling >100,000 peripheral blood mononuclear cells (PBMCs) from healthy donors and successfully detected all major subpopulations at expected proportions. Single cell profiles from frozen PBMC samples were also highly correlated to those of fresh PBMCs, making the platform suitable for profiling archived patient samples. We collected and compared >100,000 single cell transcriptomes of frozen PBMCs and bone marrow mononuclear cells (BMMCs) from healthy donors and leukemia patients. Acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) samples showed expansion of myeloid population, specifically the myeloid progenitors in AML, and more mature monocytes in CML. Comparison of diseased myeloid population to their healthy counterparts suggested enriched gene signatures associated with pathways linked to poor prognosis. These signatures were not present in comparisons of other populations, or when the transcriptome profiles were treated as bulk, highlighting the importance of being able to select specific populations of cells in single cell analysis. In contrast to AML and CML samples, chronic lymphoid leukemia (CLL) BMMCs are characterized by expansion of B cells that show abnormal cell cycle and cell death profiles relative to healthy BMMCs. Based on these results, we believe that our single cell platform and integrated mRNA analysis will lead to novel insights into the molecular characteristics of individual cancer cells and provide targets for therapeutic intervention.

TECHNOLOGIES FOR STEM CELL RESEARCH

F3001

ANALYSIS OF THE PHENOTYPICAL EFFECT OF PARKINSON'S DISEASE-ASSOCIATED MUTATIONS IN AN ISOGENIC HUMAN CELL MODEL

Ahfeldt, Tim[1], Jowett, Geraldine[1], Bell, Christina[2], Ximerakis, Methodios[1], Ordureau, Alban[2], Harper, Wade[3] and Rubin, Lee[4], [1]Harvard University, Cambridge, MA, U.S., [2]Harvard Medical School, Boston, MA, U.S., [3]Harvard Stem Cell Institute, Cambridge, MA, U.S., [4]Harvard University Department of Stem Cell and Regenerative Biology, Cambridge, MA, U.S.

Parkinson's disease (PD) is a neurodegenerative disease which disproportionately affects dopaminergic neurons of the midbrain in the substantia nigra. PD is a complex disease, with both environmental as well as genetic risk factors underlying the disease pathology. Utilizing CRISPR/Cas9 technology, we have created several human pluripotent stem cells lines (hPSCs) that harbor a knock-in fluorescence reporter driven by the endogenous Tyrosine Hydroxylase (TH) promoter. TH catalyzes the rate limiting step in the enzymatic synthesis of dopamine in midbrain dopaminergic neurons. Our targeting strategy keeps the endogenous gene product intact, while simultaneously delivering a very bright fluorescent signal. Furthermore, we have used CRISPRs in knockout mutagenesis experiments to create isogenic disease lines of several PD associated mutations. For instance, we have knocked out genes associated with the proteins PARKIN (PARK2), DJ1 (PARK7) and ATP13A2 (PARK9). We use these mutant cell lines to generate midbrain neural progenitor cells (mNPCs) and midbrain dopaminergic (mDA) neurons, using a widely accepted differentiation protocol that we have adapted to large scale spin-culture conditions at comparable efficiencies. Fluorescence-activated cell sorting (FACS) of the resulting TH positive cells allows us to generate almost pure mDA populations that are instrumental in the dissection of cell-autonomous from non-autonomous phenotypes. We are currently exploring disease mechanisms in all our lines. For example, in oxidative cell stress assays we found that isogenic disease lines are more sensitive to cell death after exposure to oxidative stress treatment. We have determined that Parkin is highly expressed in differentiated mDA neurons when compared to hPSCs or mNPCs. We analyzed mitophagy in the PARKIN and DJ1 knockout cell lines, a process that has been proposed to play a role in PD disease pathology. By combining our isogenic disease model with the ability to analyze pure populations of TH positive cells through functional assays, global transcriptional profiling and quantitative proteomics, we have cre-

ated a new experimental system to study PD disease pathology, allowing us to elucidate the mechanistic basis of neurodegeneration in mDA neurons.

Funding Source: The Harvard Stem Cell Institute, Biogen

F3003

INNOVATIVE CULTURE OF HIPSCS IN SUSPENSION ON ALGINATE-MICROCARRIER

Bur, Stephanie, Schmidt, Katharina, Schulz, André, Gentile, Luca, Neubauer, Julia and Zimmermann, Heiko, Fraunhofer IBMT, Sulzbach, Germany

Human pluripotent stem cells (hiPSCs) are one of the most promising options for regenerative medicine and tissue engineering. To provide the needed mass of high-quality hiPSCs, technologies and methodologies have to fundamentally evolve, not only to meet the clinical guidelines, but also to make therapies available to all patients via reduction of costs. To overcome these limitations, we developed a new technique for culturing hiPSCs in suspension on alginate-microcarrier. Most industrial scale-up systems are based on mechanical stirred suspension, but sensitive cells like hiPSCs were reported to spontaneously differentiate or to grow bad due to the associated high shear stress. To ensure homogeneous suspension and optimal supply of nutrients and oxygen while avoiding high shear forces, we used the Hamilton BioLevitator™ with innovative impeller-free vessels in combination with alginate-microarrier that can be easily brought in suspension because of their density similar to water. Covalent coatings with Matrigel, vitronectin or laminin were established. Moreover, the properties of the alginate-microcarrier (e.g. stiffness, elasticity) are tunable; hence this substrate resembles a physiological environment more than rigid plastic surfaces. After at least five passages no differences were found in hiPSCs cultured in suspension compared to the standard two-dimensional culture in respect to the expression of pluripotency and early differentiation markers. The whole workflow from thawing until banking was performed in this system without implementation of traditional two-dimensional culture; thereby the growth surface area in one vessel was up-scaled (maximal 400 cm^2) allowing the production of high cell mass in a small volume. Furthermore, differentiation on the alginate-microcarrier was possible. In conclusion we present an innovative new system for large-scale expansion and differentiation of hiPSCs in suspension that overcomes the current limitations in accessing high-quality hiPSCs for cell therapies.

Funding Source: BMBF Kmu-innovativ 4 and IMI EBiSC

F3005

SINGLE-CELL DIFFERENTIAL TRANSCRIPTIONAL PROGRESSION ANALYSIS FOR IDENTIFYING EARLY CELL LINEAGE REGULATORS AND MARKERS

Cordero, Pablo and Stuart, Joshua, University of California Santa Cruz, Santa Cruz, CA, U.S.

Identifying early markers and regulators of cell lineage commitment is a difficult but critical task to dissecting developmental and disease process such as differentiation and cancer. Recently, single cell transcriptomic measurements have emerged as promising tools to tackle this challenge by probing cell states with unprecedented resolution. However, it is still unclear how the key regulators and markers involved in the early transitions between these states can be extracted from these data. To address this challenge, we present an analytical framework for noisy single cell transcriptomic measurements, the Single Cell Estimation of States and Transitions (SCEST) methodology, that leverages a parametrization of Gaussian mixtures across high-dimensional curves to accurately describe the progression of cell states from single cell expression. The models inferred by SCEST allow for comparison of differential expression and regulatory network trends across all genes throughout the biological progression. When analyzing data in which one parental cell state transitions to a daughter state (e.g. during differentiation), these trends pinpoint early transition regulators. On the other hand, when analyzing transitions between a parental state and its children, the trends reveal early markers for each state. We illustrate the first scenario using single-cell neuronal differentiation data, where SCEST uncovers the DNA-binding protein SATB2 as an early regulator in the transition from neuronal progenitor to neuron. For the second scenario, we use single cell data of early hematopoiesis, where SCEST reveals early markers for the megakaryocyte-erythroid and multipotent progenitor cells in their differentiation from hematopoietic stem cells.

Funding Source: This research was made possible by a Center of Excellence for Stem Cell Genomics grant (GC1R-06673-C) from the California Institute for Regenerative Medicine.

F3007

AN EFFECTIVE IN VIVO IMAGING AND TRACKING METHOD OF TRANSPLANTED HUMAN PLURIPOTENT STEM CELLS-DERIVED HEPATOCYTES USING A NEAR-INFRARED FLUORESCENT PROBE

Han, Jiyou, Jang, Yu Jin, Uhm, Soojin, Park, Ji Young, Son, Jeong Sang, Lee, Jaehun, Lee, GyungGyu, Kim, Hyojin, Kim, Jong Seung and Kim, Jong-Hoon, Korea University, Seoul, Korea, South

In vivo imaging and tracking of transplanted somatic cells derived from human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) is an important issue when the cells were used in clinical purposes. Previously, we reported that functional hepatocytes can be efficiently obtained from hESCs and hiPSCs in high purity. Therefore, a high-resolution cell tracking system using a sensitive probe should be developed before stem cell-replacement therapies becomes a reality in near future. Here, we introduce an accurate and safe method for in vivo delivering and tracing stem cell-derived hepatocytes. We designed and synthesized a novel near-infrared (NIR) fluorescent probe, which can be applied for cell tracking in vivo, particularly for hepatocytes differentiated from hESCs. Together with the NIR fluorescence dye indocyanine green (ICG), clinically proven compound A is used as a guiding unit in this system to enhance the selective uptake and to improve the biocompatibility of this probe. Our in vitro and in vivo results clearly showed that compound A-appended ICG (A-ICG) enabled in vivolong-term monitoring of transplanted hepatocytes in a mouse model with acute liver injury. Additionally, our immunocytochemistry data (ALB and CD31) showed that liver injury was significantly recovered in A-ICG tagged cells transplanted liver, compared to the sham and control group. It might be the compound A prevent the transplanted cells to reactive oxygen damages. Our NIR cell tracking probe can provide a useful information for evaluating the efficacy and safety of transplanted stem cell-derived hepatocytes for clinical application.

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F3009

DEVELOPING O-SHAPE VESSELS FOR STABLE SUSPENSION-BASED MASS PRODUCTION OF HUMAN INDUCED PLURIPOTENT STEM CELLS.

Horiguchi, Ikki[1], Suzuki, Ikumi[2], Morimura, Takashi[2], Yoshida, Takao[2] and Sakai, Yasuyuki[1,3], [1]University of Tokyo, Japan, [2]Fukoku Co., LTD, Saitama, Japan, [3]The University of Tokyo, Japan

Suspension culture is one of the most promising approaches to obtain the enough number of human induced pluripotent stem cells (hiPSCs) for clinical applications. However, hiPSCs are sensitive to shear stress of medium flow and hard to grow up from single hiPSC in suspension condition even with ROCK inhibitor. In addition, they tend to adhere each other and form aggregates. In existing culture vessels, floating cells migrate into a center and bottom of the vessel with moderate rotary shaking condition, which is known as "Einstein's tea leaves" paradox. This migration causes excess aggregation and low growth ratio in a batch. Therefore stronger agitation than hiPSC can survive is required for hiPSC suspension culture in existing culture vessels. In this study, in order to avoid the problems caused by the paradox, we developed a novel O-shape cell culture vessel with two different sizes of petri dishes. According to a particle distribution test by suspending Cytodex 1 microbeads within rotary shaking vessels, they were well distributed with lower shaking speed than existing rotary shaking vessels. Suspension culture with mild agitation condition (20 mL of Essential 8 medium within 100 mm dish on 45 rpm rotary shaking) showed that O-shape vessels obtained uniform aggregates from single cell suspension without unacceptable over-aggregation. We also developed an O-shape culture bag as a closed culture vessel and hiPSCs were successfully cultured in the O-shape bag. These O-shape vessels can be utilized for suspension culture of cells other than hiPSCs and we also performed HEK293 cell suspension culture within the O-shape vessels. Now we are investigating on the quality of hiPSCs cultured in the vessels and tracking movement of floating cells in the vessel.

F3011

EXPANSION OF HUMAN PLURIPOTENT STEM CELLS AS AGGREGATES IN SUSPENSION CULTURES

Jervis, Eric, McLaughlin, Angela, Hukezalie, Kyle, Woodside, Steven, Thomas, Terry E., Eaves, Allen C. and Louis, Sharon A., STEMCELL Technologies Inc., Vancouver, BC, Canada

3D suspension culture of human pluripotent stem cells (hPSCs) could enable the scale-up of hPSC production. However, media optimized for 2D adherent cultures can lead to low volumetric productivity and inefficient

workflow. To overcome these limitations we developed mTeSR™3D, a defined medium based on mTeSR™1, and optimized protocols for fed-batch culture of hPSC aggregates. Shake flasks containing mTeSR™3D, were seeded with a human embryonic cell line (H1 or H9) or an induced hPSC line (WLS-1C or STiPS-M001) that was previously maintained in 2D mTeSR™1 culture. 3D cultures were maintained using either daily medium exchanges or using a fed-batch protocol whereby the culture medium was supplemented daily with a fed-batch supplement. After 3 or 4 days in suspension culture, aggregates were harvested, dissociated into small clumps with Gentle Cell Dissociation Reagent or single cell suspensions with Accutase®, and re-seeded into new flasks in mTeSR™3D. Passaging and feeding cycles were repeated for at least 5 passages. 3D cultures were assessed for viability, pluripotent marker expression, in vitro differentiation potential and karyotype. In addition, media was analyzed for lactate and glucose levels. Lactate concentration remained below inhibitory levels. With clump passaging, up to 80% of cells incorporated into aggregates within 6 hours. By day 4 post-seeding, aggregates had grown to a mean diameter of 400 μm, with a 5-fold increase in cell number. Initial karyotype analysis indicated that only cells passaged as small clumps retained a normal karyotype. Confocal imaging of optically-cleared hPSC aggregates revealed uniform staining of pluripotency markers (OCT4, TRA-1-60), high viability (>95%) and minimal early differentiation marker expression (e.g. PAX6). hPSC cultures maintained in mTeSR™3D differentiated into all 3 germ layers with high efficiency. Cells grown as aggregates could transition back to 2D cultures in mTeSR™1 and regenerate high quality colonies. The average volumetric productivities were 0.7, 3.1 and 6.9 ($x10^5$) cells/ml of medium used for 2D culture, daily 3D medium exchanges and fed-batch 3D cultures respectively. Thus, mTeSR™3D used with an optimized fed-batch culture protocol enables efficient scale-up of hPSC production with a greatly simplified workflow.

F3013

EVALUATION OF THE DEVELOPMENTAL POTENTIAL OF HUMAN INDUCED PLURIPOTENT STEM CELLS USING PORCINE PARTHENOTES AND SINGLE CELL TRANSCRIPTOME ANALYSIS

Koyano-Nakagawa, Naoko, Gong, Wuming, Arnason, Anne, Coffin, Breyen, Chapman, Christopher, Meisner, Lars, Ly, Daniel, Dutton, James, Garry, Mary and Garry, Daniel J., University of Minnesota, Minneapolis, MN, U.S.

The use of human induced pluripotent stem cells (hiPSCs) has tremendous potential for regenerative medicine by providing an unlimited source of personalized cells. A number of protocols have been established for efficient differentiation of hiPSCs to the desired lineage in vitro, such as cardiomyocytes and blood. However, the field lacks an in vivo system to evaluate the differentiation potential and quality of hiPSCs. We examined whether porcine parthenotes could be used as an experimental system to test the developmental potential of the hiPSCs. First, we utilized the in vitro culture system and examined the ability of hiPSCs to proliferate and integrate into the parthenogenetic embryos. Parthenogenetic embryos were injected with ten hiPSCs at day 4 after activation (at 8 cell ~ morula stage) and cultured up to 72 hours. During this period, parthenotes underwent blastocoel cavity formation and hatching. Cell tracing experiments demonstrated that hiPSCs proliferated and integrated into the parthenotes. hiPSCs and their derivatives were found both in trophoectoderm and embryo proper after in vitro culture, however, embryos transferred to hormonally primed gilts and allowed to develop in vivo to embryonic day 18 did not retain the hiPSCs. To evaluate the extent to which each hiPSC line is compatible with porcine embryonic development, we bioinformatically analyzed the single cell transcriptome of hiPSC lines and compared with publicly available human ES cell transcriptome and pig RNA seq data. Analysis of the "metagene entropy", a novel measure of differentiation potential, revealed that cells in the hiPSC population are in a wide range of cellular entropy states. In addition, we identified signaling pathways that are enriched in the high entropy population. We propose that these approaches can be utilized to pre-screen the compatibility of hiPSCs for human-animal chimera formation.

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F3015

DETECTION OF ENDOGENOUS CANONICAL NOTCH SIGNAL BY IN SITU PROXIMITY LIGATION ASSAY

Litwin, Hannah Charlotte[1], Fang, Trixy[1], Tsoi, Yat Long[1], Lundin, Vanessa[2], Falk, Anna[1], Lendahl, Urban[3] and Teixeira, Ana Isabel[1], [1]Karolinska Institutet, Stockholm, Sweden, [2]Boston Children's Hospital, Boston, MA, U.S., [3]Department Cell Molecular Biology, Karolinska Institute, Stockholm, Sweden

Canonical Notch signaling is a conserved cell-cell communication system with fundamental roles in development and deregulated Notch is observed in a wide range of human diseases. In order to obtain a better understanding of how the pathway is regulated in normal homeostasis and disease, improved methods are needed that enable direct detection of endogenous Notch activity levels in model systems and clinical samples. Ligand activation of Notch leads to proteolysis of the Notch receptor by gamma-secretase and subsequent release of the Notch

receptor intracellular domain (NICD), which upon nuclear translocation forms a transcriptional complex with CSL and Mastermind-like protein 1 (MAML1). Here we present an in situ proximity ligation assay (PLA) for specific detection of endogenous canonical Notch transcriptional complexes in the nucleus of single cells. PLA enables highly specific, fluorescence-based detection of proximity between epitopes recognized by primary antibodies and has the sensitivity to detect single protein-protein interactions. To validate the assay, we activated or inhibited Notch signaling with immobilised Notch ligands and a gamma-secretase inhibitor (DAPT), respectively. 293T cells cultured on immobilized Notch ligands showed significantly higher levels of nuclear PLA signals compared to cells treated with DAPT. In contrast, no enhancement of nuclear PLA signal was observed in cells in which the Notch1 or CSL genes had been ablated by CRISPR-Cas9 technology. This work provides a tool for assessing endogenous canonical Notch signaling in fixed cells and tissues at a single cell resolution.

Funding Source: The Strategic Research Area in Stem Cells and Regenerative Medicine (StratRegen) and European Research Council (ERC)

F3017

CRISPR INTERFERENCE EFFICIENTLY INDUCES SPECIFIC AND REVERSIBLE GENE SILENCING IN HUMAN IPSCS

Mandegar, Mohammad A., Gladstone Institutes, San Francisco, CA, U.S.

Developing technologies for efficient and scalable disruption of gene expression will provide powerful tools for studying gene function, developmental pathways, and disease mechanisms. Here we develop CRISPR interference (CRISPRi) to repress gene expression in human induced pluripotent stem cells (iPSCs). CRISPRi, in which a doxycycline-inducible deactivated Cas9 is fused to a KRAB repression domain, can specifically and reversibly inhibit gene expression in iPSCs and iPSC-derived cardiac progenitors, cardiomyocytes, and T lymphocytes. This gene repression system is tunable and has the potential to silence single alleles. Compared with CRISPR nuclease (CRISPRn), CRISPRi gene repression is more efficient and homogenous across cell populations. The CRISPRi system in iPSCs provides a powerful platform to perform genome-scale screens in a wide range of iPSC-derived cell types, and to dissect developmental pathways and model disease.

F3019

UNIQUE LIVE PROBES, STEM CELL BIOPROCESSES AND CELL PRODUCTS FOR REGENERATIVE MEDICINE

Oh, Steve, Bioprocessing Technology Institute, Singapore

The Stem Cell Group at the Bioprocessing Technology Institute (BTI) together with collaborators at 6 sister A*STAR Institutes over the last 16 years have developed a suite of stem cell bioprocessing technologies for the following range of applications in stem cell therapy:- 1.Live fluorescent probes for the detection of senescent adult mesenchymal stem cell (MSC) vs. proliferative populations which can be used for quality control of MSC populations in culture. These probes have been validated for human MSC derived from adult bone marrow, umbilical cord, adipose and fetal sources. 2. Reprogramming hiPSCs with a high-throughput method and selection of high quality hiPSC using a suspension protocol that enables bioprocessing and scale up from 12 well to 6 well plates and finally 25 ml stirred suspension cultures. 50 clones after lentiviral infection with the Yamanaka factors, can be selected in the first week after reprogramming and 12 isolates with high proliferative potential and expression of pluripotent markers are banked after 4 weeks of continuous passaging in suspension cultures.Expansion of human pluripotent stem cells (hPSC: both hESC and hiPSC lines) and human MSC on commercial and biodegradable microcarrier platform technologies in suspension 1 litre scale bioreactors (rocking and agitated mode) at cell densities ranging from 1 billion to 10 billion cells/litre. These yields are a factor of 4 to 10 fold higher than conventional monolayer methods.Differentiating hPSC to cardiomyocytes (90% cardiac troponin positive); neuroprogenitors (85% PSA-NCAM positive), and red blood cells (50% enucleation) by a factor of 3 to 80 fold better than conventional monolayer or embryoid body methods. Differentiating MSCs to the osteogenic (equivalent to monolayer methods) and chondrogenic lineages (3-5 fold better than EB methods) as microcarrier cell aggregate cultures.Novel non-magnetic based activation and expansion technologies for CAR-T cell immunotherapies, which has demonstrated 28 fold expansion vs. Dynabead method, 23 fold expansion of T cells in vitro. These tools and technologies can be applied broadly to a treat a range of cardiac, neurological osteogenic, chondrogenic degenerative diseases, blood and T cell immunotherapies. Several of these inventions have been filed as patents.

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F3021

FACTORS AFFECTING THE COMMERCIALIZATION OF CELLULAR BASED THERAPEUTICS: A SYSTEMATIC REVIEW

Pettitt, David[1,2], Hollander, Georg[2,3] and Brindley, David[2,4], [1]Oxford – UCL Centre for the Advancement of Sustainable Medical Innovation (CASMI), Oxford, U.K. of Great Britain and Northern Ireland, [2]University of Oxford, U.K., [3]University of Oxford Weatherall Institute of Molecular Medicine, Oxford, U.K. of Great Britain and Northern Ireland, [4]Harvard University, Boston, MA, U.S.

Cellular based therapies represent a platform technology within the rapidly expanding field of regenerative medicine and are distinct from conventional therapeutics - offering a unique approach to managing what were once considered untreatable diseases. Despite a significant increase in basic science activity within the cell therapy arena, alongside a growing portfolio of cell therapy trials and promising investment, the translation of cellular based therapeutics from “bench to bedside” remains challenging, and the number of industry products available for widespread clinical use remains comparatively low. This systematic review identifies unique intrinsic and extrinsic barriers in the cell based therapy domain. Key electronic databases were searched and manuscripts subjected to pre-defined inclusion and exclusion criteria. Two independent reviewers examined the retrieved publications, and performed data extraction. 3374 unique publications were identified. 138 of these qualified for full assessment and subsequent data extraction. A number of key themes were identified, enabling examination of current challenges and opportunities facing cell therapy development, including manufacturing, regulatory, reimbursement, ethical and clinical adoption issues. In addition to an up-to-date analysis of the current landscape, we discuss a number of pragmatic solutions to facilitate future development and translation.

Funding Source: SENS Research Foundation, Centre for the Advancement of Sustainable Medical Innovation (CASMI)

F3023

MESENTERIC ARTERIAL INJECTION OF MESENCHYMAL STEM CELLS IN COLITIS MOUSE MODEL

Lee, Seunghee[1], Kim, Yu-Lee[1], Lim, Hye-Jin[1], Kang, Kyung-Sun[2] and **Seo, Kwang-Won**[1,2], [1]Kangstem Biotech, Seoul, Korea, South, [2]Adult Stem Cell Research Center, Seoul National University, Seoul, Korea, South

Mesenchymal stem cells which are well known for immunomodulatory effects have been developed as cell therapeutics for various inflammatory diseases. Intravenous injection is non-invasive and most common route for cell therapeutics. However, most of the cells which injected through vein are trapped in pulmonary capillaries resulting in embolism. It disturbs migration of cells physically causing decrease of therapeutic efficiency and side effects. In present study, we evaluated intra mesenteric arterial injection as a route which can avoid cell trapping in capillaries of lungs and increase therapeutic effects in inflammatory bowel disease including Crohn’s disease. After induction of colitis with TNBS in mouse, we performed laparotomy and injected human MSCs through superior mesenteric artery. 3days and 14days after injection, we confirmed cell distribution by confocal imaging and realtime qPCR. Injected cells were detected in colon, small intestine, liver and spleen and not detected in lung. Taken together, mesenteric arterial injection of cells can be applied as a useful route to increase target organ specific distribution and to avoid side effects which are related with pulmonary embolism.

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F3025

IN VITRO PROPAGATION OF MULTIPOTENT NEPHRON PROGENITORS FROM MOUSE EMBRYO AND EMBRYONIC STEM CELLS

Tanigawa, Shusnuke[1], Taguchi, Atsuhiro[2], Sharma, Nirmala[3], Perantoni, Alan[3] and Nishinakamura, Ryuichi[1], [1]Institute of Molecular Embryology & Genetics, Kumamoto University, Kumamoto City, Japan, [2]IMEG Kumamoto University, Kumamoto City, Japan, [3]National Cancer Institute, National Institutes of Health, Frederick, MD, U.S.

Nephron progenitors in the embryonic kidney expand while generating differentiated nephrons. However, in mice, the progenitors cease self-renewal shortly after birth. Here we report a novel method to selectively propagate nephron progenitors *in vitro* in an undifferentiated state. Combinatorial and concentration-dependent stimu-

lation with Lif, Fgf2/9, Bmp7, and a Wnt agonist is critical for the expansion. The purified progenitors proliferated beyond the physiological limits observed in vivo, both for cell numbers and lifespan. Neonatal progenitors were maintained for a week, while progenitors from mid-gestation expanded 1500-fold for nearly 20 days and still reconstituted three-dimensional nephrons. Furthermore, progenitors generated from mouse embryonic stem cells could be expanded with retained nephron-forming potential. Thus, we have established the in vitro conditions to promote propagation of nephron progenitors, which will be essential for dissecting the mechanisms of kidney organogenesis and for regenerative medicine.

F3029

ADVANCED TECHNIQUES FOR MASS PRODUCTION OF PLURIPOTENT STEM CELLS

Amit, Michal[1,2], Roytblat, Mark[3], Shariki, Kohava[3], Mishalov-Vaisbien, Florina[3], Axelman, Elena[3], Verbuk, Mila[3] and Angel, Itzchak[3], [1]Accellta, Haifa, Israel, [2]The Ephraim Katzir Department of Biotechnology, Karmiel, Israel, [3]Accellta LTD, Haifa, Israel

Induced pluripotent stem cells (iPSCs) are cells reprogrammed from somatic cells by over expression of "stemness related" transcription factors. As with human embryonic stem cells these cells have been cultured with supportive layers in two-dimensional cultures, which allow their continuous growth as undifferentiated cells. However, any future use of human iPSCs (hiPSCs) or embryonic stem cells for cell-based therapy or for industrial purposes will require a cost-effective, scalable, reproducible and controlled culture system. We have developed a novel culture system for PSCs in suspension using ether Petri dishes, Erlenmeyer, spinner flasks, or controlled bioreactors (Xuri™ Wave Bioreactor) based on specialized media supplemented with adapted serum replacement, growth factors and interleukins. PSCs from 20 different cell lines (induced or embryonic) were cultured as either single cells or small aggregates in suspension. After prolonged culture of over 50 passages (160 doublings) the cells maintained all PSC features, including stable karyotype, pluripotency, and undifferentiated proliferative ability. While cultured in dynamic systems the cells number increased by 13.5 folds during 10 days of culture, reaching cell concentrations of over 40 million cells per ml. Cells could be induced to differentiate in suspension into desired progenitors or differentiated cell types, while cultured in suspension, by selective manipulation of the culture conditions and composition. For example, PSCs were directed to differentiate in suspension into neural progenitors (NP), demonstrated to be able to differentiate *in vitro* into the three neural lineages: astrocytes, oligodendrocytes, and mature neurons. Our innovative suspension culture systems will both enable the routine culture of hPSCs in 3D culture and facilitate the production of high quality, reproducible and homogenous mass cultures of undifferentiated stem cells and progeny needed for both regenerative medicine, clinical trials, research purposes and industrial applications

TISSUE ENGINEERING

F3033

LARGE SCALE, EFFICIENT PRODUCTION OF HPSC-DERIVED MIDBRAIN DOPAMINERGIC NEURONS IN A DEFINED, 3D BIOMATERIAL PLATFORM

Adil, Maroof M.[1], Rodrigues, Goncalo M. C.[2], Kulkarni, Rishikesh U.[1], Rao, Antara T.[1], Chernavsky, Nicole E.[1], Miller, Evan W.[1] and Schaffer, David V.[1], [1]University of California Berkeley, CA, U.S., [2]SCBL-RM, Lisbon, Portugal

Pluripotent stem cells (PSCs) offer major potential as an unlimited source of functional cells for a range of biomedical applications; however, the development of large scale cell manufacturing systems to enable this potential faces many challenges. For example, midbrain dopaminergic (mDA) neurons are a promising option for regenerative therapy for Parkinson's Disease (PD), though their production typically involves undefined components and 2D culture formats that render scalable cell production needed to treat large patient populations difficult. Here, we have developed a differentiation method using a fully-defined, 3D thermoresponsive biomaterial platform to derive mDA neurons from PSCs at substantially greater yield, quality, and maturity compared to 2D Matrigel coated surfaces. In particular, after 25 days of differentiation, ~40% of cells differentiated in 3D expressed tyrosine hydroxylase (TH), the rate limiting enzyme for dopamine production and a hallmark of mDA neurons, compared to 20% on 2D. Crucial for functional mDA neurons, ~ 5-fold more neurons differentiated in 3D generated mDA-like action potentials. Additionally, mDA neurons generated in the 3D platform demonstrated a dynamic marker expression profile that closely resembled the one exhibited by these neurons during natural development, which is indicative of an environment more favorable to mDA development in 3D. Interestingly, FOXA2 and EN1, markers known to improve survival of mDA neurons, were expressed at significantly higher levels in cells generated in the 3D platform compared to control 2D surfaces. Consistent with this expression, mDA neurons generated in 3D exhibited high viability upon implantation into rat striatum, maintained a desirable FOXA2+/TH+ phenotype, and demonstrated substantive TH+ neurite extension within and surrounding the graft. A defined, scalable, and cost-effective cell culture platform can thus generate high quality differentiat-

ed cells with strong potential to accelerate both basic and translational research.

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F3035

USING FIBRONECTIN ARCHITECTURE IN THE EMBRYONIC HEART TO ENGINEER DEVELOPMENTALLY-INSPIRED HUMAN IPS-DERIVED CARDIAC TISSUES

Batalov, Ivan, Jallerat, Quentin, Kim, Sean and Feinberg, Adam W., Carnegie Mellon University, Pittsburgh, PA, U.S.

Heart disease is the leading cause of death worldwide due in part to the limited regenerative capacity of adult myocardium. Human pluripotent stem cells differentiated into cardiomyocytes (CMs), in combination with tissue engineering strategies, provides an approach to treat heart disease by replacing the injured native tissue following myocardial infarction In order to be functional, CMs in the engineered tissue must be uniaxially aligned to enable proper action potential propagation and maximal force generation. Previous work has shown that 2-dimensional CM monolayers can be engineered as in vitro models of laminar myocardium by micropatterning the extracellular matrix protein fibronectin (FN) onto the substrate in 20 μm wide lines. While FN plays a major role in CM alignment in the developing embryonic heart, it is known that the 20 μm line pattern does not resemble the structure of native heart FN. To provide human iPS-derived CMs with a developmentally-inspired microenvironment, we designed a new biomimetic micropattern that recapitulates the FN structure in the developing embryonic heart based on confocal 3D images of the chick heart. To test this, we seeded embryonic chick CMs and human iPS-derived CMs on the biomimetic, 20 μm, and 2 μm line patterns and quantified structural alignment of the CMs based on myofibril orientation. Results showed that the chick CMs attach and align on all the patterns, showing a unique density dependent alignment on the biomimetic pattern. The iPS-derived CMs showed lower attachment to all patterns relative to the primary CMs. We improved iPS-derived CM attachment to patterned FN by conditioning them on FN-coated plates for 7 days in CDM3 media supplemented with FBS for the last 3 days. With this method, iPS-derived CM alignment was lower compared to chick CMs on all patterns. This can be attributed in part to the immature phenotype of iPS-derived CMs that appeared to have greater cell-cell versus cell-FN adhesivity than the primary cells. This implicates competing roles of cell-cell versus cell-ECM interactions on cell alignment that depends on CM origin and developmental state. Future studies are focused on improving cell-ECM adhesion of iPS-derived CMs and developing computational models to elucidate physical principles driving cardiac tissue assembly.

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F3037

ENGINEERING BIOMEMTIC SCAFFOLDS FOR SOFT-TISSUE REGENERATION

Clevenger, Tracy, Luna, Gabriel, Boctor, Daniel, Fisher, Steven and Clegg, Dennis, University of California, Santa Barbara, CA, U.S.

Soft-tissue defects can arise from cancerous resections, trauma, severe burns, congenital defects, and more. Currently, reconstructive approaches using autologous fat grafts have varied success, thus there remains an unmet medical need for improved regenerative treatments. Adipose-derived stem cells (ASCs) are capable of differentiating into various cell types including but not limited to: adipocytes, chondrocytes, osteoblasts, and endothelial cells. ASC's are easily obtained through lipoplasty, are highly abundant, robust, and capable of self-renewal. These characteristics make ASCs an ideal cell-type for treatments involving soft-tissue defects. The primary impetus behind this work is to design and develop of a synthetic scaffold that supports survival and promotes differentiation of ASCs, a critical step in establishing a predictable and stable treatment. Engineering a biomemetic scaffold that directs ASC's down a differentiation pathway, enables a supportive cellular environment or serves as a degradable implant may permit the customization of treatment options for patients. Our work has shown that incorporating peptides containing Arg-Gly-Asp (RGD) sequences into scaffolding can promote adipogenic differentiation. Here we sought to examine and characterize the effects engineering scaffold degradation has on this system. Quantifying various spatial metrics, such as nearest-neighbor distances, voronoi domains, as well as cell density demonstrate that the synthetic scaffolds show a significantly reduced surface area while the number of cells, and spatial distribution remains unchanged after 4 weeks in 3D culture. By expanding the abilities of a single synthetic scaffold to be either non-degradable or degradable without effecting the cellular population provides a greater possibility for treatments of soft-tissue defects.

F3039

RESTORING PERFUSION IN CRITICAL HINDLIMB ISCHEMIA MODEL USING PULSED FOCUSED ULTRASOUND AND MESENCHYMAL STROMAL CELLS IN AN AGED MOUSE MODEL

Frank, Joseph Alan[1], Kim, Sage[1], Milo, Blerta[1] and Burks, Scott R[1,2], [1]National Institute of Health, Bethesda, MD, U.S., [2]National Institute of Health, Bethesda, MD, U.S.

OBJECTIVES: Critical limb ischemia (CLI) is associated with a 5 year mortality rate in excess of 70% with limited effective therapies. The main goal of this study was to determine if the mechanotransductive effects of pulsed focused ultrasound (pFUS) would enhancing homing of human mesenchymal stromal cells (MSC) to CLI in an aged mouse model and reestablish perfusion compared to pFUS or MSC alone. To determine if pFUS+MSC would increase vascular cell density in muscle when compared to MSC or pFUS alone 7 weeks after induction of CLI. CLI model was created by cauterizing the external iliac artery (EIA) on C3H mice (age >10 months). Laser Doppler perfusion imaging (LDPI) was perform of lower extremities weekly for 7 weeks after surgical intervention. At 14days post surgery, mice were grouped as follows: saline (n=8), pFUS (n=7), MSC (n=8), and MSC+pFUS (n=17). Mice received 3 consecutive days of either saline, pFUS, MSC, or MSC+pFUS starting on day 14 post EIA surgery. 10^6 human MSC were administered IV to MSC alone pFUS+MSC groups. CLI mice (n=3) were euthanized after one dose of either pFUS+MSC or MSC alone to determine if there was increased cell homing to ischemic muscle. pFUS exposures was performed to the hamstring of the ischemic limb.Histological evaluation vascular cell density from ischemic limb was performed at 7 weeks post EAI. LDPI demonstrated significant ($p<0.01$) differences in perfusion with significant ($p<0.01$) increase in the pFUS+MSC (60% of contralateral limb) cohort compared to other groups at 7 weeks when treatment was delayed 2 weeks after CLI. Histological examination of the hamstring muscle revealed significant increase ($p<0.05$) in CD31+ cells and vascular density treated with pFUS+MSC compared to other groups. This study demonstrates that pFUS enhanced tropism of iv MSC to targeted muscle resulting in reperfusion and neovascularization within a CLI model compared to MSC alone. pFUS preconditioned the ischemic muscle stimulating local molecular changes when combined with iv MSC increased stem cell numbers and potency reestablishing perfusion. pFUS ability to modulate the microenvironment in CLI opens the possibilities for enhancing cellular therapies in regenerative medicine and ultimately can be translated to clinical trials.

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F3041

TISSUE ENGINEERING VASCULAR GRAFTS FOR CONGENITAL HEART DEFECT CORRECTIVE SURGERY USING HUMAN THYMUS DERIVED-MSCs: OPTIMIZATION OF GMP GRADE CULTURE SYSTEM

Iacobazzi, Dominga[1], Swim, Megan[2], Albertario, Ambra[2], Caputo, Massimo[2] and Ghorbel, Mohamed[2], [1]University of Bristol, School of Clinical Sciences, Bristol Heart Institute, Bristol, U.K., [2]University of Bristol, U.K.

Prosthetic replacement grafts are used in Congenital Heart Defect (CHD) corrective surgery. However, these grafts have limited durability and often require repeated operations because of lack of growth potential. We aim to use child's own stem cells to produce tissue engineered vascular grafts that grow, repair and remodel, thereby providing longer lasting therapeutic effect and eradicating the need for additional surgeries. We have previously identified and purified multipotent Mesenchymal Stem Cells (MSCs) from neonatal thymus and expanded them in vitro. The human thymus, surgically removed during neonatal cardiac repair, has proven to be an excellent source of MSCs owing great ex-vivo expansion rate, which is paramount for large scale engineered tissue production. However, in order to be used for clinical applications, MSCs must be isolated and cultured using Good Manufacturing Practice (GMP) standards which include the use of animal free reagents. To the best of our knowledge, culture conditions compliant with GMP have not been established for human thymus-derived MSCs (hT-MSCs). The aim of this study was to optimize GMP-compliant culture conditions from the isolation through the expansion and 3D culture of hT-MSCs for potential clinical applications. Immunophenotypic characterization, cell proliferation and senescence, in vitro differentiation capacity and 3D culture were compared. Human thymus-derived MSCs were isolated and cultured under either non-GMP procedure, using porcine derived enzymes (collagenase, trypsin) and FBS-containing medium, or under GMP conditions, using xeno-free and/or serum-free media. Culture and expansion of hT-MSCs under GMP-compliant conditions retained the typical undifferentiated MSCs markers (CD29, CD44, CD73, CD105), colony forming unit activity and multipotency when compared to culture in standard non-GMP medium. Furthermore, hT-MSCs cultured in both conditions exhibit a great capacity to engraft onto a naturally occurring scaffold and synthetize their own extracellular matrix, under both static and dynamic (bioreactor) culture systems Our optimised GMP-compliant xeno-free medium provides optimal conditions for expansion of hT-MSCs and represents the first step in preparing

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hT-MSCs to scale up for potential therapeutic use in children with CHDs.

Funding Source: The Sir Jules Thorn Charitable Trust

F3043

EFFECT OF GLUCAGON-LIKE PEPTIDE-1 ON ADIPOSE-DERIVED STEM CELLS IN REPAIR OF GOAT MANDIBLE DEFECT

Li, Shasha, Long, Ting and Yuan, Xiaoyan, Sichuan University, Chengdu, China

Adipose-derived stem cells (ADSCs) have been the most promising seed cells for tissue engineering bone research. Recently, glucagon-like peptide-1 (GLP-1) has been demonstrated to be involved in osteogenic differentiation of ADSCs in vitro. It remains unclear that the exact effect of GLP-1 on new bone formation in vivo by regulating the differentiation of ADSCs. The aim of this study was to investigate the role of GLP-1 in osteogenic differentiation of ADSCs in goat mandible defect model. Goats were divided randomly into four groups. Porous titanium treated with GLP-1 + ADSCs, GLP-1, ADSCs or not was implanted respectively into goat mandible to repair mandibular segmental defect. Animals in different groups were sacrificed to harvest the mandibles 12 weeks after implantation. Osteogenesis was evaluated by X-ray, Micro-CT, HE staining and biomechanical testing. Results obtained from all above examinations showed that the group treated with GLP-1 + ADSCs had a better osteogenesis than other groups. In conclusion, our research shed light on the positive role of GLP-1 in repairing mandibular segmental defect via promoting osteogenic differentiation of ADSCs.

F3045

A DISORIENTED DECELLULARLIZED UTERINE SCAFFOLD REGENERATES THE UTERUS BUT DISRUPTS THE TISSUE POLARITY AND ARCHITECTURE IN RATS

Miki, Fumie, Miyazaki, Kaoru, Hida, Naoko, Masuda, Hirotaka, Hihara, Hanako, Tanaka, Mamoru, Uchida, Hiroshi and Maruyama, Tetsuo, , Keio University School of Medicine, Tokyo, Japan

The use of a decellularized scaffold has been demonstrated in several organs/tissues, including the esophagus, trachea, bladder, arteries and skin, to regenerate the original organs/tissues. It has been reported that decellularlized uterine scaffold (DUS) is prepared from rat uteri through decellularization by aortic perfusion with detergents and that DUS placement onto a partially excised uterus yielded recellularization and regeneration of uterine tissues and achievement of pregnancy nearly comparable to the intact uterus. An underlying extracellular matrix (ECM) together with an acellular, perfusable vascular architecture preserved in DUS is thought to be responsible for the appropriate regeneration of the uterus when DUS is recellularized. To elucidate whether the ECM in DUS is responsible for the tissue polarity and architecture, we here placed DUS onto the partially defective uterine area in the reverse direction, in which the lumen side of DUS was outside and the serosa side was inside, and analyzed the tissue structure and mRNA expression of the receptors for estrogen or progesterone in the regenerated uterus in rats, as compared to the uteri placed with DUS in the correct direction. In the reverse group 14 of 16 uteri showed aberrant structures including ectopic location and enlargement of glands and an abnormal lining of smooth muscle layers, whereas in the correct group 8 of 16 uteri exhibited less aberrant structures only present in smooth muscle layers. Rats in both correct and reverse groups were mated with male rats 8 weeks after DUS placement and assessed for their fertility 20 days after mating. There were no significant differences in the number of fetus per uterine horn, pregnancy rate, fetal weight, and mRNA expression of estrogen and progesterone receptors between the correct and reverse groups. Thus, the disoriented placement of DUS mostly resulted in the regeneration of the structurally aberrant uterus but with reproductive function comparable to the uterus correctly placed with DUS. These results indicate that the deregulated ECM dynamisms caused by an artificial disorientation of DUS could disrupt tissue polarity and architecture of the regenerated uterus. The use of DUS in the correct orientation may be preferable when it is clinically applied to regenerative medicine for the uterus.

F3047

CHARACTERIZATION OF HUMAN RESPIRATORY EPITHELIAL STEM CELLS FOR TRACHEAL REPLACEMENT VIA A BIOENGINEERED HUMAN AIRWAY

Pellegrini, Graziella[1], Jungebluth, Philipp[2] and Genna, Vincenzo Giuseppe[1], [1]University of Modena and Reggio Emilia, Modena, Italy, [2]Karolinska Institutet, Stockholm, Sweden

Patients with primary malignant tracheal cancers cannot undergo surgery due to large tracheal involvement and only transitory treatment of symptoms is possible. Recent clinical experiences have shown that these patients can be treated by transplantation of bioengineered human airway tracts. Donor human tracheas can be decellularized and a bioengineered human trachea can be obtained by scaffold colonization by in vitro expanded autologous epithelial cells and mesenchymal-derived autologous chondrocytes. The bioengineered human trachea can be implanted to restore respiratory function in a patient with end-stage airway disease. On long term evaluation, airway epithelium, essential to prevent infections, fibrotic reactions and stenosis of transplanted human respiratory tract, was shown to be discontinuous. We optimized

culture conditions for adult human airway epithelial stem cells, to maintain their proliferative and differentiation potential. Characterization of human respiratory epithelial cells was performed in vitro, as well as progenitors cell differentiation and their in vitro maintenance. Two culture systems were evaluated for their capability to maintain expression of common epithelial stemness/proliferation markers, differentiation markers and airway epithelial cells markers. In the selected culture condition, human airway epithelial cells showed higher proliferation and migration levels, maintenance of stem/progenitor cells markers expression and differentiation in a stratified and polarized epithelium. Tracheal epithelial cells were isolated from human biopsies and cultured in vitro for several passages to evaluate their growth rate, cell yield, colony forming efficiency and proliferative potential, as the total number of cell doubling before senescence. Finally, single cell analysis performed to characterize stem and transient amplifying (TA) cells, revealed their capability to generate three clonal types previously identified in other human epithelia and their differentiation potential. In the selected culture condition, human respiratory epithelial stem and TA cells were able to produce all the different cells types required to develop a bioengineered human trachea for transplantation.

F3049

TISSUE ENGINEERED REPLACEMENT FOR TRACHEAL REGENERATION AND REPAIR: IND-ENABLING STUDIES

Tarantal, Alice F., Lee, Charles, Batchelder, Cynthia, Martinez, Michele and Belafsky, Peter, University of California, Davis, CA, U.S.

Patients with congenital or acquired severe airway stenosis have few treatment options and a severely impaired quality of life. Current treatments are limited and include the need for multiple surgeries, which in most cases will not correct the problem. To advance preclinical investigations, studies were initiated to optimize protocols for studies with rhesus monkeys. New techniques and methods were developed and refined for cellular products, tracheal decellularization/recellularization, and a biorepository of characterized stem/progenitor cells and trachea for a tailored precision medicine approach. Fresh and decellularized tracheal sections were collected for histology, biomechanical testing, and quantitative PCR. No significant loss of collagen was observed in decellularized trachea compared to native tissue, whereas a modest decline in elastin content was noted. The decellularization process was shown to remove 97% DNA and MHC proteins. Biomechanical strength testing demonstrated retention of tensile (87%) and compressive (100%) properties when decellularized scaffolds were compared with native tissue. For recellularization, autologous mesenchymal stem/stromal cells and epithelial cells were used. A consistent cell seeding protocol was developed with 100% of the external surface and ~70% luminal cellularity obtained. Studies were initiated to optimize surgical techniques for implantation in adult animals. Following surgery, clinical parameters were monitored including structure integrity by computed tomography (CT). Stents were removed after ~1 month and hematologic and clinical parameters assessed. Based on the outcomes, to date, results suggest no evidence of rejection or structural failure, and complete cellularization of the luminal surface of the implant in vivo with ciliated pseudostratified columnar epithelial cells 3 months post-implant. No abnormal findings were observed in the cartilaginous component of the trachea. While preliminary, these studies suggest that the protocols and release criteria developed are efficient and result in successful implantation in vivo. However, more studies are needed in order to fully explore a number of essential questions before this approach can be considered for human use.

Funding Source: California Institute for Regenerative Medicine (CIRM)

F3051

EFFICIENT GENE DELIVERY INTO IMPLANTABLE 3-DIMENSIONAL STEM CELL CONSTRUCTS

Yu, Xiaohua[1], Khalil, Andrew[1], Dang, Phuong[2], McDevitt, Todd[3], Alsberrg, Eben[2] and Murphy, William[1], [1]University of Wisconsin-Madison, Madison, WI, U.S., [2]Case Western Reserve University, Cleveland, OH, U.S., [3]University of California - San Francisco, CA, U.S.

Scaffold-free tissue engineering strategies offer an attractive alternative for tissue regeneration, as they largely eliminate scaffold-associated complexities, such as the need to optimize scaffold mechanics, porosity, and degradation dynamics. The high cell density within scaffold-free constructs also enables abundant cell-cell and cell-ECM interactions, which better emulates tissue morphogenesis in vivo. However, it is a significant challenge to deliver biological mediators (e.g. growth factors) into these constructs, due to the inherent transport barrier created by cellular metabolic activity and deposition of large amounts of ECM by cells. One potential method to deliver biological mediators to scaffold-free stem cell constructs is via gene delivery. Unlike recombinant protein delivery, proteins secreted by stem cells as a result of gene delivery have undergone authentic post-translational modification, and thus possess greater biological activity. However, delivery of genes into 3D cell constructs (e.g. cell aggregates, cell sheets) with non-viral vectors is extremely challenging, due to the inherent transport barrier within the constructs and relatively large sizes of most vectors. We hypothesized that incorporation of mineral coated microparticles (MCM) loaded with poly(nucleic

acids) (e.g. plasmid DNA and mRNA) into 3D stem cell constructs would enable efficient 3-dimensional gene delivery. Human mesenchymal stem cells (hMSC) formed into 3D cell aggregates or cell sheets were readily transfected by incorporating pDNA-loaded MCMs. Transfected hMSCs distributed uniformly throughout the cell sheet. After optimizing the transfection efficiency by varying the pDNA dosage and the amount of MCMs used, we achieved hMSC transfection efficiency up to 30% within 3D cell sheets, which is 5-fold higher than current standard protocols on 2D substrates, and more than 20-fold higher than standard protocols in 3D culture formats. Finally, we demonstrated that delivery of a plasmid encoding bone morphogenetic protein-2(BMP-2) via this approach enhanced osteogenic differentiation of hMSC within the cell sheets. The technology described herein may have utility as a tool to achieve scaffold-free 3D stem cell transfection with high efficiency and uniformity.

Funding Source: National Institute of Health

F3053

'SMART' SCAFFOLDS FOR IMPROVED TISSUE REGENERATION

Matosevic, Sandro, Pasley, Shannon, Torres, Fabiana and Zylberberg, Claudia, Akron Biotech, Boca Raton, FL, USA

Three-dimensional scaffolds have emerged as promising vehicles for tissue and organ regeneration. They are increasingly being used as substrates for cell attachment and to provide suitable mechanical properties for cell expansion. One of the big challenges to the use of such materials in clinical settings is the ability of these scaffolds to provide suitable microenvironments for cellular expansion and tissue neogenesis while avoiding biocompatibility issues. Work in our lab has focused on generating three-dimensional scaffolds with smart surfaces to aid in cellular attachment, expansion and delivery. These scaffolds are based on electrospun nanofibers and hydrogels, and have properties that allow their delivery as both injectable biomaterials as well as tissue constructs. By fine-tuning biological and mechanical properties of these scaffolds we are able to generate composite structures with superior cell attachment characteristics, which is critical for improving the active delivery of cells to target sites. Such scaffolds with 'smart' surfaces are tunable to direct the expansion of different cell types and have physical properties – including porosity and biodegradability – which can be edited to match the delivery constraints of *in vivo* systems.

REGENERATION MECHANISMS

F3055

HUMAN MESENCHYMAL STEM CELLS GENETICALLY ENGINEERED TO PRODUCE BDNF ATTENUATE NON-MOTOR FEATURES AND DELAY STRIATAL ATROPHY IN A HUNTINGTON'S TRANSGENIC MOUSE MODEL

Fink, Kyle[1], Pollock, Kari[1], Dahlenburg, Heather[1], Nelson, Haley[1], Cary, Whitney[1], Hendrix, Kyle[1], Deng, Peter[1], Torrest, Audrey[1], Gutierrez, Josh[1], Nacey, Catherine[1], Pepper, Karen[1], Gruenloh, William[1], Bauer, Gerhard[1], Annett, Geralyn[1], Tempkin, Teresa[1], Wheelock, Vicki[1] and Nolta, Jan[2], [1]University of California, Davis, Sacramento, CA, U.S., [2]University of California Davis, Sacramento, CA, U.S.

Huntington's disease (HD) is a terminal neurodegenerative autosomal dominant disease that may present behavior deficits before the age of 20 (juvenile HD) and more commonly in midlife with psychiatric, behavior, motor and cognitive impairment. HD is neuropathologically characterized by a selective loss of medium spiny neurons resulting in striatal atrophy. Brain-derived neurotrophic factor (BDNF) has been shown to prevent cell death and to stimulate the growth and migration of new neurons in the brain, making it a lead candidate for the treatment of Huntington's disease (HD). Mesenchymal stem cells (MSC) isolated from human bone marrow were transduced with a lentivirus designed to overproduce BDNF. The MSC/BDNF product was manufactured following Good Manufacturing Practice Standard Operating Procedures to facilitate translation to future clinical trials. No alterations in cell growth, differentiation capacity, cell size or phenotype were observed after transduction by the BDNF vector, and the karyotype remained stable. MSC/BDNF were intrastriatally transplanted in two strains of HD transgenic mice: YAC 128 and R6/2 to establish primary efficacy of this therapeutic. MSC/BDNF treatment decreased striatal atrophy in YAC128 mice. MSC/BDNF treatment also significantly reduced anxiety as measured in the open field assay. A significant correlation between striatal size and anxiety-like behaviors was observed suggesting the validity of these endpoints for our planned future cellular trial. Both MSC and MSC/BDNF treatments induced a significant increase in neurogenesis-like activity in R6/2 mice. Our genetically modified MSC/BDNF cells set a precedent for stem cell-based neurotherapeutics and could potentially be modified for other neurodegenerative disorders such as amyotrophic lateral sclerosis, Alzheimer's disease, and some forms of Parkinson's disease. These cells provide a platform delivery system for future studies involving corrective gene editing strategies.

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F3057

DESIGN OF NOVEL TIE2 INHIBITORS TO ELUCIDATE TIE2 FUNCTIONALITY IN MAINTAINING STEM CELLS

Ferreccio, Amy, University of Washington, Seattle, WA, U.S.

Cancer and adult stem cells are resistant to radiation and chemical-induced apoptosis. Recent studies in Drosophila adult stem cells have shown that germ line stem cells in Drosophila are protected and able to bypass apoptosis by the activation of the Tie pathway, initiated by PVF1 ligand, which is secreted from dying daughter cells. Tie then activates microRNA Bantam, which inhibits pro-apoptotic HID/DIAP1, intercepting caspase cascade activation. It is of great value to understand if stem cells are protected by dying daughters also in mammalian system and in particular, if Tie-2 controls the process. Preliminary data show that while human naïve stem cells survive in media that has been exposed to irradiated feeders (IR CM), they do not survive in media exposed to non-irradiated feeders (non-IR CM), suggesting that irradiated fibroblasts secrete components essential for hESC survival. We have initiated candidate and unbiased screens to identify these survival signals. Importantly, while mammalian Tie-2 ligand Ang4 did not show growth rescue, Ang1 significantly increased hESC growth in non-IR CM. Western blot analysis shows an elevated amount of Ang1 in media from irradiated feeders compared to that of non-irradiated. Our working hypothesis is that Ang1 activation of Tie2 receptor is needed for naïve stem cell survival in non-IR CM. To dissect this hypothesis in detail, we will generate novel, computational design based inhibitors and activators of Ang1-Tie2 interface and test them in hESC survival assay. We have already optimized Ang1 F-domain and Tie2 binding assay using yeast surface display method. We are now in the process of designing and testing high affinity inhibitors of the surface using combination of computational and mutagenesis based methods. Re-iterative cycles in the yeast cell surface display method will be utilized to obtain competitors, >100 fold higher affinity binders than normal Ang1. The novel binders without dimerization domains will be optimized for inhibitors and high capacity oligomerization domains will be utilized to switch the inhibitors' mode of action to activation. We will test the functionality of these inhibitors and activators in hESCs, endothelial cells and breast cancer stem cells. The most potent inhibitors will be tested in future in vivo mouse tumor models.

F3059

SINGLE-CELL RNA-SEQ ANALYSIS OF ADULT SALIVARY GLAND PROGENITORS

Hauser, Belinda R[1], Kelly, Michael C[2], Burns, Joesph C[2], Kelley, Matthew W[2] and Hoffman, Matthew P[1], [1]NIDCR/NIH, Bethesda, MD, U.S., [2]NIDCD/NIH, Bethesda, MD, U.S.

Salivary gland hypofunction after irradiation for head and neck cancer severely impacts the oral health of patients. We propose to regenerate salivary gland function using adult salivary progenitor cells. Keratin 5-positive (K5+) and K14+ cells are progenitors during fetal development of murine submandibular glands (SMGs) as determined by genetic lineage tracing studies; however, the relationship between these progenitors in the adult gland is unknown. Here we used K5-venus and K14-RFP expressing mice to FACS sort subpopulations of K5+, K14+, K5+;K14+ and K5-;K14- from adult SMGs. We then used microfluidic-based single-cell analysis to isolate, process, and profile RNA expression in individual cells. Our goal is to further characterize these subpopulations to identify markers that could be used to isolate them to investigate their function during regeneration. In total, 156 single-cell libraries were sequenced with an average read depth of over 1 million reads. Using bioinformatic analyses we defined 5 groups based on the 60 most variable genes through unbiased clustering. Using the molecular signatures database (MsigDB) we further characterized the phenotype of the clusters, which included mesenchymal cells, cells enriched in epithelial tissue stem cell markers, epithelial ductal cells, and markers of hematopoietic/bone marrow cells. Bioinformatics analyses also suggest subpopulations within the clusters express genes involved in regulating progenitor cell lineage such as Kit, Hs3st3, Sox9, and Itga6. RNAseq analysis has identified gene regulatory networks that may be useful to direct progenitor cell lineage and expansion for the regeneration of salivary glands after either irradiation damage or for bioengineering of salivary tissue.

F3061

THERAPEUTIC MECHANISMS OF HUMAN ADIPOSE-DERIVED MESENCHYMAL STEM CELLS IN A RAT TENDON INJURY MODEL

Lee, Sang Yoon, Chung-Ang University Hospital, Seoul, Korea, South and Chung, Sun G., Seoul National University College of Medicine, Seoul, Korea, South

There has been no in vivo study to demonstrate that MSCs could function as differentiated cells after transplantation. We aimed to investigate whether MSCs could differentiate into the tenogenic lineage and secrete their own proteins using a xenogeneic MSC transplantation model. Bilateral Achilles tendons of 57 Sprague Dawley rats were given

full-thickness rectangular defects at the tendon insertion site to the midsubstance. They were randomly assigned to 3 groups after the modeling: 1) human adipose-derived mesenchymal stem cells (hASC) implantation with fibrin glue (cell group), 2) fibrin glue implantation with cell media (fibrin group), and 3) identical surgical procedure without any treatment (sham group). Two and 4 weeks after modeling and implantation, all groups were evaluated by morphological, biomechanical, and histopathological analyses. Viability of tagged hASC was observed by immunofluorescence staining and protein expressions (collagen type I/III and tenascin-C) were evaluated by immunohistochemistry and Western blot analyses. The incomplete healing rate of cell group (11.1%) was lower than the rates of sham (25.0%) and fibrin (22.2%) groups. The cross sectional areas of tendons in cell group were decreased (P = 0.008) while those in sham group were increased (P = 0.005) from 2 to 4 weeks. At 2 weeks, ultimate tensile strength and stiffness of cell group were significantly higher than those of sham group (P = 0.037 and P = 0.010, respectively). Tagged hASCs remained viable in host rat tendon for 4 weeks as confirmed by immunofluorescent staining. Cell group showed higher optical densities in immunohistochemistry than those of sham and fibrin groups in human-specific collagen type I at both 2 (P = 0.004 and P = 0.006, respectively) and 4 weeks (P = 0.033 and P = 0.011, respectively) and in human-specific tenascin-C at 2 weeks (P = 0.009 and P = 0.028, respectively). Transplantation of hASCs enhanced rat tendon healing biomechanically, being superior to sham and active controls. Implanted hASCs to the rat tendon defect model survived for at least 4 weeks and secreted human-specific collagen type I and tenascin-C. These findings suggest that transplanted MSCs can differentiate into the tenogenic lineage and contribute their own proteins to tendon healing.

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F3063

CRYOPRESERVED MIDBRAIN DOPAMINE NEURONS DERIVED FROM HUMAN IPSC REVERSE BEHAVIORAL DEFICITS IN PARKINSONIAN ANIMALS

Wakeman, Dustin R.[1], Hiller, Benjamin M.[1], Marmion, David J.[1], McMahon, Christopher W.[2], Ma, Junyi[2], Corbett, Grant T.[1] and Kordower, Jeffrey H.[1], [1]Rush University, Chicago, IL, U.S., [2]Cellular Dynamics International, Madison, WI, U.S.

Large-scale manufacturing and cryopreservation of neurons that can be efficiently prepared with minimal manipulation is a major hurdle for clinical translation of pluripotent stem cell based therapies for neurodegenerative disorders like Parkinson's disease. To address this obstacle, midbrain dopamine neurons were derived from human induced pluripotent stem cells (iPSC-mDA) via floor plate induction and cryopreserved in large production lots for biochemical screening and transplantation studies. Upon thaw, cryopreserved iPSC-mDA neurons retained high viability with a gene expression profile similar to the human substantia nigra. Biochemical analysis confirmed a protein expression signature consistent with the midbrain floor-plate lineage. In addition, iPSC-mDAs released dopamine upon depolarization with KCl stimulation. Furthermore, electrophysiological recordings revealed firing of both spontaneous and evoked action potentials with active Na^+ and K^+ channels responsive to pharmaceutical inhibition. In order to test therapeutic efficacy, cryopreserved iPSC-mDA neurons were transplanted without sub-culturing into the 6-OHDA-lesioned rat and MPTP-non-human-primate models of Parkinson's disease. Grafted neurons demonstrated robust survival, extensive fiber outgrowth, and innervation of host parenchyma in both rodent (1-month) and macaque (3-months). Immunohistochemistry of grafted neurons confirmed co-expression of human midbrain lineage, A9 subtype dopamine neuron markers (FoxA2, TH, Girk2, human-Nuclei, and human-NCAM). Furthermore, a behavioral study in 6-OHDA-lesioned parkinsonian rats revealed statistically significant reversal in functional deficits in both amphetamine and apomorphine induced rotational asymmetry for up to 6-months post-transplantation with complete iPSC-mDA neuron graft reinnervation of the host striatum. Critically for safety, no proliferation in transplanted cells was observed by Ki-67 staining at any time points tested in the 6-OHDA-lesioned rat or MPTP-macaque. The results demonstrate excellent graft survival, maintenance of the midbrain dopaminergic phenotype, and lack of neural overgrowth in parkinsonian rats and monkeys, as well as indicate considerable promise for the development of pluripotent cell-based therapies in PD.

ETHICS AND PUBLIC POLICY; HISTORY OF STEM CELL RESEARCH; SOCIETY ISSUES; EDUCATION AND OUTREACH

F3067

UNPROVEN STEM CELL INTERVENTIONS AND ENCOURAGING COLLABORATION BETWEEN REGULATORS AND DESPERATE PATIENTS WITHIN THE CLINICAL TRIAL PROCESS

Matthews, Kirstin RW, Rice University, Houston, TX, U.S. and Iltis, Ana, Wake Forest University, Winston Salem, NC, U.S.

Stem cells have been touted by scientists as the new future for medicine, but with limited therapies currently

available, clinics around the world are marketing unproven interventions utilizing stem cells to allegedly "cure" diseases ranging from Autism to Multiple Sclerosis. The continued marketing and use of experimental stem cell-based interventions is problematic and unsustainable. Central problems include the lack of patient protection, regulation of clinical sites, and clinician licensing. These interventions lack evidence of safety and efficacy; patients may be wasting money and time; and they may be forgoing other opportunities for interventions that have not been shown to be safe and effective. The landscape of stem cell tourism should prompt a re-evaluation of current policy approaches to studying stem cell-based interventions with respect to the design, initiation, and conduct of clinical trials in the US as well as abroad. To understand this issue and ways to promote change, we reviewed and analyzed several national and local policy initiatives that tried to provide expanded access to patients. These policies include Japan's 'Pharmaceuticals, Medical Devices and Other Therapeutic Products Act', the UK's 'Saatchi Bill', and the US 'Right to Try' movement. While these new policies address patients' concerns about access, they also increase overall risks to patients. This original research will identify key goals for developing a public policy and regulatory approach that engages relevant stakeholders, prevents patients from seeking unproven interventions, and minimizes risks. Stakeholders, including scientists, clinicians, regulators and patient advocates, need to work together to find a compromise to encourage patients to stay in their home country and within the clinical trial process instead of seeking unproven treatments abroad.

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F3069

WHAT DID YOU DO WITH MY EMBRYO? ISSUES SURROUNDING GIVING RESULTS TO PEOPLE WHO DONATED THEIR EMBRYO TO STEM CELL RESEARCH

Jonlin, Erica C., University of Washington, Seattle, WA, U.S.

Human embryos used for human embryonic stem cell research are commonly excess frozen embryos leftover from fertility clinics. Individuals and couples no longer needing or wanting these frozen embryos for reproductive purposes may opt to donate the embryos to research. In the process of consenting to the donation, donors are informed of the various uses of the embryos, the primary use being the derivation of human embryonic stem cell lines. The potential donors are further informed of the many uses of hESC lines, including basic research in regenerative medicine, as well as the development of a differentiated tissue that could be used therapeutically for patients with serious, degenerative diseases. During the consent process some donors ask if they will ever find out if their embryo led to derivation of a line. They often express the hope that their embryo "will do some good" and help someone. Currently, donors are told that they will not learn the results of the research on their embryo, and that not learning the results of research on leftover human specimens is typical in tissue research. This explanation is often disappointing to the embryo donors. This poster discusses both the general ethical and historical underpinnings for giving or not giving results to tissue donors, as well as the specific pros and cons of informing embryo donors of the results of the research with their embryos.

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F3071

ACCEPTABILITY OF HUMAN-ANIMAL CHIMERIC EMBRYO RESEARCH USING HUMAN INDUCED PLURIPOTENT STEM CELLS: A SURVEY AMONG THE PUBLIC AND RESEARCHERS

Sawai, Tsutomu[1], Hatta, Taichi[2] and Fujita, Misao[2],
[1]Kyoto University, Center for iPS Cell Research and Application, Kyoto, Japan, [2]Center for iPS Cell Research and Application (CiRA), Kyoto, Japan

Since 2001, in Japan, the Guidelines for the Handling of Specified Embryos based on the Act on Regulation of Human Cloning Techniques have permitted human-animal chimeric embryo (HACE) research using human induced pluripotent stem cells (hiPSCs) for the purpose of "creating human organs" in animals; nonetheless, it has imposed restrictions on the research. Recently, a ministry working group of HACE research in Japan has almost finished discussing the science of the research. According to the working group, HACE research can be divided into the following: Phase 1 in which HACEs are created by injecting hiPSCs into days-old animal embryos; Phase 2 in which the HACEs are gestated in animals and the animals are brought to term; Phase 3 in which the animals are used for organ transplants. Each phase has different purposes such as exploration of pluripotency of hiPSCs, disease modeling and drug discovery, and organ transplants. However, other issues concerning the research such as further evaluations of ethical, legal and social implications, are yet to be tackled. In order to consider whether HACE research is acceptable and then form a social consensus for promoting the research in Japan, attitudes toward HACE research among the public and researchers should be taken into account. This study aims to ascertain how the public and researchers evaluate HACE research using hiPSCs, and whether there are differences in opinions between the two groups. Upon approval from the ethics committee at Kyoto University, the data will be collected from

500 individuals from the general public registered with a research company and approximately 500 researchers from Center for iPS Cell Research and Application (CiRA) in February 2016 by online survey. Focusing on the three phases of HACE research, we ask the public and researchers about acceptability of HACE research using hiPSCs, and explore their differences. In this poster presentation, we will present our research findings. It is expected that they contribute to the decision-making process of public policy on HACE research using hiPSCs.

LATE BREAKING ABSTRACT

F4001

DINACICLIB TREATMENT EFFICIENTLY ELIMINATES RESIDUAL IPS CELLS IN IPS-DERIVED BIOENGINEERED CARDIAC TISSUE

Alsayegh, Khaled N[1,2] and Matsuura, Katsuhisa[2], [1]King Abdullah International Medical Research Center, King Saud bin Abdulaziz University for Health Sciences, King Abdulaziz Medical City., Jeddah, Saudi Arabia, [2]Tokyo Women's Medical University, Tokyo, Japan

Induced Pluripotent Stem Cells (iPSCs) hold great potential for being a major source of cells for regenerative medicine. Since their discovery, human iPSCs were successfully differentiated to numerous functional cell types that closely resembled those found in primary human tissue. However, one of the remaining issues that need to be resolved before the full potential of iPSCs can be seen in clinic, is the risk of teratoma formation. The presence of undifferentiated cells in iPS-derived tissue may cause tumor formation in patients following transplantation. Therefore there is a pressing need to develop highly efficient techniques that can completely eliminate remaining iPSCs. The need for such techniques substantially increases when treating major organs like the heart, where a minimum of 1-2 x 10^9 of iPS-derived cardiac cells are required. In this study we found that treatment of bioengineered iPS-derived cardiac tissue with the pan-CDK inhibitor, dinaciclib, eliminates residual iPS cells. Dinaciclib is currently undergoing phase I/II clinical trials for treatment of advanced breast cancer and non-small cell lung carcinoma. We found that treatment of human iPS cells with dinaciclib induced apoptosis and cleavage of caspase-9 in as early as 6 hours after treatment, while not affecting the viability of iPS-derived cardiac tissue. Additionally, dinaciclib significantly upregulated p53 at the protein level and induced a reduction in the levels of the antiapoptotic protein, MCL1 in human iPSCs. To ascertain whether or not dinaciclib-induced apoptosis in hiPS cells was p53 dependent, we used a specific short-hairpin RNA (shRNA) to downregulate p53. We found that a 75% p53 knockdown prevented dinaciclib induced apoptosis and therefore, dinaciclib-induced apoptosis in human iPS cells seem to be p53-dependent. The levels of LIN28 transcripts in iPS-derived tissue has recently been shown as a sensitive marker for detection of residual iPS cells. We found that following treatment of bioengineered cardiac tissue with dinaciclib, LIN28 levels were significantly reduced from 5.8% in DMSO treated control to 0.8% (p-value = 0.0005). In conclusion, treatment with dinaciclib is an efficient technique to eliminate residual iPS cells in iPS-derived cardiac tissue.

F4003

COMPREHENSIVE GENE EXPRESSION ANALYSIS OF HUMAN IPSC-DERIVED NEURONAL PROGENITOR CELLS AND NEURONS

Panicker, Leelamma[1], Spencer, Michelle[1] and **Yin, Dezhong**[2], [1]ATCC Cell Systems, Gathersburg, MD, U.S., [2]ATCC, Gaithersburg, MD, U.S.

Human iPSC-derived neural progenitor cells (NPCs) and neurons are an attractive *in vitro* model to study neurological development and to model diseases. However, there is a lack of NPC lines and media that support differentiation into multiple types of neurons for disease modeling and drug screening. Here we investigated expression of genes associated with different types of neurons by qRT-PCR during 3 weeks of dopaminergic differentiation. Known early neuron makers, MAP2 and Tuj1 genes reached peak expression at 2 weeks while expression of dopaminergic neuronal genes (TH, Nurr1, VMAT2, AADC) was significantly increased in a time-dependent manner ($P < 0.05$). Furthermore, expression of genes associated with GABAergic (GABRB3), glutamatergic (vGLUT1, vGLUT2, GLS2), and cholinergic (ChAT) neurons was also induced and peaked at 3 weeks during dopaminergic neuron differentiation in the two NPC lines tested. This shows that our NPC lines and dopaminergic differentiation media are capable of producing GABAergic, glutamatergic and cholinergic neurons in addition to dopaminergic neurons. However, there was no significant induction of motor neuron gene (EN1, LIM3, Hb9) expression during dopaminergic differentiation. To validate that our NPC lines and NPC dopaminergic neuron differentiation media are suitable for drug screening, we conducted neurotoxicity screenings in the two NPC lines and NPC-derived neurons by using resazurin viability and high content imaging assays. We found that paclitaxel, a microtubule-stabilizing chemotherapeutic agent, significantly induced neurotoxicity ($P < 0.05$) in the two NPC lines tested, but not in NPCs-derived neurons. Vincristine, amiodarone, and chlorhexidine significantly decreased viability of both NPCs and neurons, whereas piperine and hydroxyurea did not induce any significant neurotoxicity in either NPCs or neurons. This study demonstrates that our iPSC-derived NPC lines and dopaminergic differentiation media are suitable for neurodegenerative disease modeling and drug screening.

F4005

INTRACEREBRAL TRANSPLANTATION OF HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURAL PRECURSOR CELLS CAN RESTORE NEUROLOGICAL DEFICITS IN A RODENT MODEL OF SUBACUTE-STAGE ISCHEMIC STROKE

Noh, Jeong-Eun[1], Lee, Suji[1], Ko, Jung Jae[1], Oh, Seung-Hun [2], Ju, Ji Hyeon[3], and **Song, Jihwan[1]**, CHA Stem Cell Institute, Department of Biomedical Science, CHA University, Gyeonggi-do, Korea, [2]Department of Neurology, CHA Bundang Medical Center, CHA University, Gyeonggi-do, Korea and [3]Department of Internal Medicine, St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea

There is increasing evidence that stem cells may provide therapeutic benefits in stroke via various mechanisms. However, whether such cells can be converted to functional neurons that survive and give rise to behavioral recovery after transplantation in the stroke-injured cerebral cortex and striatum is not clear. In the present study, we have transplanted neural precursor cells derived from human induced pluripotent stem cells (hiPSC-NPCs), which were generated from adult human peripheral blood cells, into a rodent model of subacute-stage middle cerebral artery occlusion (MCAo). hiPSC-NPCs were transplanted intracerebrally at one week after MCAo, and the transplanted animals were examined up to 12 weeks using various behavioral tests. We observed that the animals showed significant behavioral improvements in rotarod, stepping, mNSS, staircase, and apomorphine tests compared with control groups (media-injected or fibroblast injected groups). Histological analyses indicate that the transplanted group exhibited robust survival and migration of cells towards the lesion at 12 weeks post-transplantation. While most of the cells still remain as Nestin-positive, some were differentiated into MAP2-positive mature neurons, GABAergic neurons, DARPP32-positive medium spiny neurons, and so on. We also found that the transplanted animals exhibited significant increase in the number of proliferating neuroblasts in the subventricular zone (SVZ), and in the angiogenesis in penumbra area of ischemic striatum. Taken together, these results strongly suggest that neural precursor cells from adult human peripheral blood-derived induced pluripotent stem cells (hiPSC-NPCs) may serve as a useful candidate to treat stroke in the future.

F4007

FABRICATION OF SALT-INDUCED ELECTROSPUN PATTERNED (SIEP) BUNDLED FIBERS AND THEIR EFFECTS ON NEURAL CELL RESPONSES

Cho, Mira[1], Kim, Seung-Hyun[2], Jin, Gyuhyung[2], KIM, Yoojin[2], Park, Kook In[3] and Jang, Jae-Hyung[2], [1]Yonsei University, Seoul, Korea, South, [2]Department of Chemical and Biomolecular Engineering, Yonsei University, Seoul, Korea, South, [3]Department of Pediatrics, College of Medicine, Yonsei University, Seoul, Korea, South

Surface topography in the patterned fibrous matrix has been a significant factor for spatial manipulation of the cell behaviors (e.g. cell adhesion, orientation, proliferation, and differentiation). Especially for neural engineering approaches, regulation of cell structures in terms of striated alignment or directional growth of axons are key elements for the functional recovery of damaged nervous systems. Thus, understanding the structural parameters of patterned fibrous scaffolds that can effectively promote neural regeneration can provide crucial clues for designing advanced tissue engineering scaffolds. In this study, salt-induced electrospun patterned fiber bundles (i.e., SiEP-bundles) which consist of the longitudinally stacked multiple fibers were fabricated, and their capabilities of spatially stimulating the responses of neural cells, including PC12 cells, chick dorsal root ganglia (DRG), and human neural stem cells (hNSCs) were analyzed by comparing them with conventionally fabricated 2-dimensional fibrous sheet having either randomly oriented fibers or individually aligned fibers. The SiEP-bundles possessed remarkably distinctive morphological and topographical characteristics: multi-complexed infra-structures with nano- and micro-scale fibers, rough surfaces, and soft mechanical properties. Importantly, the SiEP-bundles resulted in spatial cellular elongations corresponding to the fiber directions and promoted neurite extensions along the patterned fibers. Furthermore, the residence of hNSCs on the topographically rough grooves of the SiEP-bundles boosted neuronal differentiation. These findings can provide crucial clues for advanced fibrous scaffolds that can spatially regulate cellular behaviors, potentially offering powerful strategies for regenerating the peripheral or central nervous system.

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F4009

DETAILED FUNCTIONAL AND GENE EXPRESSION PROFILING UNCOVERS THE EARLY WOLFFIAN DUCT MATURATION PROCESSES

Taguchi, Atsuhiro, IMEG Kumamoto University, Kumamoto City, Japan and Nishinakamura, Ryuichi, Institute of Molecular Embryology & Genetics, Kumamoto University, Kumamoto City, Japan

Kidney derives from embryonic metanephros which develops by the reciprocal interaction of the mesenchymal blastema; metanephric mesenchyme (MM) and the epithelial primordium; ureteric bud (UB). Although both of them are reportedly derived from common Osr1+ intermediate mesoderm, the mechanisms how they acquire distinct cellular identities during early embryogenesis are largely unknown. Recently we find that the origin of MM and UB are segregated along the antero-posterior axis within the intermediate mesoderm at E9.5. Further examination at the earlier stages utilizing the T-GFPCreER mice indicated the timing of differentiation from T-positive immature state into T-negative state determines the antero-posterior positioning within the intermediate mesoderm. Here we further analyzed the functional capacity of early stage UB precursors; i.e. the Wolffian duct (WD) by developing the kidney reconstitution assay which evaluates the dichotomous branching capacity of the WD. The results showed the WD isolated from E10.5 and E11.5 reconstructed branching UB regardless of its antero-posterior position. On the other hand, the WD from E9.5 or E8.5 embryo failed to branch both from the rostral or caudal source. Further gene expression examination by microarray analysis identified the distinct groups of gene sets which are categorized into “cellular identity markers”, “tip identity markers” and “maturation markers”. In summary, our results indicate the functional maturation process of Wolffian Duct takes place regardless of the antero-posterior axis. Whereas, the gene expression profile suggests the distinct cellular character between stalk and tip positioning.

F4011

RAPAMYCIN HAS SUPPRESSIVE EFFECTS OF DOXORUBICIN MEDIATED CARDIOTOXICITY THROUGH AUTOPHAGY SIGNALING REGULATION IN ENDOGENOUS CARDIAC PROGENITOR CELLS

Park, Ji Hye, Pusan National University School of Medicine, Yangsan/ Beomeo-ri, Mulgeum-eup, Korea, South

Although doxorubicin (DOXO) is widely used for chemotherapy against various solid cancer and childhood cancer, but using the DOXO is limited by serious cardiac toxicity through loss of cardiomyocyte. Recently reported researches suggested that endogenous cardiac stem/progenitor cells (eCPC) play important roles in cardiomyocyte homeostasis. Furthermore, most recently reported research suggested that DOXO occurred eCPC depletion. However, these underlying mechanisms have not been fully demonstrated. In addition, autophagy has emerging signaling pathway for regulation of cellular bioactivities, such as proliferation, differentiation and senescence. Thus, in this study, we first examined whether autophagy signaling regulation could rescue to DOXO-mediated eCPC dysfunction. For this study, c-kit positive eCPCs were isolated from infant-derived cardiac tissues as previously reported. To determined optimal DOXO concentration in eCPC, we used eCPC viability using MTS assay. As shown result, over the 500nM of DOXO treatments were significantly reduced eCPC viability. Next, immunoblotting was used to detect expression of regucalcin(SMP30; calcium regulator protein), mTOR, and LC3 (autophagy maturation related protein). SMP30 and LC3 expressions were time dependently reduced after DOXO treatment in eCPC. However, mTOR expressions were significantly increased after treatment with DOXO in eCPC. Next, we examined whether administered with rapamycin (mTOR inhibitor) could rescue SMP30 and LC3 expression. After treatment with rapamycin, reduced SMP30 and LC3 expressions were significantly increased in DOXO-induced eCPC. Additionally, intracellular Ca^{2+} levels were analyzed by Fluo-8 assay, and reduced Fluo-8 level in DOXO-treated eCPC groups were significantly reduced fluorescence intensities after treatment with rapamycin. From the above results, rapamycin could be rescue autophagy formation and SMP30 expression DOXO-treated eCPC through mTOR inhibition and intracellular Ca^{2+} handling. Thus, rapamycin might be suppressive effects of DOXO-mediated cardiotoxicity through autophagy signaling regulation in eCPC.

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F4013

CRITICAL ROLE OF WNT SIGNALING IN FOLLICULAR MELANOCYTE STEM CELLS

Sun, Qi[1], Hu, Hai[1], Takeo, Makoto[1], Lee, Wendy[1], Taketo, M. Mark[2], Millar, Sarah[3] and Ito, Mayumi[1], [1]New York University School of Medicine, New York, NY, U.S., [2]Kyoto University, Kyoto, Japan, [3]University of Pennsylvania, Philadelphia, PA, U.S.

Wnt signal controls stem cell behavior during homeostasis, regeneration and cancer in a variety of tissues in the body. How this signaling pathway impacts stem cells in the melanocytic lineage is not completely understood. Our study reveals that extrinsic Wnt ligands produced

by epithelial niche cells are required for the function of melanocyte stem cells (McSCs) that are responsible for hair pigmentation. Upon exposure to Wnt ligands at the initiation of hair regeneration, McSCs give rise to mature melanocytes that produce pigment for the hair. Loss and gain of function of Wnt signaling in McSCs revealed that temporal activation of Wnt signaling promotes McSC differentiation and simultaneously maintains their self-renewing capacity. We also found that McSCs undergo prolonged Wnt activation during aging. This causes the loss of their self-renewing capacity similar to what is seen with constitutive Wnt activation in McSCs. In contrast, once McSCs undergo malignant transformation induced by oncogenic mutations, constitutive Wnt activation promotes their differentiation without diminishing the self-renewing capacity. This results in an aggressive melanoma with a highly differentiated melanocytic phenotype. These results show that the Wnt signaling pathway is critical to fine-tune the balance between self-renewal and differentiation of McSCs, and these regulatory mechanisms are dysregulated during cancerous transformation of McSCs.

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F4015

JMJD2A REGULATES PITUITARY TRANSCRIPTION FACTORS AND IS REQUIRED FOR FEMALE FERTILITY

Sankar, Aditya[1], Kooistra, Susanne Marije[1,2], Gonzalez, Javier Martin[1], Bagger, Frederik Otzen[3], Ohlsson, Claes[4], Poutanen, Matti[4,5] and Helin, Kristian[1],
[1]University of Copenhagen, Denmark, [2]University of Groningen, Netherlands, [3]EMBL, Cambridge, U.K., [4]University of Gothenburg, Sweden, [5]University of Turku, Finland

The modification state of histones affects chromatin structure and transcriptional competence. Jmjd2a is a histone demethylase with specificity toward di-and tri-methylated H3K9 and H3K36. However, there is little understanding of its role in normal development and homeostasis. Here, we report that female mice deficient for Jmjd2a are infertile. Jmjd2a$^{-/-}$ females show normal reproductive-organ anatomy, ovulation and intra-ovarian steroidogenesis post fertilization. However, they display major defects in timed sexual activity, pre-implantation embryo development, implantation rates, embryonic stem cell establishment and the capacity to maintain pseudopregnancy. Significantly lower levels of circulating LH, PRL, P4 and TST in blood serum point towards a disrupted pituitary-gonad signaling axis. In line with this hypothesis, we show reduced expression of several prominent immediate-early genes (IEG) transcription factors such as c-Fos, Egr1, Ier2 and Junb while accompanied by increased expression of Egr1 co-repressor Nab2. c-Fos and Egr1 which have well-documented functions in maintenance of fertility whose null mutants share several phenotypes observed in Jmjd2a null females. Widespread stimulation of IEGs is achieved through immune signals. As an epigenetic mechanism, we find that the ligand specific GM-CSF receptor Csf2ra is an unique Jmjd2a target showing strong gain of H3K9me3 seen at its TSS in Jmjd2a null females. GM-CSF driven IEG priming (specifically for Egr1 among others) has been previously demonstrated in humans and mice. We propose that female infertility in Jmjd2a is a complex phenotype – effected by immune response driven de-regulation of pituitary TFs critical for maintenance of the pituitary-gonad signaling axis.

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F4017

A NOVEL ADENO-ASSOCIATED VIRAL VARIANT ENGINEERING FOR ENHANCED TRANSDUCTION OF NEURAL STEM CELLS

KIM, Yoojin[1], Cho, Mira[2] and Jang, Jae-Hyung[1],
[1]Department of Chemical and Biomolecular Engineering, Yonsei University, Seoul, Korea, South,
[2]Yonsei University, Seoul, Korea, South

Efficient gene delivery technology for stem cell engineering would make tremendous contribution to the future biomedical fields. A novel adeno-associated viral variant, AAVr3.45, was developed by directed evolution method in the purpose of effective targeting of non-permissive cells, especially neural stem cells (NSCs). To improve the transduction efficiency by inducing more direct interaction between AAVr3.45 and NSCs, AAVr3.45 was complexed with catechol conjugated heparin; heparin and catechol can cause interaction with heparin binding site on AAVr3.45 capsid and cell membrane, individually. Finally, catechol conjugated heparin complexed AAVr3.45 shows highly enhanced transduction to NSCs. Moreover, we found that AAVr3.45 has better neutralizing antibody evading ability compared to AAV2 does, which is the main origin of AAVr3.45's development, despite the small difference of one point mutation and peptide insertions between them. We demonstrated the reason of AAVr3.45's resistant from neutralization by gene correction and confirmed that the point mutation at the specific site of AAVr3.45 plays an important role in inspiring strengthened features. These promising properties of AAVr3.45 suggest the possibility of its future creative applications in tissue engineering, such as in vivo stimuli-responsive NSCs engineering using AAVr3.45 delivered by scaffolds.

F4019

REGULATION OF MAMMALIAN DNA METHYLATION BY TRANSCRIPTION FACTOR BINDING

Schmidt, Juliane[1], Krebs, Arnaud[1], Stadler, Michael B.[1], Burger, Lukas[1], Akalin, Altuna[2] and Schübeler, Dirk[1], [1]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland, [2]Max Delbrück Center for Molecular Medicine, Berlin, Germany

Transcriptional regulation in mammals is realized through intricate interactions between transcription factors and chromatin. DNA methylation constitutes a repressive chromatin modification leading to stable transcriptional silencing of repetitive elements, the inactive X chromosome and imprinted regions. Whole-genome bisulfite sequencing in mammals has revealed widespread cytosine methylation with notable hypomethylation at cis-regulatory elements. Hypomethylation can be found within so-called CpG islands and distal CpG-poor regions. Previous investigations have shown, that some DNA-binding factors directly reduce local methylation at these distal sites. However, how DNA-binding factors mediate methylation changes remained unclear. We have studied the regulation of DNA methylation by the transcriptional repressor RE1-silencing transcription factor (REST) in mouse embryonic stem cells. While the full-length protein is necessary and sufficient to reduce methylation at its binding sites, several REST mutants lack this ability. We have profiled DNA methylation, chromatin accessibility and nucleosome positioning and characterized differences between mutants. Eventually, we describe endogenous transcription factors that show similar behavior.

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F4021

A GENE REGULATORY NETWORK ESTABLISHES RETINOIC ACID MEDIATED FEEDBACK TO BALANCE NEURAL AND MESODERM SPECIFICATION AND CONTROL EMBRYO ELONGATION

Gouti, Mina[1,2], Delile, Julien[1], Stamataki, Despina[1], Huang, Yali[3], Wymeersch, Filip[3], Wilson, Valerie[3] and Briscoe, James[1], [1]The Francis Crick Institute, London, U.K., [2]Max Delbruck Center, Berlin, Germany, [3]MRC Centre for Regenerative Medicine, Institute for Stem Cell Research, Edinburgh, U.K.

Transcriptional networks, regulated by extracellular signals, control cell fate decisions and determine the size and composition of developing tissues. One example is the network controlling bipotent neuromesodermal progenitors (NMPs) that fuel vertebrate embryo extension. Here we use single cell transcriptomics to define the molecular signature of NMPs and compare *in vitro* generated NMPs with their *in vivo* counterparts. The analysis reveals a striking similarity between the two populations and allows the reverse engineering of the transcriptional network responsible for NMP induction and differentiation. Targeted perturbations identify a network comprising the transcription factors Cdx1,2,4, T/Bra, Sox2, Msgn1, and Tbx6, that integrates Wnt and Retinoic Acid (RA) signalling to regulate entry to and exit from the NMP state. RA, produced by newly generated mesodermal cells provides feedback that promotes NMP identity and induces neural differentiation thereby ensuring the balanced production of neural and mesodermal tissue. Together the data identify a network architecture that coordinates the generation of different cell types from bipotential progenitors and facilitates orderly axis elongation.

F4023

PEAKS AND VALLEYS IN CELL BEHAVIOR AND GENE EXPRESSION DURING THE AGING OF THE SUBVENTRICULAR ZONE OF MUS MUSCULUS

Apostolopoulou, Maria[1], Kiehl, Thomas R[1], Winter, Mark[2], Cardenas, Edgar[2], Goderie, Susan K.[1], Wang, Yue[1], Cohen, Andrew[2] and Temple, Sally[1], [1]Neural Stem Cell Institute, Rensselaer, NY, U.S., [2]Drexel University, Philadelphia, PA, U.S.

Neurogenesis in the subventricular zone (SVZ) decreases markedly with aging, thought to occur by a unidirectional decline. However, after conducting an unbiased analysis of SVZ transcriptome at 2, 6, 18 and 22 months, we found that in addition to trends following age, numerous genes had surprising maximal or minimal expression at 18 months, including progenitor and cell cycle-related genes. In vivo, transit amplifying Type C cell number and proliferation also exhibit a nadir at 18 months. We then followed the proliferation and differentiation of isolated SVZ cells in vitro. Lineage analysis of 944 clones showed that age-related declines in neurogenesis were recapitulated. Moreover, even after isolation from the niche, Type C cells show U-shaped cell proliferation rates with age. Our findings indicate that age-related changes in the SVZ are not only monotonic but can exhibit peaks and valleys, and that programmed changes in progenitors are key drivers of neurogenic aging.

F4025

PROSPECTIVE ANALYSIS OF MOUSE LIMB MESENCHYMAL STROMAL POPULATIONS DURING EMBRYONIC DEVELOPMENT AND POSTNATALLY

Nusspaumer, Gretel[1], Jaiswal, Sumit[2], Barbero, Andrea[3], Ronen, Dana[2], Christofori, Gerhard[2], Martin, Ivan[3] and Zeller, Rolf[2], [1]Department of Biomedicine/ University of Basel, Switzerland, [2]University of Basel, Switzerland, [3]University Hospital Basel, Switzerland

Our work shows the relationship among the most relevant mouse mesenchymal stromal populations prospectively identified so far. We addressed the mesenchymal stromal compartment during limb embryonic development, early postnatally and during adulthood. The analysis was performed by multicolor flow cytometry analysis in Sox-9$^{IRES-EGFP}$ mice and in the PrxCre; b-Actin >ST> EGFP double transgenic line. The different mesenchymal stromal populations were sorted early postnatally (day 2-4) and in adult animals (10-13 weeks) and the expression of key genes associated with chondrogenesis, osteogenesis and hematopietic stem cell maintenance and differentiation was compared. We identified four new subsets within PaS progenitors that were tested in tri-lineage differentiation assays. Chondrogenic molds obtained with two PaS subsets were subcutaneously engrafted in nude animals and the explants were analyzed after 8 weeks.

F4027

REGIONAL SPECIALIZATION OF THE ADULT MENINGES SUPPORTS DIFFERENT BRAIN AREAS

Allen, Christina, Neural Stem Cell Institute, Rensselaer, NY, U.S.

The meninges are a protective outer covering for the brain and consist of three layers, the dura mater, arachnoid mater, and pia mater. During brain development, the meninges secrete growth factors that stimulate neural proliferation and ensure proper migration in the forebrain and hindbrain. The pia mater, combined with the basement membrane, serve as a scaffold to migrating neurons. Adult meninges continue to provide neural protection and survival to the central nervous system during trauma, infection, and disease. We have recently found that adult meninges have regionally distinct genetic identities. Expression levels of IGF1&2 in posterior meninges are significantly more abundant than in anterior meninges. These differences suggest that meninges provide a specific and varied mitogenic role to neighboring cells. We have also shown that anterior and posterior meninges promote functional differences when placed with anterior and posterior cortical cells. Variations between anterior and posterior meninges in the survival of cortical cell cultures are attributed to differences in the diffusible factors released by the meninges. Disruption to the dispersion of these growth factors could have detrimental effects on disease susceptible cells like neurons within the entorhinal cortex, those cells that initially and preferentially die in Alzheimer's disease, making it plausible that meninges might play a role in the initiation or progression of neurodegenerative disease. We hypothesize that adult anterior and posterior meninges provide distinct functional support to sub-adjacent brain cells. Thus, detrimental impacts on neuronal cell types could result without proper support from regionally defined meninges.

F4029

UNDERSTANDING HOW DISTINCT TUMOR CELL CLONES COMMUNICATE IN GLIOBLASTOMA

Nister, Monica[1], Guo, Min[1], Zhao, Jian[1], Jin, Shao-Bo[1], Heller, Susanne[2], van Vliet, Marjolein[1], Liu, Tong[1], Lendahl, Urban[1] and Hägerstrand, Daniel[1], [1]Karolinska Institutet, Stockholm, Sweden, [2]Uppsala University, Uppsala, Sweden

Tumor cell heterogeneity constitutes a major challenge in cancer treatment. Interactions between genetically different tumor cell subclones affect overall tumor growth. To identify factors that contribute to interclonal effects we have used the U343 cell culture system, which consists of a panel of cell clones derived from a single human glioblastoma tumor. Here we show that U343MG cells have invasive capacity in vitro and express elevated mRNA levels of mesenchymal genes, including SNAI2, LAMC1 and FN1. In contrary, other clones are less invasive and express high mRNA levels of the stem cell factor SOX2 and the astrocytic intermediate filament protein GFAP. Co-culture and conditioned media experiments illustrate how different tumor cell subclones affect each other´s growth, differentiation, and stemness via secreted and/or cell-attached factors. For example, U343MG elicited differentiation and growth inhibitory effects on GFAP+ clones via activated Notch signaling. Gene expression, proteomic and functional genomic approaches will pinpoint the specific signaling pathways that elicit inter-clonal effects. Further studies on cell-to-cell communication in glioblastoma may provide novel therapeutic targets.

F4031

DIFFERENTIAL GLUCOSAMINE UTILIZATION BY EMBRYONIC STEM CELLS FROM MOUSE STRAINS WITH DIFFERENTIAL SUSCEPTIBILITY TO DIABETIC PREGNANCY-INDUCED NEURAL TUBE DEFECTS

Loeken, Mary R. and Jung, Jin Hyuk, Joslin Diabetes Center, Boston, MA, U.S.

Maternal diabetes increases birth defects in humans and experimental animals, a diabetic complication referred to as 'diabetic embryopathy'. Strain differences in risk for diabetic embryopathy have been found both in mice and rats. We have shown that FVB mouse embryos are susceptible to neural tube defects (NTDs) and inhibition of Pax3, a gene required for neural tube closure, in diabetic pregnancy, while C57Bl/6J (B6) embryos are resistant. We propose to use these strain differences to identify pathways responsible for diabetic pregnancy-induced NTDs. The high K_M glucose (Glc) transporter, Glut2, is essential for diabetic embryopathy in susceptible strains. The normal function of Glut2 in embryos may be to take up exogenous glucosamine (GlcN) for protein glycosylation, thereby sparing fructose 6-PO_4 + glutamine (gln) from GlcN synthesis. We hypothesized that differential transport and metabolism of Glc and/or GlcN may be responsible for strain-dependent risk for diabetic embryopathy. We tested this using embryonic stem cells (ESC) derived from FVB or B6 blastocysts. ESC were grown undifferentiated (UD), modeling embryonic day (E) 3.5 inner cell mass, or as differentiating (D) neuronal precursors, modeling E 8.5 neuroepithelium. Like maternal hyperglycemia in vivo, high Glc media inhibited Pax3 expression by D FVB ESC, but high glucose did not inhibit Pax3 expression by D B6 ESC. There were many differences in protein levels of several Glc transporters (-1, 2, -3), hexokinases (-1, -2, -4), and gln transporters (Asct2, Snat1, Lat1, Snat5) between FVB and B6 ESC at each stage, and between UD and D stages of each line. GlcN increased numbers of pluripotent (alkaline phosphatase (AP) –positive) FVB ESC, but only increased AP staining of B6 ESC. GlcN increased O-glycosylation by both lines, but increased N-glycosylation only by FVB ESC. GlcN increased activity of the pentose shunt pathway, which is important for control of oxidative stress in FVB but not B6 ESC. These results suggest that B6 embryos may be less dependent on exogenous (maternal) GlcN than FVB embryos, but this differential utilization and synthesis of GlcN may protect from increased Glc uptake and embryopathy during maternal diabetes.

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F4033

STEM CELL COLLECTION AND PRACTICES IN A TERTIARY CARE HOSPITAL IN INDIA

Kaura, Ashima, DMC&H, Ludhiana, India

The incidence of stem cell collection of cord blood and placental tissue after delivery in a tertiary care hospital in India was 8%. A study was conducted to find out its low incidencence.All deliveries that took place in this hospital from January 2014 to January 2016 were given questionaires which included the demographic profille of the patients,their awareness about stem cell collection and cost effectiveness.It was found that out of 2468 deliveries 197 got the collection done (8%).Only 12.3% patient had the knowledge of the stem cells which was obtained by social media(30.2%),through professionals (70.4%) and friends (22%).Of the social media online information was obtained by 60.3% patients,television contributed Information to 2.2% and magazines 2%.72.4% patients refused collection of stem cells due to lack of funds. 75.6% patients were educated and 40% patients belonged to upper and middle class income group and these two groups were more inclined towards stem cell collection. India is a developing country where stem cell collection after delivery is limited to upper class who can afford and are aware of its usefulness ,metropolitan cities and private health sector.Stem cell collection advertisement is not a part of the government policy so reaching out to general masses is difficult .Availability of funds to underprivelidged can increase the collection rate.

F4035

CANINE MESENCHYMAL STEM CELL VIABILITY IS AFFECTED BY TIME AND TEMPERATURE OF COOLING

Alvarenga, Marina Landim[1], Daroz Guastali, Midyan[2], De Vita, Bruna[2], de Paula Freitas Dell'Aqua, Camila[2], da Cruz Landim- Alvarenga, Fernanda[2] and Assad Hassun Filho, Pericles[1], [1]OMICS Animal Biotechnology, Botucatu, Brazil, [2]FMVZ- UNESP, Botucatu, Brazil

Mesenchymal stem cells (MSCs) represent a great therapeutic promise due to their immunosuppressive and regenerative capacities. As allogeneic canine MSCs do not evoke a strong immune response, their clinical use for cell-based transplantation may provide an alternative for treatment of several disorders. Regardless of the success achieved in cryopreservation storage of dogs MSCs, many important technical issues remain, concerning mainly transport of these cells for clinical use. In this context, the present experiment aimed to compare different times and temperature of cooling during dogs MSC transport. For such, samples of adipose tissue were collected from 3 health dogs, processed and cultured in DMEM high glucose supplemented with 20% fetal bovine serum,

antibiotics and antimycotics at 37.5ºC in a humidified atmosphere, containing 5% CO_2 in air. At the end of third passage MSCs were trypsinized, ressuspended in 5mL of fresh culture media and incubated in 15mL falcon tubes in a cooling device designed to transport semen (Botuflex®, Botupharma, Botucatu, Brazil). The cells were kept at a constant temperature of 23 (room temperature), 15 and 5ºC for a period of 24, 48 and 72 hours. At the end of each incubation period the cells were analyzed by flow cytometry (FC) for cell viability and apoptosis with Alexa fluor 488 and PI. After 24 of incubation the cooled samples presented higher viability compared to the cells kept at room temperature, (67.8, and 90% for cells kept at room and cooled temperature respectively). When the cooled time was extended to 48 hours, cell viability was also lower at room temperature (58.8, 79 and 86.1% for cells maintained at 23, 15 and 5ºC respectively). The same pattern was maintained after 72 hours of incubation (53.6, 66.6 and 75.8 for room, 15 and 5ºC respectively). The number of apoptotic cells was also increase at room temperature in all groups with values of 29.5, 35 and 29.5 for cell incubated for 24, 48 and 72 hours respectively. Higher viability and lower apoptosis rate was observed when cells were kept at 5ºC either for 24 or 48 hours. The results showed that even during short periods of transport the control of temperature is important to maintain high cell viability, since the incubation of MSC at room temperature significantly increase apoptosis rates.

F4039

HUMAN NEURON-BASED HIGH-THROUGHPUT SCREENING PLATFORMS FOR NEURODEGENERATIVE DISEASES

Du, Zhong-Wei[1], **Hendrickson, Michael**[1] and Zhang, Suchun[1,2], [1]BrainXell, Inc., Madison, WI, U.S., [2]University of Wisconsin-Madison, Madison, WI, U.S.

Drug screening for neurodegenerative diseases often employs fibroblasts or lymphocytes immortalized from patients and ectopic reporter transgenes, leading to high failure rates in clinical trials. The selective vulnerability of subtype-specific neurons is a defining feature of neurodegenerative disease. Therefore, subtype-specific neurons are more relevant target cells for drug screening. In this study, we established disease iPSC reporter lines by CRISPR technology to target only one copy of a sensitive reporter (Nanoluc) into the endogenous disease related gene locus, such as the SMN2 gene for spinal muscular atrophy or the NEFL gene for amyotrophic lateral sclerosis. Combined with our recently developed method to produce large-scale and pure subtype-specific neurons from iPSCs, we have established a neuron based high-throughput screening (HTS) platform. The HTS platform is optimized in a 384-well format, validated with previously identified compounds, and applied to a primary screening with the NIH clinical collection library. These results suggest that human stem cells can be readily engineered and formatted for phenotypic and mechanistic HTS screening for neurodegenerative diseases.

Funding Source: This study was supported by NIH-NINDS (NS085689), ALSA grant (15IIP194), and in part by a core grant to the Waisman Center from the National Institute of Child Health and Human Development (P30 HD03352).

F4041

THE EFFECTS OF HUMAN IPSC-DERIVED CORTICOSPINAL MOTOR NEURON TRANSPANTED INTO RAT CERVICAL HEMISECTION INJURY

Weimann, James M., Doulames, Vanessa and Plant, Giles W., Stanford Medical School, Stanford, CA, U.S.

Cervical spinal cord injury (SCI) accounts for over half current injuries and contributes significantly to life-long morbidity and mortality. The transplantation of neural stem cells and neuronal progenitors has the potential to rebuild circuits and repair functional deficits after SCI. Implantation of embryonic neural tissue has been well established over decades of research, however, this mixture of cells requires further elucidation with respect to their survival, integration and promoting axonal elongation. In addition, NPCs derived from embryonic stem cell and induced pluripotent stem cells have also shown some behavioral efficacy. We have used human induced pluripotent stem cells (iPSC) patterned towards cortical pyramidal neurons and implanted these cells, which are enriched for corticofugal neurons, into a cervical hemi-lesion in immunodeficient rats. Cells were characterized prior to transplantation and following 6 & 12 weeks. We find that the vast majority of transplanted cells survive and express markers of cortical neurons. In addition, extensive axon outgrowth within the cord can be observed. We have also characterized extensive growth of host axons into and beyond the graft. The goal of this project is to pattern hiPSC-dervied neurons towards a corticospinal motor neuron fate and to use these neurons to act as cellular relays in the lesion site. An array of behavioral test demonstrates partial recovery of forepaw function after 3 months post-transplantation. In addition, inactivation of the transplanted human neurons 30 weeks post-transplant reverses the behavioral gains in the rats. These results suggest that transplantation of human iPSC-derived cortical neurons into a lesion site can ameliorate some of the motor deficits associated SCI.

F4043

GSK3 INHIBITION INDUCED MORE EXPANDABLE AND PLURIPOTENT HUMAN HINDBRAIN PROGENITORS VIA GBX2 ACTIVATION

Su, Zheng Hui[1], Zhang, Yanqi[1], Liao, Baojian[2] and Pan, Guangjin[3], [1]Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences., Guangzhou, China, [2]GIBH, GuangZhou, China, [3]Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou, China

Differentiation of neural progenitor cells (NPCs) from human pluripotent stem cells (hPSCs) provides an unlimited cell source for replacement therapy for neural disease and drug screen platform. Dual SMAD inhibition (SB431542/Dosomophrin, S/D) has been proven efficient and stable for hPSCs neural differentiation, however, with limited proliferation ability and unclear differentiation mechanism. In this report, we showed that in addition to dual SMAD inhibition, blocking Glycogen synthase kinase3(GSK3) signaling by CHIR99021 (S/D/C) generated hindbrain biased NPCs(S/D/C-NPC). S/D/C-NPC possess metencephalic region identity, with significantly expandable property and differentiation bias to posterior neurons. Through human neural regionally induction system, we found that: (1) OTX2 and GBX2 palyed the key roles during human neural regional pattern at very early stage of induction. The high expression level of OTX2 in human pluripotent stem cells is one of determining factors for the phenomenon that the default neural differentiation goes to the rostral lineage. (2) GSK3 inhibition activated WNT/beta-catenin pathway which further activated GBX2. And then GBX2 antagonized OTX2, resulting in metencephalic formation. (3) OTX2 promoted PAX6 expression during rostral differentiation, while in hindbrain formation, it was the expression of SOX1 that elevated rather than PAX6. Our study based on the neural induction "default model" suggested that, artificially manipulating relevant signaling pathways could produce different NPCs. These manipulating might not exist in normal brain development, but could bring broad practical usage in clinical therapy.

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F4045

MECHANISMS TO ENHANCE TRANSCRIPTIONAL REPROGRAMMING OF FIBROBLASTS TO FUNCTIONAL CARDIOMYOCYTES

Zhou, Huanyu, Morales, Maria Gabriela, Hashimoto, Hisayuki, Dickson, Matthew, Bassel-Duby, Rhonda and Olson, Eric, University of Texas Southwestern Medical Center, Dallas, TX, U.S.

After a heart attack, millions to billions of cardiomyocytes are lost. Because the adult mammalian heart possesses little regenerative potential, a precipitous loss of cardiac function ensues. Patients with heart failure could benefit from repopulating injured areas of the heart with functional cardiomyocytes. To date, cellular transplantation has been therapeutically unsuccessful. Various combinations of kinase and cardiogenic transcription factors including Akt1,Gata4, Hand2, Mef2c and Tbx5 (AGHMT), can reprogram fibroblasts into induced cardiac-like myocytes (iCLMs) in vitro and in vivo. This direct lineage reprogramming offers a new approach to repopulate cardiomyocytes in the heart, but the technique thus far has been slow and inefficient in adult fibroblasts. Only 1% of adult fibroblasts displayed spontaneous beating after three weeks of induction by AGHMT. This indicates that there are "barriers" in adult fibroblasts that hinder cardiac reprogramming. We try to optimize methods for reprogramming fibroblasts to cardiomyocytes in vitro and in vivo. To identify additional regulators of this process, we carried out an unbiased screen of ~1,100 open reading frames (ORFs) encoding transcription factors and cytokines for the ability to enhance reprogramming by AGHMT. This screen, carried out in high-throughput, led to the discovery of 64 new activators of reprogramming and 263 barriers to this process. Many of these genes could not have been anticipated to impinge on the mechanisms of fibroblast-to-cardiomyocyte reprogramming and they offer opportunities to discover new facets to this mechanism. In the future, we intend to continue to explore the ways in which these various proteins interact to govern cardiac cell fate.

F4049

SECRETED PHOSPHOLIPASES A2 ARE STEM CELL NICHE FACTORS WITH DISTINCT ROLES IN HOMEOSTASIS, INFLAMMATION AND CANCER

Schewe, Matthias[1], Franken, Patrick[2], Sacchetti, Andrea[2], Cormier, Robert T[3], Lambeau, Gerard[4] and Fodde, Riccardo[1,2], [1]ErasmusMC, Rotterdam, Netherlands, [2]Erasmus MC, Department of Pathology, Rotterdam, Netherlands, [3]University of Minnesota Medical School, Duluth, MN, U.S., [4]IPMC CNRS, Sophia-Antipolis, France

Secreted phospholipases A2 (sPLA2s) hydrolyze membrane phospholipids to generate lipid mediators such as arachidonic acid which can be converted to eicosanoids. Group IIA sPLA2 is a genetic modifier of intestinal tumorigenesis expressed in the small intestine by Paneth cells from which secretion is enhanced upon inflammation. Group X sPLA2 is expressed in the colon by Paneth/goblet-like secretory cells. We show that both sPLA2s represent stem cell niche factors with specific context-dependent functional roles in the intestine. During homeostasis group IIA/X sPLA2s control homeostasis by inhibiting Wnt signaling through Yap1 from within the intracellular compartment. However, upon inflammation they are secreted into the intestinal lumen from where they support tissue regeneration by promoting prostaglandin synthesis and Wnt signaling. Notably, genetic ablation of both sPLA2s confers improved regenerative response to inflammation but also increased susceptibility to colon cancer due to the release of their Wnt-inhibitory role in homeostasis. This “trade-off” effect points to the critical role that sPLA2s are likely to exert as intestinal stem cell niche factors and genetic modifiers of inflammation and colon cancer in man.

Funding Source: KWF Dutch cancer society

F4051

GENERATION OF TRANSGENIC FUS MODELS IN THE ADULT ZEBRAFISH

Mashkaryan, Violeta[1], Cosacak, Mehmet Ilyas[2] and Kizil, Caghan[1,2], [1]CRTD, TU Dresden, Germany, [2]German Centre f. Neurodegenerative Diseases (DZNE), Helmholtz Association, Dresden, Germany

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease (ND) that leads to a death of motor neurons. Among many mutations in different ALS-associated genes, mutations in FUS (Fused in Sarcoma) are known to be the cause of some forms of familial ALS. The majority of the identified mutations are located in the C-terminus of FUS. We are establishing and characterizing transgenic FUS zebrafish models that carry a particular point mutation at the nuclear localization signal of C-terminus. Two lines that carry either the full length or the C-terminus of the mutant FUS were generated. This particular isoform of FUS is known to cause an aggressive form of ALS in humans with an early onset of the disease. Using zebrafish as a model organism we are able to study the molecular pathways of the CNS regeneration in the context of NDs thus having an excellent tool for the understanding of neural stem cell biology underlying the regenerative processes. The addition of transgenic zebrafish model of FUS mutations can potentially lead to a development of novel therapeutic approaches for ALS and other NDs by activation of neural stem cell potential that is evolutionary lost in the mammalian organisms.

F4053

L-PROLINE DRIVES NEURAL DIFFERENTIATION FROM MOUSE EMBRYONIC STEM CELLS VIA EARLY PRIMITIVE ECTODERM-LIKE CELL, DEFINITIVE ECTODERM AND NEURECTODERM POPULATIONS

Shparberg, Rachel Adina, Mason, Timothy and Morris, Michael B., Bosch Institute and Discipline of Physiology, School of Medical Sciences, University of Sydney, NSW, Australia

In order to build a functional nervous system, cells of the mammalian embryo transition through primitive ectoderm, definitive ectoderm and neurectoderm populations before becoming terminally differentiated neural cells. Unlike the mesendoderm lineages where marker expression and signal requirements are relatively well understood, the molecular mechanisms that drive definitive ectoderm (and subsequently, neurectoderm) differentiation are poorly defined, partly due to a lack of lineage characterization. Therefore, the aim of our study is to develop a protocol using mouse embryonic stem cells (mESCs) to recapitulate the stepwise progression of neural lineage commitment via embryologically relevant populations including primitive ectoderm, definitive ectoderm and neurectoderm populations. D3 and 46C-Sox1-GFP mESCs were cultured as embryoid bodies (EBs) for 9 days using concentration- and time-dependent additions of L-proline and nodal (mesendoderm) inhibitor, SB431542. EBs were seeded in serum-free conditions from day 9 and allowed to differentiate for a further 6 days, after which they were assessed for neural cell production. A significant increase in the number of neural EBs was observed in L-proline-treated EBs (D3: 60.2% +/- 14.1%; 46C: 53.8% +/- 7.3%, n > 3) compared to basal medium controls (D3: 16.1% +/- 5.1%; 46C: 9.4% +/- 4.4%, n > 3). This was confirmed by immunofluorescence staining of the neural markers Nestin, BLBP and NeuN. qPCR showed L-proline-treated EBs sequentially differentiated from mESCs (Rex1$^+$ and Nanog$^+$) to primitive ectoderm (Dnmt3b$^+$ and Fgf5$^+$)

and then definitive ectoderm (Penk1^{+}) by days 3 and 5, respectively. By day 7, Sox1 and Nestin (neurectoderm) expression significantly increased (at the expense of the mesendoderm marker Mixl1^{+}) compared to undifferentiated mESCs in both cell lines. Taken together, we have developed a protocol that models embryonic neural lineage commitment in vitro using the unique growth factor-like properties of the amino acid L-proline. This protocol will allow for future investigations into the molecular mechanisms driving neural lineage development in the early embryo.

F4055

USING IPS CELL-DERIVED OLIGODENDROCYTE PROGENITOR CELLS TO RESCUE A MOUSE MODEL OF KRABBE DISEASE

Tian, E[1], Chao, Jianfei[2], Ye, Peng[2] and Shi, Yanhong[2], [1]City of Hope, Duarte, CA, U.S., [2]City of Hope National Medical CenterBeckman Research Institute, Duarte, CA, U.S.

Krabbe disease (KD) is a devastating neurological disorder involving the central and peripheral nervous system. It is caused by an autosomal recessive deficiency in the gene encoding the lysosomal enzyme GALC. Currently, the only therapy for KD is hematopoietic stem cell (HSC) transplantation using bone marrow or cord blood. However, HSC transplantation is only partially effective, likely because relatively few HSC-derived cells can cross the blood-brain barrier to rescue therapeutic defect in central nervous system. Therefore, alternative therapeutic approaches are urgently needed. In our study, we generate KD iPSCs from patient dermal fibroblasts and differentiate iPSCs into pre-oligodendrocyte progenitor cells (pre-OPC). These pre-OPCs were corrected by WT-GALC in vitro. Our results show that corrected pre-OPCs had significantly increased GALC enzymatic activity. After transplantation into Krabbe disease mouse model (twitcher mouse), those cells were able to restore the body weight and motor function of twitcher mice. The study will provide a novel method for therapy of the Krabbe disease in the near future.

POSTER SESSION III EVEN

PRE-CLINICAL AND CLINICAL APPLICATIONS OF MESENCHYMAL CELLS

F1002

INTRATHECAL TREATMENT USING AUTOLOGOUS MESENCHYMAL STEM CELL NEURAL PROGENITORS (MSC-NP) MAY IMPROVE ESTABLISHED DISABILITY IN MS: RESULTS OF A PHASE I OPEN-LABEL TRIAL

Harris, Violaine[1], Blackshear, Leslie[1], Joo, Gloria[1], Vyshkina, Tamara[1], Stefanova, Valentina[1] and Sadiq, Saud[2], [1]Tisch MS Research Center of New York, New York, NY, U.S., [2]Tisch Multiple Sclerosis Research Center of New York, New York, NY, U.S.

Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the CNS associated with a progressive clinical course and significant physical disability. There is a critical unmet need to develop therapies that enable repair and neuroprotection. Mesenchymal stem cell-neural progenitors (MSCNPs) are a bone marrow-derived cell population with regenerative potential. Preclinical studies in the EAE mouse model of MS showed that 3 doses of MSCNPs delivered intrathecally (IT) resulted in improved neurological function associated with suppression of local inflammatory response and trophic support for damaged cells at the lesion site. The objective of this FDA-approved phase I clinical trial was to determine safety and tolerability of autologous MSCNPs administered IT in 3 doses of up to 10 million cells per injection, spaced 3 months apart. Twenty MS patients with established disability and relatively stable disease were enrolled. MSCNPs expanded from autologous bone marrow were batch-tested for quality, sterility, and chromosomal stability. Primary safety outcomes included adverse event assessments. Secondary outcomes to observe trends in efficacy included a patient-based quality of life assessment, neurological exam, MRI, evoked potentials, and urodynamic testing. All of the 20 enrolled study participants have received autologous IT-MSCNP treatment and the follow up assessments are ongoing. There were no serious adverse events, but minor adverse events such as transient headache or fever occurred in approximately 65% of patients. Improvement in motor function as assessed by patient self-report, neurological examination, or walk speed over 25 feet was seen in 70% of patients. Sustained improvement in disability scores (EDSS) was seen post treatment in the majority of patients. In addition, 60% of patients with bladder dysfunction at baseline demonstrated symptomatic improvement supported by significant increases in urodynamic volumes post-treatment. These interim results indicate

that IT MSCNP treatment is safe and well-tolerated, and may reverse established disability as assessed by motor strength and function as well as bladder function in a majority of treated patients. The MSCNP trial is the first of its kind to test IT administration of neural progenitors as a regenerative therapy in MS.

F1004

HUMAN PLATELET LYSATE PATHOGEN REDUCED THROUGH ADDITIVE-FREE UV-C LIGHT IRRADIATION RETAINS ITS OPTIMAL EFFICACY FOR THE EXPANSION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS

Viau, Sabrina[1], Chabrand, Lucie[2], **Eap, Sandy**[2], Goudaliez, Francis[1], Sumian, Chryslain[1] and Delorme, Bruno[2], [1]Macopharma, Tourcoing, France, [2]Macopharma, Mouvaux, France

We recently developed and characterized a standardized and clinical grade human Platelet Lysate (hPL) that constitutes an advantageous substitute for fetal bovine serum for human mesenchymal stem cell (hMSC) expansion required in cell therapy procedures, avoiding xenogenic risks (virological and immunological) and ethical issue. Because of the progressive use of pathogen reduced (PR) labile blood components, we evaluated the impact of the novel procedure THERAFLEX UV-Platelets for pathogen reduction on hPL quality (growth factors content) and efficacy (as a medium supplement for hMSC expansion). This technology is based on short-wave ultraviolet light (UV-C) and has the main advantage not to need the addition of any photosensitizing additive compounds (that might secondary interfere with hMSCs). We applied THERAFLEX UV-Platelets procedure on fresh platelet concentrates (PCs) suspended in platelet additive solution and prepared hPL from these treated PCs. We compared the quality and efficacy of PR-hPL with the corresponding non-PR ones. We showed no impact on the content in 5 cytokines tested (EGF, bFGF, PDGF-AB, VEGF and IGF) and a significant decrease in TGF-b1 (-21%, n=16, $p<0.01$). We performed large scale culture of hMSCs during 3 passages and showed that hPL or PR-hPL at 8% triggered comparable hMSC proliferation than FBS at 10% plus bFGF (n=3). Moreover, after proliferation of hMSCs in hPL or PR-hPL containing medium, their profile of membrane marker expression, their clonogenic potential and immunosuppressive properties were maintained, in comparison with hMSCs cultured in FBS conditions. The potential to differentiate in adipogenic and osteogenic lineages of hMSCs cultured in parallel in the 3 conditions remained also identical. In conclusion, we demonstrated the feasibility to use UV-C treatment to subsequently obtain pathogen reduced hPL, while preserving its optimal quality and efficacy for hMSC expansion for cell therapy applications.

F1006

COMBINED SYSTEMIC THERAPY OF MESENCHYMAL STEM CELLS AND PARATHYROID HORMONE INDUCES REPAIR OF MULTIPLE NONUNION RIB FRACTURES

Pelled, Gadi[1,2], Cohn Yakubovich, Doron[2], Sheyn, Dmitriy[3], Shapiro, Galina[2], Tawackoli, Wafa[1], Da, Xiaoyu[1], De Mel, Sandra[1], Yalon, Eran[2], Schary, Yeshai[2], Sirhan, Afeef[2], Ley, Eric[1], Gazit, Dan[1,2] and Gazit, Zulma[1,2], [1]Cedars-Sinai Medical Center, Los Angeles, CA, U.S., [2]The Hebrew University-Hadassah Faculty of Dental Medicine, Jerusalem, Israel, [3]Cedar-Sinai Medical Center, Los Angeles, CA, U.S.

Rib fractures are associated with high morbidity and mortality in up to 20% of elderly patients. Current treatment is limited to pain control, which often does not prevent functional disabilities and even death. Hence there is an unmet clinical need for new therapies for rib fractures. We have recently shown that intermittent administration of parathyroid hormone (PTH) enhanced the targeting of I.V. injected mesenchymal stem cells (MSCs) to vertebral bone fractures in osteoporotic rats and mini-pigs. Yet, rib nonunion fractures pose unique challenges due to the constant movement of fracture edges during respiration. Here we hypothesized that multiple rib nonunion fractures would heal better following combined systemic administration of human MSCs and PTH compared to either treatment alone. Thirty-two athymic rats were operated to create two 5mm segmental bone defects in adjacent ribs. Next the animals were divided to four treatment groups: 1. I.V. injections of human MSCs and subcutaneous PTH injections for 21 days; 2. I.V. injections of MSCs; 3. PTH injections for 21 days and 4. Negative control (PBS injections). MSCs were labeled with a Luciferase reporter gene and a lipophilic fluorescent dye prior to injection. In vivo bioluminescence and microCT imaging were used to track cell migration and quantify bone regeneration at the bone defects, respectively. Eight weeks post surgery the animals were sacrificed and rib samples were subjected to histology and immunofluorescence. Our results showed that injected MSCs migrated to rib fractures and new bone volume at the defect site was two fold higher in the group treated MSCs and PTH compared to all other groups, as early as week 4-post surgery. Moreover, by Week 8, 35% of the nonunion fractures in the MSC+PTH group healed completely compared to 6.25% in the PTH group and none in the other groups. Histological analysis revealed mainly fibrosis at the defect sites in all groups but the MSC+PTH group in which new bone formation was evident. Immunostaining against CXCR4, SDF-1 and osteogenic markers' was used to investigate the mechanism of MSC migration and contribution to bone repair. We conclude that a combined treatment of systemic MSC administration and PTH therapy exerts a synergistic os-

teogenic effect that could be highly attractive as a therapy for traumatic rib fractures.

F1008

CYTOKINE-PRIMED HUMAN MESENCHYMAL STEM CELLS ATTENUATE PARKINSON'S DISEASE VIA THE ANTIOXIDATIVE ACTIVITY OF IDO CATABOLITES

Cao, Wei, Wang, Ying and Shi, Yufang, Institute of Health Sciences, Shanghai Institutes for Biological Sciences,CAS, Shanghai, China

Mesenchymal stem cells (MSCs) are considered a promising candidate for cell therapy against Parkinson's disease (PD). Recent studies have demonstrated that MSCs can be activated to produce large amounts of immunosuppressive factors and growth factors by the stimulation of various inflammatory cytokines. Here we show that pretreating human MSCs with Interferon (IFN)-g and Tumor necrosis factor (TNF)-α significantly enhanced the neuroprotective effects of these cells on dopaminergic neuron loss in 6-hydroxydopamine (6-OHDA)-induced PD mice. This enhanced effect was found to be exerted not through modulation of neuro-inflammation, but rather through direct effects on oxidative stress. Administration of cytokine-primed MSCs (pMSCs) resulted in a substantial increase of anti-oxidant enzyme activities and a marked decrease of free radical levels in pMSC-treated PD mice compared to control mice. Mechanistically, we found that a tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO), which was highly induced by the stimulation of IFN-g and TNF-a in human MSCs, was primarily responsible for the therapeutic effects of pMSCs on PD mice. When IDO expression was silenced with specific siRNAs, the protective effect of pMSCs was abrogated. To determine which IDO metabolites may play a role in pMSC-mediated effects, we examined which metabolites human MSCs were capable of producing. Interestingly, we found that in response to inflammatory cytokines, MSCs converted tryptophan almost completely to the metabolites kynurenine (KYN), kynurenic acid (KYNA) and anthranilic acid (AA). Among these metabolites, KYNA and KYN were demonstrated to be effective in treating PD mice. This study reveals that cytokine-primed MSCs can effectively modulate oxidative stress status and ameliorate the disease severity of PD mice in an IDO-dependent manner. New evidences described here lend strong rationale for re-thinking the role and underlying mechanism of MSCs in the treatment of PD.

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F1010

ENHANCEMENT OF ANTI-INFLAMMATORY EFFECTS OF UMBILICAL CORD DERIVED MESENCHYMAL STEM CELLS THROUGH PRE-TREATMENT WITH PRANOPROFEN.

Ikeyama, Yoshifumi, Nishida, Hiroyuki, Mura-Uno, Eiko, Tsuda, Tomohiro, Yoshino, Mihoko, Ngo, Trung Xuan and Sakaki-Yumoto, Masayo, Rohto Pharmaceutical Co., Ltd., Kyoto, Japan

Mesenchymal stem cells (MSCs) are known to reside in various tissues, and have high proliferative capacity and a potential to differentiate into several cell types including osteocytes, chondrocytes and adipocytes. Increasing evidence has recently highlighted the potential of MSCs in the modulation of immune response by suppressing function of T cells, B cells, dendritic cells, macrophages, and natural killer cells. Since hyper activated immune response may cause multiple organ failures or septic syndromes, the immunomodulatory effects of MSCs are also expected to be utilized as a cell therapy drug in the tissue regeneration process, graft versus host disease (GVHD) and other chronic inflammatory diseases. Based on the previous reports, we first evaluated the immunomodulatory effects of commercially available umbilical cord derived MSCs (UC-MSCs) through co-culture system with mouse macrophage RAW264.7 cells. As reported, IL-6 secretion from RAW264.7 cells upon LPS treatment was markedly suppressed when co-cultured with MSCs or with anti-inflammatory reagent dexamethasone. We then seek for a method to establish highly immunosuppressive population from UC-MSCs. We performed the screening of drugs and various culture conditions for the population, and found that pre-treating UC-MSCs with pranoprofen, a kind of NSAIDs, enhanced the immunomodulatory effects of UC-MSCs on the RAW264.7 cells. We are currently analyzing the cytokine secretion and gene expression of UC-MSCs upon pranoprofen treatment to elucidate the mechanisms of how MSCs suppress immune response of macrophage. Further analysis will enable us to modify the potential of MSCs toward the development of highly effective cell therapy drugs.

F1012

ATTACKING PROSTATE CANCER WITH A PRODRUG-DOPED CELLULAR TROJAN HORSE

Levy, Oren, Harvard Medical School/Brigham and Women's Hospital, Cambridge, MA, U.S.

Despite considerable advances in prostate cancer research, there is a major need for a systemic delivery platform that efficiently targets anti-cancer drugs to sites of disseminated prostate cancer while minimizing host toxicity. In this proof-of-principle study, human mesenchymal stem cells (MSCs) were loaded with poly(lactic-co-gly-

colic acid) (PLGA) microparticles (MPs) that encapsulate the macromolecule G114, a thapsigargin-based prostate specific antigen (PSA)-cleavable prodrug . G114-particles (~950nm in size) were internalized by MSCs, followed by the release of G114 as an intact prodrug from loaded cells. Moreover, G114 released from G114 MP-loaded MSCs selectively induced death of the PSA-secreting PCa cell line, LNCaP. Finally, G114 MP-loaded MSCs inhibited tumor growth when used in proof-of-concept co-inoculation studies in CWR22 PCa xenografts, suggesting that cell-based delivery of G114 did not compromise the potency of this pro-drug in-vitro or in-vivo. To our knowledge, this is the first demonstration of a therapeutically efficacious cell-based drug delivery platform, which inhibits cancer growth in-vivo without the need of genetic engineering. We envision that upon achieving efficient homing of systemically infused MSCs to cancer sites, this MSC-based platform may be developed into an effective, systemic 'Trojan Horse' therapy for targeted delivery of therapeutic agents to sites of metastatic PCa.

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F1014

HUMAN MESENCHYMAL STEM CELLS INDUCE THE EXPRESSION OF CD4+ CD25+ FoxP3+ PD-1+ REGULATORY T CELLS IN IN VITRO AND IN VIVO

Mun, Chin Hee, Division of Rheumatology, Yonsei University College of Medicine, Seoul, Korea, South

Mesenchymal stem cells (MSCs) have profound immunomodulatory properties. The immune modulation of MSCs is related to inhibition of immune cell proliferation and activation. T cells critical effector immune cells in affecting and regulating immune response and can differentiate into one of several subtypes, including TH1, TH2, TH17 or regulatory T cells (Tregs). In this study, we investigated the immunomodulatory property of MSCs on T cells.We co-cultured human bone marrow-derived MSCs (BM-MSCs) and mouse CD4+ T cells directly. The effect of BM-MSCs on T cell differentiation was assessed by T cell subtype markers by flow cytometry, and supernatants for induced production of cytokines. Gene and protein expressions were analyzed by qRT-PCR and western blot, respectively. In addition, immunohistochemistry was performed in inflammatory tissues in collagen induced arthritis (CIA) mice. The expression of CD4+CD25+FoxP3+ PD-1+ Tregs were highly induced in co-culture condition. Human BM-MSCs significantly induced the Tregs by increasing programmed cell death-1 (PD-1) and neuropilin-1 (Nrp-1) expressions in vitro. However, human BM-MSCs did not induce the FoxP3+ Tregs in the splenic CD4+ T cells from PD-1-/-mice. Moreover, immunohistochemical analysis of inflamed tissues in BM-MSC treated CIA mice showed significant immunopositive staining for PD-1 on T cells.Our data showed that human BM-MSCs induced the PD-1+ Tregs in both in vitro and in vivo.

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F1016

CANINE ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS AMELIORATE SEVERE ACUTE PANCREATITIS BY INHIBITING INFLAMMATION IN RATS

Song, Woo-Jin[1], Kim, Hyun-Wook[1,2], Li, Qiang[1], Han, Sei-Myung[1], Jeon, Kee-Ok[1], Park, Sang-Chul[1], Ryu, Min-Ok[1], Lee, Jiye[1], Lee, Bo-Yeon[1], Kim, Kun-Ho[1], Kyeong, Kweon[1], Chae, Hyung-Kyu[1], Kim, Hyeon-Jin[1], Kim, Su-Yeon[1], Yang, Hye-Mi[1] and Youn, Hwa-Young[1], [1]College of Veterinary medicine, Seoul National University, Seoul, Korea, South, [2]Haemaru Referral Animal Hospital, Seoungnam, Korea

Severe acute pancreatitis (SAP) is a severe form of acute pancreatitis which is one of the leading causes of gastrointestinal diseases in industrialized countries. The mortality rate for SAP is approximately 30% and it has not decreased in the past decade despite improvement in supportive care. Recently, effects of adipose tissue-derived mesenchymal stem cells (ATMSCs) on treatment of several inflammation models have been studied and ATMSC therapies have increased the possibility of improving inflammatory diseases. Aim of this study was to investigate the therapeutic effects of canine adipose tissue-derived mesenchymal stem cells (cATMSCs) on rats with SAP. We isolated cATMSCs and induced rat SAP model by retrograde pancreatic duct injection of 3% sodium taurocholate solution. CM-DiI-labeled cATMSCs (1×10^7 cells/kg) were administered into the tail vein and pancreas tissues were collected 3 days after cell infusion. Histopathological analysis, quantitative real-time PCR analysis, and immunoflourescence staining of the samples were performed. Greater numbers of infused cATMSCs were detected in pancreas of rats with SAP than of sham-operated rats. Infused cATMSCs reduced pancreatic edema, inflammatory cell infiltration and acinar cell necrosis. cATMSCs significantly reduced expression of inflammatory cytokines and increased expression of anti-inflammatory cytokines such as IL-4 and IL-10 in pancreas tissues in SAP rats. In addition, SAP rats given cATMSCs not only

decreased CD3+ T cells and but also increased expression of Foxp3+ (a marker of regulatory T cells) in pancreas tissues. In conclusion, cATMSC reduced SAP by inhibiting inflammation in pancreas tissues of SAP rats. Transplantation of ATMSCs might be developed as a potential therapy strategy of SAP.

F1018

HUMAN PLACENTA-DERIVED MULTIPOTENT CELLS (PDMCS) PROTECT AGAINST KLEBSIELLA PNEUMONIAE-INDUCED PNEUNOMIA BY ENHANCING POLYMORPHONUCLEAR GRANULOCYTES (PMN) FUNCTIONS

Wang, Li-Tzu[1,2], Chao, Ying-Yin[2], Lee, Wei[1], Huang, Li-Yueh[2], Liu, Ko-Jiunn[2], Siu, Leung-Kei[2] and **Yen, B. Linju**[2], [1]National Defense Medical Center, Taipei City, Taiwan, [2]National Health Research Institutes, Zhunan Maoli County, Taiwan

Human mesenchymal stem cells (MSCs) are multilineage somatic progenitors with strong immunomodulatory properties which have been well-demonstrated for T lymphocytes and dendritic cells/macrophages. However, interactions with neutrophils (polymorphonuclear granulocytes or PMNs)—the most abundant population of human leukocytes—are less well understood. Therefore, we investigated the interactions of human placenta-derived multipotent cells (PDMCs), a population of fetal-stage MSCs, with PMNs through in vitro studies and a mouse model of Klebsiella pneumoniae (KP)-induced pneumonia. KP is a highly virulent gram-negative bacterium and a leading cause of community- and hospital-acquired infections, especially pneumonia. We found that after co-culture with PDMCs, PMN expression of CD11b—a marker of activation—as well as phagocytosis of FITC-labeled KP was significantly increased. Moreover, PMN oxidative metabolism was significantly increased as well, which manifested as increased anti-bacterial activity and improved killing of KP. To ascertain the therapeutic efficacy of PDMCs on bacterial infections, we infected wild type mice with KP by intratracheal inoculation with subsequent intravenous administration of PDMCs. Surprisingly, while injection of PDMCs reduced the influx of PMNs in KP-infected lung tissue, respiratory burst activity was simultaneously enhanced. PDMC treatment also decreased bacterial counts both locally in lung tissue and systemically in the bloodstream. Most importantly, administration of PDMCs significantly increased survival rates in this mouse model of KP pneumonia. Taken together, we found that PDMCs enhance PMN functions in vitro and in vivo towards KP without increasing overall lung inflammatory damage. Our data strongly implicate a possible therapeutic role for PDMCs towards gram-negative bacterial infections.

MESENCHYMAL STEM CELL DIFFERENTIATION

F1024

EXOSOME-DERIVED HUMAN UMBILICAL CORD PLASMA ATTENUATED LIVER FIBROSIS IN MICE LIVER FIBROSIS MODEL

Huang, Yu-Jen, National Taiwan University, Taipei, Taiwan and Wu, Yao-Ming, National Taiwan University Hospital, Taipei, Taiwan

Liver fibrosis is a reversible disease, which is characterized by excessive extracellular matrix (ECM) accumulation in liver architecture during the liver healing process, and eventually led to liver dysfunction. Being the main producer of ECM in liver fibrosis, hepatic stellate cells (HSCs) would become fibrogenic myofibroblast-like cells through transdifferentiation, which carried the ability of high proliferation and production of excessive ECM proteins (specifically collagen type I). During the past decades, efforts has been devoted to develop the novel and safe methods to attenuate the liver fibrosis. Among them, exosome therapy has been pointed out with the possibility of regenerative medicine, but the implied function over liver fibrosis was not well recognized. In this study, to verify the therapeutic potential of exosome derived umbilical cord plasma, mice liver fibrosis was evaluated with the CCL4-induce liver fibrosis mice model. We found that exosome derived umbilical cord plasma could decrease fibrosis score and reduce collagen deposition in fibrotic liver, with inhibition of TGF-β, a fibrogenesis mediator, with subsequently suppression of TIMP-1 and TIMP-2 gene expression, and enhancement of MMP-9 activity. Further exploring, exosome derived umbilical cord plasma suppressed HSCs from proliferation and collagen production in vitro. Taken together, our result highlighted the therapeutic potential of the exosome derived umbilical cord plasma with the mediation of MMPs/TIMPs balance via the modulated and suppressed TGF-β signal pathway in liver fibrosis.

F1026

IN VIVO LABELING OF MESENCHYMAL DERIVATIVES ENABLES THE MOLECULAR CHARACTERIZATION OF BONE MARROW SINUSOIDAL CELLS

Kimura, Kenichi[1,2], Fuegemann, Christopher J.[1], Facchini, Raffaella[3], Hesse, Michael[1], Woll, Petter S.[3], Reinhardt, Julia[4], Luis, Tiago[3], Ohneda, Osamu[2], Kastenmüller, Wolfgang[5], Hölzel, Michael[4], Jacobsen, Sten E.[3], Fleischmann, Bernd K.[1] and Breitbach, Martin[1], [1]Institute of Physiology I, University of Bonn, 53105 Bonn, Germany, [2]Department of Regenerative Medicine and Stem Cell Biology, University of Tsukuba, 305-8575 Tsukuba, Japan, [3]Haematopoietic Stem Cell Laboratory, Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Oxford, U.K., [4]Department of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Bonn, Germany, [5]Institute of Experimental Immunology, University of Bonn, Bonn, Germany

Mesenchymal stem cells (MSCs) are multipotent cells residing in the bone marrow (BM) and other tissues. To enable identification and tracking of MSCs in vivo, we established a CD73-EGFP reporter mouse which facilitates enrichment of MSC-like cells with typical marker expression and tri-lineage differentiation potential. In fetal and adult bones EGFP+ mesenchymal progenitor cells emerged at the proliferative sites of peri- and endochondral ossification, whereas in other solid organs the EGFP+ MSCs showed a perivascular distribution pattern. Purification of the CD73-EGFP fraction from bone lining cells led to a strong enrichment in colony-forming units-fibroblast with robust stemness enabling the direct isolation of non-manipulated MSCs. Of particular importance is the unexpected finding that our live reporter specifically labels the sinusoidal endothelial cell population in the BM, which is a critical component of the hematopoietic stem cell (HSC) niche. Immunohistochemistry on bone sections demonstrated that c-kit+/Sca1+ hematopoietic stem/progenitor cells mostly localized adjacent or in very close vicinity to the EGFP+ sinusoidal structures. As molecular characterization of this specific cellular entity has been impossible to date due to the lack of specific genetic models or marker molecules, we purified sinusoidal and non-sinusoidal endothelial cells from the BM taking advantage of the cell subtype specific transgene labeling. RNAseq and subsequent gene set enrichment analysis revealed that sinusoidal endothelial cells possess a distinct mesenchymal signature and showed higher expression of E-selectin and Vcam-1 compared to arteriolar endothelium, both adhesion molecules that are directly involved in HSC signaling, highlighting endothelial cell heterogeneity within the same organ. Taken together, we provide a genetic mouse model that labels MSCs and specifically sinusoidal cells within the bone marrow endothelial compartment. This allowed for direct visualization and molecular characterization of this important component of the HSC niche. Future efforts should be also directed towards intravital microscopy in order to directly visualize the sinusoidal cells and track them and interacting cells during immunological challenges such as mobilization, inflammation, and infection.

F1028

DIFFERENCE IN IMMUNOMODULATION AND MULTIPOTENT POTENTIAL EXISTS AMONG HUMAN ADIPOSE TISSUE-, BONE MARROW-, AND UMBILICAL CORD-DERIVED MESENCHYMAL STEM/STROMAL CELLS

Kwan, Cyndi L., Noon, Allison and **Ni, Jessie H.-T.**, Irvine Scientific, Santa Ana, CA, U.S.

Mesenchymal stem/stromal cells (MSCs) have the capacity to differentiate into cells within the mesodermal tissue lineage that have the potential to treat a variety of regenerative or reparative applications due to their unique immunosuppressive properties. MSCs are able to interfere with different pathways of immune response by means of direct cell interaction and by indirect soluble factor secretion. However, due to endogenous differences that exist amongst the variety of tissue sources to derive MSCs and ex vivo expansion culture variability, there could be unknown/limiting factors in employing MSCs for therapeutic applications. In this study, we evaluated human adipose tissue- (AD-), bone marrow- (BM-), and umbilical cord-derived (UC-) MSCs cultured in serum-containing medium (10% FBS) and in PRIME-XV® xeno-free, serum-free medium (XSFM) to characterize them according to the ISCT guidelines and to determine their immunosuppressive abilities. For this study, MSCs were conditioned into both media prior to expansion and characterization. The MSCs were cultured through three passages and assessed for morphology, cell viability, cell marker expression, and potency including differentiation and immunomodulation potential. No significant difference was noted for cell growth over three passages and for the expression profile of CD45, CD105 and CD90 between AD-, BM-, and UC-MSCs cultured in serum-containing medium and XSFM. Initial differences in cell morphology can be seen with superior quality found in cells cultured in XSFM. All MSCs cultured in both medium retained its differentiation potential except for UC-MSCs. UC-MSCs cultured in both medium were not able to exhibit adipogenic differentiation potential. All MSCs cultured in both medium retained their immunomodulatory potential. BM-MSC in serum-containing media was observed to have a relatively weaker immunomodulatory potential in comparison to AD- and UC-MSCs. The immunnoldulatory potency of BM-MSC was improved and comparable to AD-and UC-

MSCs when cultured in XSFM. Overall, this study demonstrated that the desired improvements in MSC potential can be accomplished with expansion medium development without compromising the expected MSC profile while maintaining the endogenous difference that exist as the result of tissue origin.

F1030

NEURAL PROGENITORS DIFFERENTIATION POTENTIAL COMPARISON BETWEEN STEM CELLS FROM HUMAN PERMANENT AND DECIDUOUS TEETH

Sritanaudomchai, Hathaitip[1], Gonmanee, Thanasup[2], Thonabulsombat, Chareonsri[2] and Vongsavan, Kutkao[3], [1]Department of Oral Biology, Bangkok, Thailand, [2]Department of Anatomy, Bangkok, Thailand, [3]Department of Pediatric Dentistry, Bangkok, Thailand

Functional recovery from injuries to the brain or spinal cord represents a major clinical challenge. Human dental pulp stem cells (DPSCs) from permanent teeth and stem cells from human exfoliated deciduous teeth (SHED) are derived from ectomesenchymal origin, a potential to differentiate into neural lineage. This investigation was aimed to compare the neural progenitor differentiation potential of DPSCs and SHED. Stem cells were isolated and characterized from 4 permanent dental pulps (patients aged 20-25 years) and 4 deciduous dental pulps (patients aged 5-10 years). DPSCs and SHED were differentiated into neural progenitors, the free-floating structures or called neurospheres, in the presence of EGF, FGF and B27 in serum-free condition. A single sphere was immunostained with nestin, a marker for neural progenitor cells. The size and total number of neurospheres were determined on day 7 of differentiation using ImageJ software. SHED exhibited higher proliferation rates than DPSCs. However, both stem cells contained a cell population expressing the CD44, CD105 and CD146 mesenchymal stem cell markers and had multipotent nature. DPSCs and SHED were successfully differentiated into neural progenitor cells or forming neurospheres. At 7-days, neurospheres derived from DPSCs and SHED had generally a dark core and a well-defined spherical shape. Cells from the spheres were positive for nestin. The total numbers of neurospheres (diameter > 30 μm) from both stem cells gradually decreased. In parallel, a higher percentage of large neurospheres (diameter > 200 μm) was observed in all four lines of DPSCs compared with SHED. These results suggest that neurosphere derived from DPSCs may have more potential to differentiate into neural lineages. Human dental pulp stem cells from permanent teeth may provide an alternative cell source to treat neurodegenerative disorders.

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F1032

MICRORNA REGULATION OF MESENCHYMAL PROGENITOR CELL FATE IN MOUSE SKELETAL MUSCLE

Wosczyna, Michael N, Gan, Qiang, Brett, Jamie, Wang, Theodore, Perez, Edgar and Rando, Thomas A., Stanford University School of Medicine, Stanford, CA, U.S.

The appearance of fatty and fibrotic infiltration in aged skeletal muscle causes a decline in muscle function and an increase in metabolic disorders. However, a means to mitigate this detrimental process remains absent, as the cellular and molecular mechanisms that lead to the accumulation of these adverse tissues are not well understood. Recently identified mesenchymal progenitor cells (MPCs) have been postulated as the foremost cellular mediators of intramuscular adiposis and fibrosis. In young tissue MPCs do not covert to unfavorable fates, but instead, remain as progenitors and are assumed to support the processes of tissue homeostasis and regeneration. To begin to understand and ultimately control MPC fate determination, we have developed progressive cell isolation techniques and coupled these with bioassays of skeletal muscle adipogenesis/fibrogenesis and deep sequencing to dissect this process. Bioinformatical and functional testing have identified multiple miRNAs that are capable of modulating cell fate. In particular, gain and loss-of-function experiments have proven these miRNAs to be key regulators of MPC adipogenesis. We are currently using in silico and experimental methods to identify the transcript targets of these miRNAs and to elucidate the miRNA dependent genetic circuits responsible for MPC adipogenesis. We ultimately aim to leverage these findings to develop therapeutic targets to mitigate age-related skeletal muscle decline.

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MESENCHYMAL CELL LINEAGE ANALYSIS

F1036

HUMAN-INDUCED PLURIPOTENT STEM CELL-DERIVED MESENCHYMAL STEM CELLS RESCUE ANTHRACYCLINE-INDUCED CARDIOMYOPATHY: ROLES OF MIRO1 EXPRESSION AND TUNNELING NANOTUBE

Qizhou, Lian and Yuelin, Zhang, University of Hong Kong, Hong Kong, Hong Kong

Apart from paracrine action, direct mitochondrial transfer (MT) from induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) to injured cells plays an important role for tissue repair, although the underlying

mechanisms remain elusive. We sought to investigate the effects and mechanisms of MT of human iPSC-MSCs in an in-vitro and in-vivo model of anthracycline-induced cardiomyopathy (AIC). Compared with bone marrow-derived MSCs (BM-MSCs), iPSC-MSCs were more effective in transferring functional mitochondria to doxorubicin (Dox)-injured neonatal mice cardiomyocytes during co-culturing, and significantly restored their mitochondrial respiratory function. Systematic screening of mitochondrial motor molecules revealed that intrinsic mitochondrial Rho-GTPase 1 protein (Miro1) is highly overpresented in iPSC-MSCs compared with adult BM-MSCs. The effects of depletion of Miro1 by siRNA or replenishing Miro1 expression indicated intrinsic Miro1 is a critical regulator that directly contributes to higher efficiency of MT in iPSC-MSCs. Moreover, iPSC-MSCs are more responsive than BM-MSCs to pro-inflammatory cytokine tumor necrosis factor-α (TNF-α)-induced formation of tunneling nanotubes (TNT) for MT due to their higher level of TNFαip2 expression. Inhibition of TNFαip2 in iPSC-MSCs abrogated TNT formation and reduced MT and decreased cardioprotective effects. Compared with BM-MSCs, transplantation of iPSC-MSCs into an AIC mouse model resulted in more human mitochondrial retention and bioenergetic preservation in mice heart tissues, accompanied with significantly attenuated heart failure, and decreased oxidative stress and cardiomyocytes apoptosis. Efficacious transfer of functional mitochondria from human iPSC-MSCs to injured cardiomyocytes, due to higher intrinsic expression of Miro1 and responsiveness to TNF-α-induced-nanotube formation, plays as a novel role in cardio-protection against AIC.

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F1038

STROMAL CELL-DERIVED EXTRACELLULAR MATRIX PROMOTES CORD BLOOD-DERIVED MESENCHYMAL STEM CELL PROLIFERATION WHILE RESTRICTING NEURAL LINEAGE DIFFERENTIATION

Zamilpa, Rogelio[1], Navarro, Mary[1], Alvarado, Lucero[1], Flores, Iris[1], Mallinson, David[2] and **Griffey, Sy**[1], [1]StemBioSys Inc, San Antonio, TX, U.S., [2]Sistemic Ltd, Glasgow, U.K.

The critical role of mesenchymal stem cells (MSCs) during tissue repair and regeneration has propelled the use of MSCs for research and development as well as clinical applications. Although MSCs are globally distributed in tissues, they are found at very low frequencies which require a robust cell expansion paradigm. Umbilical cord blood-derived MSCs (UCB-MSCs) are low frequency MSCs with inherent neurogenic potential. To facilitate the expansion of UCB-MSCs, a cell culture substratum referred to as High Performance Micro Environment (HPME) was generated using stromal cells. Scanning electron microscopy and mass spectrometry analyses demonstrated that the HPME is an intricate, three-dimensional, protein network composed primarily of extracellular matrix proteins such as collagen types I, VI, XII, and fibronectin. Expansion of UCB-MSCs on HPME significantly increased the cell yield by a minimum of 2 fold per passage compared to cells expanded on tissue culture plastic ($p<0.05$). Concurrently, the absolute number of stage specific embryonic antigen (SSEA)-4 positive cells significantly increased when the cells were cultured on HPME. Using miRNA microarray analysis, 9 differentially expressed miRNAs were identified between cells expanded on HPME compared to tissue culture plastic. Among the identified miRNAs, miR-7 and miR-29b were upregulated in the HPME expanded cells. MiR-7 and miR-29b maintain self-renewal capacity and stimulate cell proliferation, respectively. In addition, miR-30a, which is upregulated in senescent UCB-MSCs, was found downregulated in the HPME cultures. Interestingly, miR-324, which promotes neural lineage differentiation, was downregulated in the HPME cultures. These results suggest that the HPME is an ideal platform for UCB-MSC expansion because it increases cell proliferation and self-renewal while restricting cell differentiation.

HEMATOPOIETIC CELLS

F1040

DISRUPTION OF NON-HOMOLOGOUS END-JOINING DRIVES GENOMIC INSTABILITY IN HEMATOPOIETIC STEM CELLS

Bakker, Sietske T.[1], Zhang, Si Yi[2] and Passegué, Emmanuelle[2], [1]Eli and Edyth Broad Center for Regeneration Medicine and Stem Cell Research, San Francisco, CA, U.S., [2]Department of Medicine, Division of Hematology/Oncology, The Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California San Francisco, San Francisco, CA, U.S.

Hematopoietic stem cells (HSCs) are responsible for life-long maintenance of blood production, and can drive leukemia development upon acquisition of mutations. At steady state, HSCs are mostly kept in the quiescent phase of the cell cycle, which protects them from genotoxic stress associated with cellular respiration and DNA replication, and are highly resistant to death signals due to an enhanced pro-survival wiring of their apoptosis machinery. However, quiescent HSCs are also restricted to the usage of the error-prone non-homologous end-joining (NHEJ) for DNA repair that, in turn, renders them susceptible to mutations. In contrast, cycling HSCs can use the error-free homologous recombination (HR) repair pathway and better maintain their genome integrity upon

DNA damage. Here, we use mice deficient for the Prkdc (DNA-Pkcs) gene to probe the importance of the NHEJ repair pathway for HSC function. We find that NHEJ is essential to maintain HSC numbers both at steady state and following regenerative challenges like chemotherapeutic treatments or aging, but is dispensable for HSC self-renewal activity upon transplantation. In addition, we show that NHEJ disruption causes exquisite toxicity in quiescent HSCs at low levels of DNA damage (0.5Gy of ionizing radiation), and directly increases HSC apoptosis due to an inability to repair DNA breaks and constitutive activation of DNA damage signaling. This higher sensitivity to DNA damage can only be partially rescued in cycling NHEJ-deficient HSCs through engagement of the HR pathway. In addition, some rare quiescent NHEJ-deficient HSCs can survive low levels of DNA damage but acquire even more mutations (i.e., translocations and small insertion/deletions) likely through engagement of the highly error-prone alternative NHEJ pathway. Our results identify NHEJ as an essential DNA repair mechanism that ensure the survival of quiescent HSCs and is, despite its low repair fidelity, an important gatekeeper of HSC genome integrity. Moreover, they raise concerns for the use of NHEJ inhibitors as sensitizer for cancer treatments, as they would likely increase the risk of mutations in quiescent HSCs and the development of therapy-related leukemia.

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F1042

ESTROGEN AFFECTS REDOX BALANCE IN HEMATOPOIETIC STEM CELLS

Chapple, Richard[1], Kitano, Ayumi[2], Lin, Angelique[2], Jiang, Yajian[2], Murdaugh, Rebecca[2], Hu, Tianyuan[2], Luu, Thien[2] and Nakada, Daisuke[2], [1]Baylor College of Medicine, Pearland, TX, U.S., [2]Baylor College of Medicine, Houston, TX, U.S.

Quiescence is a defining characteristic of hematopoietic stem cells (HSCs), and is required for the maintenance of the stem cell pool. Recently, our lab has shown that HSCs are recruited out of quiescence upon exposure to 17β-estradiol (E2). To determine if E2-induced proliferation negatively influences HSC self-renewal, we competitively transplanted purified HSCs from E2-treated animals. Interestingly, E2 exposure enhances overall engraftment rates, with particularly robust reconstitution in both myeloid and platelet lineages. Furthermore, secondary transplantation revealed stable engraftment and lasting contribution among all lineages. Given that E2 activates HSC proliferation without affecting long-term self-renewal, we hypothesized that E2 elicits protective mechanisms against cell cycle induced cytotoxic stress. To this end, we profiled E2-treated HSCs for reactive species. Reactive oxygen species (ROS) levels were unchanged after E2 treatment. However, a subset of reactive nitrogen species (RNS) called nitric oxide (NO) was significantly induced. Chemical inhibition of NO production revealed that NO is necessary for the E2-mediated effects on HSC function. Currently, we are exploring the antioxidant effects of E2-induced NO production in HSCs. NO is a potent regulator of mitochondria, and thus may decrease ROS production in HSCs. In support of this notion, E2 reduces ROS levels in mouse models of oxidative stress. Currently, we are more thoroughly characterizing the role of NO on mitochondrial function in HSCs by measuring mitochondrial membrane potential, oxygen consumption, and nitrosylation of respiratory chain complexes. These results hold promise for E2 and/or NO as conditioning agents, which may enhance engraftment upon transplantation as a result of mitigating oxidative stress.

F1044

EFFECT OF CELL CULTURE SURFACES ON MONOCYTE-DERIVED DENDRITIC CELL DIFFERENTIATION AND MATURATION

Fekete, Natalie[1], Wargenau, Andreas[1], Clark, Sarah[2], Tufenkji, Nathalie[1] and Hoesli, Corinne[1], [1]McGill University, Montreal, QC, Canada, [2]Saint-Gobain Fluid Systems, Northborough, MA, U.S.

Human monocytes can be used as progenitor cells to obtain dendritic cells (DCs), the most potent antigen-presenting cells in the immune system. DC-based cancer vaccines are currently under intense investigation in a number of phase II/III clinical trials and are available as an FDA-approved immunotherapy product. For clinical-scale production, traditional open polystyrene-based culture flasks are often replaced by closed cell culture bag systems. Importantly, monocyte-derived DCs are cultured in suspension in hydrophobic fluoropolymer-based bags, typically fluorinated ethylene propylene (FEP), in contrast to the adhesion cultures traditionally used on polystyrene surfaces (PS). The objective of this study was to characterize the effect of different commercially available culture surfaces on cell fate decisions for DC-based immunotherapies. To this end, we cultured human monocytes on both surfaces in defined, serum-free medium and induced their differentiation into DCs with IL-4 and GM-CSF. Full maturation of DCs was achieved within 2 days of exposure to LPS and TNF alpha. A loss of CD14 surface expression with concomitant upregulation of the antigen-presenting cell markers CD1a, CD40, CD80, CD83 and CD86 was observed within five days of culture. Moreover, soluble factors, such as ICAM-1, CCL5 and EGF-R were detected in the medium collected from cultures on all test surfaces. Monocytes partially adhered to both polystyrene and fluoropolymer surfaces and showed comparable levels of cell viability and aggregation. As proteins are known to facilitate cell adhesion to surfaces, we compared protein

adsorption onto fluoropolymer and polystyrene surfaces and found comparable kinetics, revealing that cells interact with protein-modified surfaces in vitro. In summary, we found that fluoropolymer and polystyrene surfaces differ in their chemical composition, surface topography, wettability and other physico-chemical properties, but not in their protein adsorption profiles. Monocytes were able to efficiently differentiate into mature DCs in both fluoropolymer bags in suspension and as adherent cultures on polystyrene surfaces. The long-term goal of this project is to understand cell-surface interactions at a molecular level to tailor culture materials and surfaces for cell therapy applications.

F1046

SYNERGISTIC RELATIONSHIP OF CELL CYCLE PARAMETERS OF HEMATOPOIETIC STEM/ PROGENITOR CELLS BETWEEN RADIATION-INDUCED LATE EFFECTS AND SENESCENCE

Hirabayashi, Yoko[1], Tsuboi, Isao[2], Kusunoki, Yoichiro[3] and Aizawa, Shin[2], [1]Natl Inst of Health Sciences, Tokyo, Japan, [2]Nihon University School of Medicine, Tokyo, Japan, [3]Radiation Effects Research Foundation, Hiroshima, Japan

Immediately after a 2-Gy whole-body irradiation (WB-IR), the decrease in the number of blood cells seemed much more severe for mice irradiated at 6 weeks of age (IR-6W) than for those irradiated at 6 months of age (IR-6M). However, irrespective of irradiation time, peripheral blood cells and mature hematopoietic progenitor cells (CFU-GM) showed complete recovery within 4-6 weeks, although immature hematopoietic stem/progenitor cells (HSCs/ HPCs) remained low in number more than a year after the irradiation. In addition, Ccnd1, Fyn, and PiK3r1 showed upregulated expressions only in the HSCs of the irradiated mice at 21 months of age compared with the control mice. Accordingly, the chronological changes in the cell cycle status in cells in the lineage-negative, c-kit-positive, and stem cell antigen 1 (Sca1)-positive (LKS) fraction as the HSC-enriched fraction and CFU-GM as mature HPCs were evaluated on the basis of bromodeoxyuridine (BrdUrd) incorporation ratio in vivo until 21 months of age after 2-Gy WB-IR. As a result, in the case of cells in the LKS fraction, we found that the ratios of BrdUrd incorporation in the irradiated groups with 2-week BrdUrd treatment were significantly higher than that in the control group at each observation time point. However, simultaneously, the generation doubling time in the irradiated groups seems to be longer than that in the control group, as determined from the increase in BrdUrd-incorporation ratio from two to six weeks of BrdUrd treatment. Because the prolonged generation doubling time and upregulated expressions of Ccnd1 and Fyn were also observed in nonirradiated elderly mice, a single WB-IR seemed to enhance the aging phenotype. In the case of CFU-GM, both irradiated groups showed higher incorporation ratios more than one year after irradiation. Interestingly, the cycling fraction of the CFU-GM in the IR-6W group was suppressed but that in the IR-6M group was not suppressed 4 weeks after irradiation. Accordingly, although the radiation-induced numerical suppression of HSCs/HPCs seemed to be much milder in the IR-6M group than in the IR-6W group, both groups were revealed to suffer from the same radiation-induced late effects that perturbed homeostasis to keep hematopoiesis at numerically normal levels, which may cause changes that lead to the development of leukemia.

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F1048

CD82/KAI1 MAINTAINS THE DORMANCY OF LONG-TERM HEMATOPOIETIC STEM CELLS THROUGH INTERACTION WITH DARC-EXPRESSING MACROPHAGES

Hur, Jin[1], Choi, Jae-Il[2], Lee, Hwan[2], Nham, Pniel[2], Kim, Tae-Won[2], Chae, Cheong-Whan[3], Baek, Sung Hee[4] and **Kim, Hyo-Soo**[2], [1]Seoul National University Hospital Division of Cardiology Department of Internal Medicine, Seoul, Korea, South, [2]Seoul National University Hospital, Seoul, Korea, South, [3]Seoul National University Hospital, Seoul, Korea, South, [4]Seoul National University, Seoul, Korea, South

Hematopoiesis is regulated by crosstalk between long-term repopulating hematopoietic stem cells (LT-HSCs) and supporting niche cells in the bone marrow (BM). Here, we examine the role of CD82/KAI1 in niche-mediated LT-HSC maintenance. We found that CD82/KAI1 is expressed predominantly on LT-HSCs and rarely on other hematopoietic stem-progenitor cells (HSPCs). In *Cd82*$^{-/-}$ mice, LT-HSCs were selectively lost as they exited from quiescence and differentiated. Mechanistically, CD82-based TGF-β1/ Smad3 signaling leads to induction of CDK inhibitors and cell cycle inhibition. The CD82 binding partner DARC/ CD234 is expressed on macrophages and stabilizes CD82 on LT-HSCs, promoting their quiescence. When DARC^{+} BM macrophages were ablated, the level of surface CD82 on LT-HSCs decreased, leading to cell cycle entry, proliferation, and differentiation. A similar interaction appears to be relevant for human HSPCs. Thus, CD82 is a functional surface marker of LT-HSCs that maintains quiescence through interaction with DARC-expressing macrophages in the BM stem cell niche.

F1050

ù-3 (N-3) AND ù-6 (N-6) POLYUNSATURATED FATTY ACIDS STIMULATE THE BONE MARROW STEM CELL POPULATION IN MICE

Limaye, Lalita, NCCS, Pune, Maharashtra, India, Kale, Vaijayanti P., Natl Centre for Cell Science, Pune, India and Limbkar, Kedar, NCCS, Pune, India

Stem cell therapy holds great promise in the treatment of numerous diseases and for regenerative medicine. A key issue in stem cell therapy is the control of stem cell fate for which safe and practical methods are limited. Essential fatty acids, namely ω-6 (n-6) and ω-3 (n-3) polyunsaturated fatty acids (PUFA), and their metabolites are critical components of cell structure and function, and have shown to influence stem cell fate. Being natural compounds, they can be used as nutritive supplements in regenerative medicine. Very few reports talk about their effect on hematopoiesis. Earlier we have shown that that PUFAs promote differentiation of cord blood HSCs to megakaryocytes in vitro. To check their effect in vivo, we fed mice with n-3 or n-6 PUFAs daily for ten days and analysed their bone marrow cells and peripheral blood for hematopoiesis and thrombopoiesis. PBS fed mice served as controls in all experiments. We observed that PUFAs enhanced bone marrow mononuclear cells (BM-MNCs) , side population (SP) cells as well as Lin^{-}Sca-1+c-Kit+(LSK) cells. In vitro functionality of PUFA fed bone marrow cells was found to be higher as compared to PBS fed cells as detected by Colony forming Unit (CFU) assay, Long term culture initiating assay(LTC –IC) and Extended LTC-IC assays. In vivo assays like Colony forming unit spleen assay(CFU-S) and long term engraftment assays further confirmed that PUFAs stimulated haematopoiesis.Megakaryocyte colonies and platelet count was higher in PUFA fed mice. A combination of docosahexanoic acid(Omega 3) and Arachidonic acid (omega6) was found to be effective. GC–MS analysis of bone marrow cells showed higher amount of PUFAs in fed vs unfed mice. Upregulation of mRNA levels of enzymes involved in PUFA metabolism like cyclo-oxygenases, fatty acid desaturases etc. was observed by real time PCR in BM MNCs. Similar trend was seen in the down stream metabolites of PUFAs like PGE1, LTB4 etc. as detected by ELISA. Feeding of Bone marrow transplanted recipient mice with PUFAs showed enhanced engraftment as compared to transplanted mice that were fed with PBS. Taken together, PUFAs enhance haematopoiesis and thrombopoiesis in mice and help in recovery of hematopoietically compromised mice. Our novel findings will pave ways for incorporating a dietary supplement in treatment of haematological malignancies.

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F1052

STUDY OF THE IMPACT OF MECHANICAL FORCES IN THE DEVELOPMENTAL ORIGIN OF MOUSE HEMATOPOIETIC STEM CELLS

Morgado-Palacin, Lucia[1], Bagnaninchi, Pierre[2], Souilhol, Celine[2], Rybtsov, Stanislav[2] and Medvinsky, Alexander[2], [1]MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, U.K., [2]University of Edinburgh, Edinburgh, U.K.

Hematopoietic stem cells (HSCs) arise in the aorta-gonad-mesonephros (AGM) region around E10.5 during embryo development. HSCs preferentially emerge from the ventral hemogenic endothelium of the dorsal aorta. Surrounding niche cells of the dorsal aorta enhance HSCs generation by providing a competent microenvironment. Several signalling pathways as well as inductive interactions in the AGM microenvironment, such as spatial-defined stem cell factor (SCF), Sonic-Hedgehog (Shh) or BMP expression, contribute to HSCs development. However, very little is known about whether mechanical cues generated in the AGM play a role in HSCs emergence and maturation. During embryo development cells are subjected to different mechanical stresses that impact on organ size and tissue morphogenesis. By using optical coherence phase elastography we have reported different mechanical properties along the dorso-ventral axis of E10.5 and E11.5 embryos, being the ventral part of the AGM stiffer than the dorso-lateral areas. Mimicking a stiffer environment by compressing ex vivo AGM explants is sufficient to boost HSCs long-term reconstitution capacity and progenitor potential. This increased HSCs performance occurs, importantly, in the absence of exogenous growth factors. SCF expression, which is involved in maturation of AGM-derived HSCs, correlates with stiffer anatomical regions in the AGM and is upregulated in response to compression. This suggests that compression could be positively modulating HSCs emergence by, at least, increased SCF signalling in niche cells. Clarifying physical forces that govern development of HSCs will allow us to refine protocols for efficient generation of HSCs *in vitro*, which currently constitutes a major bottleneck to further downstream applications in regenerative medicine.

F1054

ETS1 PROMOTES THE FORMATION OF DEFINITIVE HEMOGENIC ENDOTHELIUM IN HUMAN PLURIPOTENT STEM CELL CULTURES VIA UPREGULATION OF NOTCH SIGNALING

PARK, MI AE[1], Kumar, Akhilesh[2], Jung, Ho Sun[2], Moskvin, Oleg[2], Swanson, Scott[3], Thomson, James[3] and Slukvin, Igor I.[4], [1]National Primate Research Center, University of Wisconsin , Madison, WI, U.S., [2]National Primate Research Center, University Of Wisconsin, Madison, WI, U.S., [3]Morgridge Institute for Research, Madison, WI, U.S., [4]Universityof Wisconsin Madison, Madison, WI, U.S.

Hemogenic endothelium (HE) has been identified as a unique transitional stage of blood development from mesoderm in the embryo. Although blood formation from HE occurs at both extraembryonic and several embryonic sites, HSCs are predominantly formed from HE in the ventral wall of the dorsal aorta. Thus, identification of the regulators that lead to HE formation with definitive hematopoietic potential should help to design strategies to recapitulate HSC development from human pluripotent stem cells (hPSCs). In our hPSC studies, we defined HE as epithelioid cells with primary endothelial characteristics that are lacking hematopoietic colony-forming potential and surface markers, but are capable of generating blood and endothelial cells when cultured on a matrix or feeder layer; and showed that HE can be precisely separated from non-HE and emerging blood cells based on the lack of CD73 and CD43 expression. Here, using ETS1 conditional and knockout H1 hESC lines, we discovered that ETS1 induction at the mesodermal stage of differentiation dramatically enhances the formation of HE with lymphoid potential and its capacity to produce red blood cells with high expression of BCL11a and b-globin. RNAseq and flow cytometric analysis revealed profound upregulation of SOXF genes and DLL4 expression in HE following ETS1 induction. Blocking NOTCH signaling with neutralizing DLL4 antibodies or DAPT abrogated ETS1 effect on HE. Together, these findings establish a pathway linking ETS1 to SOX and NOTCH signaling in the HE cells to promote definitive hematopoiesis.

F1056

CHIMERISM AFTER BONE MARROW TRANSPLANTATION IN NON-CONDITIONED MICE

Rozman, Primoz, Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

Long-term engraftment of allogeneic bone marrow (BM) cells can only be successful after prior myeloablation. Engraftment in non-conditioned mice was possible only in autologous settings and the procedure requires immunocompatible grafts with high numbers of BM cells that can be a challenge to prepare and to transplant successfully. Our aim was to develop a protocol for transplantation of high numbers of nuclear cells in order to achieve highest possible chimerism after the transplantation of male BM cells into non-conditioned female BALB/c mice, and to develop a reliable method for assessment of chimerism in the recipient BM and different organs. The BM cells were isolated by crushing the main bones, collecting the cell suspension and performing red blood cell (RBC) lysis. Between 39 to 82 million of the nucleated BM cells were transplanted in four consecutive separate doses. Chimerism at 2, 6, and 12 weeks after bone marrow transplantation (BMT) was measured using the quantitative real-time PCR (qPCR) method. Two weeks after BMT, we detected 3.4–4.7% of the male donor cells in the female recipient's bone marrow (n=4). Six weeks after BMT, the percentage of the male donor cells was higher, 8.3–17.7% (n=3), and it stayed at the same level 12 weeks after BMT (12.7–14.2%; n=2). Twelve weeks after BMT, chimerism was also present in the blood (11.6–14.2%, n=2), spleen (3.6–4.1%, n=2), and lungs 0.7–1.5%, n=2). Our study confirmed some of the previous reports on chimerism obtained after BMT in non-conditioned recipients. In addition, it showed that the qPCR method is an accurate and a reliable method for chimerism detection in this setting.

F1058

GROWTH FACTOR RECEPTOR-BOUND PROTEIN 10 (GRB10) REGULATES HEMATOPOIETIC STEM CELL(HSC) SELF-RENEWAL AND REGENERATION VIA CONTROL OF RAC1 SIGNALING

Yan, Xiao[1], Himburg, Heather[2], Doan, Phuong[3], Quarmyne, Mamle[1], Pohl, Katherine[1], Zhang, Yurun[1], Tran, Evelyn[1], Zhao, Liman[1], Chao, Nelson JEN AN[3] and Chute, John[1], [1]University of California, Los Angeles, CA, U.S., [2]University of California, Los Angeles, Los Angeles, CA, U.S., [3]Duke University, Durham, NC, U.S.

Discovery of the mechanisms which govern HSC regeneration is impeded by difficulty in isolating HSCs early following genotoxic injury, such as total body irradiation (TBI). We performed gene expression analysis of BM ckit^{+}sca-1^{+}lin^{-} (KSL) cells at the earliest detectable point of regeneration (day +14) following 700cGy compared to non-irradiated BM KSL cells. We identified growth factor receptor-bound protein 10 (Grb10), a co-receptor which regulates Insulin Receptor/IGF-1 signaling, to be significantly overexpressed by regenerating HSCs compared to steady-state HSCs (3.3-fold, p<0.0001). Heterozygous 8 week old Grb10$^{m/+}$ (1 mutant allele, 1 wild type allele) mice displayed approximately 5-fold increased HSC repopulating capacity compared to Grb10$^{+/+}$ mice, as measured by competitive repopulation assays (p=0.003 for %CD45.2^{+}

donor cells). At day+10 after 550cGy, Grb10$^{m/+}$ mice contained significantly increased numbers of BM SLAM$^+$KSL cells (p=0.04), compared to Grb10$^{+/+}$ littermates. Similarly, mice transplanted with BM cells from irradiated Grb10$^{m/+}$ mice displayed 5-fold increased donor hematopoietic repopulation at 20 weeks post-transplantation compared to mice transplanted with BM cells from irradiated, Grb10$^{+/+}$ mice (p=0.006). Mechanistically, Grb10 ablation significantly promoted cell proliferation, both in mice transplanted with Grb10$^{m/+}$ donor cells and in ckit$^+$lin$^-$ cells of Grb10$^{m/+}$ mice at 24h after 700cGy TBI, compared to Grb10$^{+/+}$ controls (p = 0.01 and p = 0.006, respectively). Interestingly, a member of RhoGTPases, Rac1, was overactivated in Grb10$^{m/+}$ lin$^-$ cells compared to Grb10$^{+/+}$ lin$^-$ cells, both in steady state and following irradiation (p = 0.001 and p < 0.0001, respectively). In accordance with this, pharmacological inhibition of Rac1 fully muted the cell cycle increase in Grb10$^{m/+}$ mice after irradiation (p = 0.002). Additionally, Grb10$^{m/+}$ BM cells displayed enhanced migration capability, which can be abrogated by Rac1 inhibition (p =0.04 and p = 0.02, respectively). Taken together, our results suggest that Grb10 regulates HSC migration and self-renewal in steady state and HSC regeneration following myelotoxic stress, via regulation of Rac1 signaling. Selective modulation of Grb10 signaling has the potential to augment HSC self-renewal and regeneration in vivo.

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F1060

COMPREHENSIVE ANALYSES OF HEMATOPOIETIC STEM CELLS AND THEIR NICHE NETWORK IN BONE MARROW

Zhao, Meng[1], Venkatraman, Aparna[1], TAO, Fang[2], Li, Zhenrui[1], Perry, John[1], Qian, Pengxu[1], He, Xi (CiCi)[1] and Li, Linheng[1], [1]Stowers Institute for Medical Research, Kansas City, MO, U.S., [2]Stowers Institute for Medical Research, KANSAS CITY, MO, U.S.

Hematopoietic stem cells (HSCs) are preserved by niche cells in the bone marrow (BM). Although a few HSC regulating signals have been studied in respect to their BM niches, how different niche cells integrate their signals to maintain various HSC states has remained largely uncovered. In this study, by combining transcriptomic analysis with functional approaches, we have revealed the HSC-niche network interactions in the context of marrow architecture. 1) We initially defined various functional HSC states. Both reserved HSCs and primed HSCs can support hematopoiesis for more than 40 weeks, whereas short-term HSCs can support hematopoiesis only 8 weeks. However, upon chemotherapeutic stress, the function of reserved HSCs was preserved, but primed HSC function was compromised. 2) Transcriptome analysis showed that reserved HSCs have less cell cycle activity, lower metabolism with low IGF-mTOR activity, and a unique DNA damage repair system with higher non-homologous end joining (NHEJ) activity. 3) To analyze the HSC-niche network interaction, we performed transcriptomic analysis of 4 different HSC populations and 13 different niche components in BM using murine reporter lines. From bioinformatics analysis, we observed a comprehensive ligand-receptor interaction network between HSCs and their niches, which builds a foundation to understand how niche cells provide unique and shared signals to regulate HSCs in different stages. 4) Despite being the first proposed HSC niche cell marker, the identity of N-cadherin+ cells and their role in regulating HSCs have remained unknown and controversial. By using a newly generated N-cadherin-*Cre-Ert* based cell ablation mouse line, we provided for the first time functional evidence that N-cadherin$^+$ stromal cells contribute to maintain functional HSCs in the BM. More interestingly, by using the N-cadherin reporter mouse line, we found that N-cadherin$^+$ stromal cells have mesenchymal stem cell features. Endosteal N-cadherin$^+$ stromal cells directly give rise to osteoblastic cells, and the central marrow N-cadherin$^+$ stromal cells predominantly overlap with Lepr$^+$ stromal cells. Overall, our study for the first time provides a comprehensive view to understand the role of HSC niche cells with their unique and shared signals in regulating different subpopulations of HSCs.

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CARDIAC CELLS

F1064

IPS-DERIVED CARDIOMYOCYTES FOR SAFETY PHARMACOLOGY ASSESSMENT: SUPPORT OF THE COMPREHENSIVE IN VITRO PROARRHYTHMIA ASSAY

Luerman, Gregory[1], **Kfoury, Elena**[1], Horai, Hirofumi[2], D'Angelo, Jean Marc[3] and Kettenhofen, Ralf[4], [1]Axiogenesis Inc, Plymouth Meeting, PA, U.S., [2]Hamamatsu Photonics, Hamamatsu, Japan, [3]Hamamatsu Photonics, Massy, France, [4]Axiogenesis AG, Köln, Germany

A major hurdle to overcome in drug development is drug-induced hERG block leading to QT-prolongation and their association with potentially life threatening ventricular cardiac arrhythmias, a.k.a. Torsade-des-Pointes (TdP). The FDA/HESI/CSRC has sponsored the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative - a new model for a mechanistic approach to cardiac safety testing that improves upon existing methodologies (i.e. hERG testing) to better predict proarrhythmic activity in order to reduce the unnecessary attrition of “safe” compounds in the drug development process through the use

of ion channel screening, computer modeling, and stem cell derived cardiomyocytes. Outside of CiPA and arrhythmia concerns, long-term structural cardiotoxicity due to chronic dosing are currently one of the major reasons for late stage drug attrition. Thus, the development of more predictive cardiotox screening platforms to enhance drug discovery during early preclinical development is of great value to potentially reduce costly downstream attrition. Here we present work with hiPS-derived cardiomyocytes that compares the effects of CiPA reference compounds with varying cardiac risk (e.g. E-4031, nifedipine, flecainide, etc.) amongst medium (microelectrode arrays) throughput and HTS compatible 384 well fluorescent calcium and voltage sensitive dye assays. Compounds with high, medium, low or no cardiac risk demonstrated almost identical effects between the two test platforms on multiple electrophysiological parameters and occurrence of proarrhythmic events. These data demonstrate the predictivity functionality of iPS-derived cardiomyocytes as a HTS-scalable tool for comprehensive cardiac risk assessment.

F1066

A ROBUST METHOD FOR LARGE-SCALE DERIVATION OF HUMAN IPSC-CARDIOMYOCYTES FOR FUNCTIONAL GENOMIC APPLICATIONS

D'Antonio-Chronowska, Agnieszka, Garcia, Melvin, Okubo, Jonathan, D'Antonio, Matteo and Frazer, Kelly A, University of California San Diego, La Jolla, CA, U.S.

The large panels of iPSC lines becoming available provide an unprecedented opportunity for studying the role genetic variants play in molecular phenotypes (RNA expression, histone modifications, DNA methylation) underlying individual variation in cardiac cell biology and disease. However, in order to be able to elucidate the genetic components of these molecular phenotypes we must eliminate all variables that could confound our findings, such as variability between derivations resulting in obtaining different mixed cell sub-populations. As part of the NHLBI NextGen consortium we used a standardized method (from fibroblasts using Sendai virus) to generate a panel of well-characterized iPSCs from 222 individuals (143 belong to one of 41 families and 79 are unrelated). 41 individuals have cardiovascular disease ranging from common conditions to rare diseases. These iPSC lines are currently being distributed through WiCell Research Institute. Here we present a robust standardized protocol for large-scale derivation of cardiomyocytes iPSC lines (24 lines per month / 3 member team). To date we have used this protocol for 160 different differentiations (45 different lines from 35 individuals) obtaining high-quality cardiomyocytes and are currently differentiating the remaining iPSC lines in our resource. As part of our protocol we developed a method to digitally measure iPSC confluency, which ensures the iPSC lines are at optimal confluency at the time differentiation is initiated. We have also optimized both the concentration of IWP2 and the process of metabolic cardiomyocyte purification using Sodium L-Lactate. Using our optimized protocol we obtain on average 3.08×10^8 and up to 5.72×10^8 hiPSC-CM from the cultures of 450cm^2 (three T150 flasks). The average density of hiPSC-CM is 6.8×10^5 / cm^2 of original cell culture surface and the purity measured by flow cytometry as cTNT positive cells is on average 90.16% and up to 99.5%. We are able to freeze and thaw the derived cardiomyocytes for use in downstream analysis. Thus, our protocol affords sufficient amounts of high-quality derived cardiomyocytes to examine genetic variants associated with molecular phenotypes in the naïve state as well as with electrophysiological traits and gene expression / epigenetic changes in response to stress-triggered drug effects.

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F1068

THE EFFECT OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELL ON CARDIAC MITOCHONDRIAL RESPIRATION IN A MURINE MODEL OF ACUTE MYOCARDIAL INFARCTION DURING NECROTIC PHASE

Goldenberg, Regina[1], Irion, Camila[1], Santos-Silva, Lucas[1], Melo, Teby[1], Christie, Michelle[1], Suhett, Grazielle[2], Martins, Eduarda[1], Galina, Antonio[1] and Vieyra, Adalberto[1], [1]Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [2]4 Hospital Israelita Albert Einstein, São Paulo, Brazil

Acute myocardial infarction (AMI) remains the leading cause of death worldwide. Myocardial ischemia induces mitochondria dysfunction that impact negatively on functional recovered and cellular viability. Cell therapy have demonstrated a significant promise for cardiovascular research. This study aimed to evaluate the effect of Mesenchymal stem cells from adipose tissue (AD-MSC) on the mitochondrial respiration (MR) of infarcted cardiac tissue in an *i*n situ model. Female Wistar rats underwent permanent left anterior descending coronary (LAC) ligation. Two hours later, treated group (AD-MSC group, n=7) received 1×10^6 AD-MSC (from Wistar rats) by intramyocardial route and non-treated group (PBS group, n=10) received PBS. Age matched sham procedure (n=8) were used as a control. AMI was confirmed by electrocardiogram (ECG). Infarction scar size (ISS) was measured by triphenyltetrazolium chloride staining. Activity of the MR in saponin-permeabilizaded fibers was measure 24 hours after surgery using a high resolution respirometry. Cardiac muscle fiber were obtained from infarcted zone (IZ)

and border zone (BZ). A titration protocols of multiple substrates and inhibitors were used to assess MR. Density of mitochondria was measure through citrate synthase activity (CS). Infarcted rats that showed pathologic Q wave and ST-elevation in ECG were included in the study. ISS show no difference between PBS and AD-MSC group. Treated and non-treated IZ slices from infarcted rats presented a decreased in basal respiration, complex I, succinate (CI+CII), rotenone, oxygen flux coupled to ATP syntheses and leak control radio (L/E) when compared to sham group. IZ from PBS group presented a MR significantly altered in malonate, cytochrome C and proton Leak (L). Non-treated BZ slice presented a decreased in CI+CII respiration, L, malonate and oxygen flux coupled to ATP syntheses. ADP respiration and electron transport capacity (ETC) decreased in IZ and BZ slices from AD-MSC and PBS group comparing to sham. Residual oxygen consumption had no difference and no changes were observed between PBS and AD-MSC groups in all of the parameters. CS activity did not differ between groups. This results suggest that LAC ligation was responsible to alter MR in cardiac tissue but AD-MSC did not prevent this alteration in 24 h after the surgery.

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F1070

FUNCTIONAL SCREENING FOR EMBRYONIC GENE PROGRAMS THAT STIMULATE ADULT MOUSE CARDIOMYOCYTE PROLIFERATION

Judd, Justin, Lovas, Jonathan and Huang, Guo, University of California, San Francisco, San Francisco, CA, U.S.

Neonatal mice and adult lower vertebrates exhibit striking regeneration of the heart in response to injury. Despite early promise of resident cardiac stem cells, the mechanism of regeneration in both cases has been shown with genetic lineage tracing experiments to derive mostly from pre-existing differentiated cardiomyocytes that have dedifferentiated and regained the ability to proliferate. Adult mammalian cardiomyocytes in general are terminally differentiated and lack the ability to re-enter the cell cycle, potentially explaining why adult mammals cannot regenerate the heart. Thus, induction of cardiomyocyte proliferation in adult mammals may significantly contribute to adult heart regeneration. Here, we perform a functional screen to identify genes that can stimulate proliferation of primary adult mouse cardiomyocytes. A pool of candidate genes was selected from microarray data that exhibited high expression in proliferative embryonic cardiomyocytes, but were downregulated in senescent adult myocardium. The pool of candidate genes were packaged in adenovirus vectors and used to infect isolated adult mouse cardiomyocytes en masse. Lineage tracing transgenic lines were used to label cardiomyocytes unambiguously, despite potential changes in gene signatures that could otherwise make antibody staining difficult to interpret. Staining for EdU revealed the pool of candidate genes is able to stimulate cell cycle re-entry in up to 40% of adult mouse cardiomyocytes. Future work will require delivery of candidate gene subpools to identify the minimum number of candidate genes required to induce cell cycle re-entry and the utilization of confocal and epifluorescent live imaging to search for gene programs sufficient for the completion of karyokinesis and cell division.

F1072

THE ELECTROPHYSIOLOGICAL VARIABILITY OF THE CARDIAC NAV1.5 CHANNELS BETWEEN HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Lee, Sujeong, Lee, Hyang-Ae and Kim, Ki-Suk, Korea Institute of Toxicology, Korea Research Institute of Chemical Technology, Daejeon, Korea, South

The cardiac voltage-gated sodium channels encoded by the gene SCN5A have been revealed that their localization and activities are finely regulated by multiple domains of Na_V1.5 in cardiomyocytes. In human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs), the SCN5A can be measured and lead to the maximum upstroke velocity (V_{max}) of action potentials conducting the inward sodium currents (I_{Na}). The malfunction of I_{Na} caused by side effects of drugs has been linked to life-threatening arrhythmias as well as conduction disturbances. Therefore, it is very important to evaluate the potential effects of drugs on I_{Na} in various human cell lines. To elucidate pharmacological suitability of the I_{Na} assay in hPSC-CMs, we investigated the effect of class 1 anti-arrhythmic drugs which act as Na_V1.5 blockers on V_{max} and I_{Na} of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs, purchased from Cellular Dynamics International) and compared their sensitivity with the human embryonic stem cell-derived cardiomyocytes (hESC-CMs, combined matrigel mattress with enhanced GiWi differentiation protocol) by using whole-cell patch clamp recordings. The tetrodotoxin (TTX), a Na_V1.5 antagonist, showed the dose-dependent decrease of V_{max} at 0.3 and 1 μM both in hiPSC-CMs and hESC-CMs; however, the inhibitory potency for I_{Na} was higher in hiPSC-CMs than in hESC-CMs. Each class 1 anti-arrhythmic drugs which include quinidine (1A), lidocaine (1B), flecainide (1C) also decreased the V_{max} at the similar concentrations in hiPSC-CMs and hESC-CMs, but their inhibitory potencies for I_{Na} were much higher in hiPSC-CMs than in hESC-CMs. This is the first study to report the substantial differences in endogeneous I_{Na} sensitivity between hiPSC-CMs and hESC-CMs. Although the I_{Na} assay in hPSC-CMs appear to be functional, as TTX and class 1 anti-arrhythmic drugs commonly decreased the V_{max} conserving drug actions (Class 1C >> Class 1A >>

Class 1B), the density of I_{Na} is highly varied between hiPSC-CMs and hESC-CMs, which support the notion that the hPSC-CMs are composed of different pools of $Na_V1.5$ that might vary during cardiac differentiation. Future advances are needed to generate homogeneous electrical properties of I_{Na} by enhancing the maturation of CMs derived from hPSCs.

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F1074

CHARACTERIZATION OF DOXORUBICIN TOXICITY IN HUMAN PLURIPOTENT STEM CELL DERIVED-CARDIOMYOCYTES

Maillet, Agnes[1], Tan, Kim[1], Chai, Xiaoran[2], Mehta, Ashish[3], Sadananda, Singh[1], Shim, Winston[3] and Brunham, Liam[1], [1]ASTAR, singapore, Singapore, [2]Duke-NUS Graduate Medical School, singapore, Singapore, [3]National Heart Centre Singapore, Singapore, Singapore

Anthracyclines, such as doxorubicin (DOX) are widely used in the treatment of numerous cancers. However, their clinical utility is limited by a dose-dependent risk of cardiotoxicity and congestive heart failure. Despite decades of study, the precise mechanisms involved in DOX-induced cardiotoxicity remain incompletely understood. Recently, human pluripotent stem cell (hPSC)-derived cardiomyocytes have emerged as a valuable tool for modeling drug toxicity in cardiac cells. The goal of this study was to investigate the features of DOX-induced toxicity in hPSC-derived cardiomyocytes. Up to 90% of differentiated hES cells stained positive for cardiac specific markers indicating a high degree of efficiency of directed differentiation. We found that doxorubicin causes dose-dependent increases in apoptotic and necrotic cell death, reactive oxygen species production, mitochondrial dysfunction and increased intracellular calcium concentration. We characterized genome-wide changes in gene expression caused by doxorubicin using RNA-seq, as well as electrophysiological abnormalities caused by doxorubicin with multi-electrode array technology. Finally, we show that CRISPR-Cas9-mediated disruption of *TOP2B*, a gene implicated in DIC in mouse studies, significantly reduces the sensitivity of hPSC-CMs to doxorubicin-induced cell death. Our results establish the characteristics of doxorubicin toxicity in hPSC-derived cardiomyocytes. This model recapitulates many of the cardinal features of doxorubicin-induced cardiotoxicity observed in humans and represents a novel cellular model system that could be used to gain insight into the molecular mechanisms of doxorubicin-induced cardiotoxicity, investigate the impact of genetic variants on doxorubicin-response, and enable personalized medicine approaches to predicting risk of toxicity in individual patients.

F1076

UNRAVELLING THE REGULATORY MECHANISMS OF CARDIAC HYPERTROPHY DEVELOPMENT

Ovchinnikova, Ekaterina[1,2], Hoes, Martijn[2], Voors, Adriaan[2], van der Mei, Henny[2], van der Meer, Peter[2] and Berezikov, Eugene[1], [1]European Research Institute for the Biology of Ageing, Groningen, Netherlands, [2]University Medical Center Groningen, Groningen, Netherlands

Despite the decades of the extensive research, the mechanisms behind the development and regulation of heart failure (HF) are poorly understood. Cardiac hypertrophy is one of a major manifestations of HF. The primary cause of cardiac hypertrophy is mechanical stress. In this project we aim to study gene regulatory networks that underlie cardiac hyperthrophy development. In particular, we focused on advancing an in vitro model of mechanical stress on human embryonic stem cells (hESCs)-derived cardiomyocytes and characterization of the interplay between signalling pathways and cardiac microRNAs. In order to follow changes in the hESCs- derived cardiomyocytes we complemented our research with atomic force microscopy to evaluate dysregulation of hESC-cardiomyocytes mechanobiology, confocal microscopy to study cardiomyocyte cell size and cytoskeleton reorganization, and measure HF-associated mediators in cell culture media to evaluate levels of agents associated with cardiac remodeling. Our preliminary data show that hESCs-derived cardiomyocytes subjected to mechanical stretching exhibit features of a hypertrophic state and mimic the cardiac remodeling that accompanies HF progression. First of all, confocal microscopy revealed increase in cardiomyocytes size. Secondly, concentrations of HF-related mediators (such as cardiac troponin and brain natriuretic peptide) were measured in growth media of control and stretched hESCs-derived cardiomyocytes and significantly higher concentrations were detected upon stretching. Finally, we performed systematic analysis of changes in expression of protein-coding genes and microRNAs using RNA sequencing. RNA Seq- and small RNA-Seq-based gene expression analysis resulted in identification of many genes and microRNAs previously implicated in HF as well as novel potential targets. The integrated analysis of the generated datasets revealed the dynamic interplay between signalling pathways, cardiac microRNAs and microRNAs targets underlying cardiac hyperthrophy development. The obtained findings broaden our understanding of the molecular mechanisms behind HF and can serve as a launch platform for the development of new pharmacological approaches.

F1078

RECAPITULATION OF CLINICAL INDIVIDUAL SUSCEPTIBILITY TO DRUG-INDUCED QT PROLONGATION IN HEALTHY SUBJECTS USING IPS CELL-DERIVED CARDIOMYOCYTES

Shinozawa, Tadahiro[1], Nakamura, Koki[2], Shoji, Masanobu[3], Morita, Maya[3], Kimura, Maya[3], Furukawa, Hatsue[3], Ueda, Hiroki[4], Shiramoto, Masanari[5], Matsuguma, Kyoko[5], Kaji, Yoshikazu[5], Ikushima, Ippei[5], Yonou, Makoto[5], Liou, Shyh-Yuh[4], Nagai, Hirofumi[3], Nakanishi, Atsushi[3], Yamamoto, Keiji[3] and Izumo, Seigo[3], [1]Drug safety research laboratories, Fujisawa, Japan, [2]Takeda Pharmaceutical company, Tokyo, Japan, [3]Takeda Pharmaceutical company, Kanagawa, Japan, [4]Takeda Pharmaceutical company, Osaka, Japan, [5]Souseikai Global Clinical Research Center, Fukuoka, Japan

For prediction of drug-induced serious adverse event (SAE) in clinical trials, an "in vitro clinical trial" using a panel of cells derived from human induced pluripotent stem cells (hiPSCs) of individuals with differing susceptibility could facilitate major advances in translational research models in terms of safety and pharmaco-economics. However, it is unclear whether hiPSC-derived cells can recapitulate interindividual differences in drug-induced SAE susceptibility, especially in healthy subjects. To address this question, we evaluated individual differences in SAE susceptibility based on an in vitro model using hiPSC-derived cardiomyocytes (hiPSC-CMs). hiPSCs were generated from blood samples of ten healthy subjects with different susceptibility to moxifloxacin (Mox)-induced QT prolongation. The study design was the same as that in regulatory "thorough QT" (TQT) studies. Global gene expression analysis showed comparable gene expression related to cardiac ion channel activity in hiPSC-CMs in each subject. The correlation between the concentration–response relationship for QT prolongation in the individuals and that from field potential duration (FPD) prolongation in hiPSC-CMs generated from these individuals was evaluated. Different Mox-induced FPD prolongation values were observed in the hiPSC-CMs from each individual, and the QT interval was significantly and positively correlated with FPD values at clinically relevant concentrations (r=0.64-0.84) in multiple analyses including concentration–QT analysis. Genomic analysis showed no significant differences in known Mox-binding sites between individuals or in SNPs of genes related to cardiac ion channels. In conclusion, individual differences in the susceptibility to Mox-induced QT prolongation could be recapitulated in hiPSC-CMs derived from healthy subjects. To our knowledge, this is the first report to provide proof-of-concept for in vitro clinical trials and shows the potential of in vitro TQT studies as an alternative to clinical regulatory studies. Notably, it was demonstrated that iPS technology could be an effective tool to investigate the mechanism underlying individual differences in susceptibility with unknown genotype variations.

F1080

SMALL MOLECULE DIFFERENTIATION OF CARDIOMYOCYTES FROM HUMAN AMNIOTIC FLUID CELLS: POTENTIAL APPLICATION IN PRENATALLY DIAGNOSED CONGENITAL HEART DISEASE

Walker, Kendal Antoinette, Jiang, MS, Guihua, Herron, Todd J., Di Bernardo, Julie, O'Shea, K. Sue and Kunisaki, Shaun, University of Michigan, Ann Arbor, MI, U.S.

Prenatally diagnosed congenital heart disease (CHD), which includes hypoplastic left heart syndrome and ventricular septal defects, is the most common type of birth defect associated with high morbidity and mortality. Since amniotic fluid mesenchymal stem cells (AF-MSCs) can be obtained in a minimally invasive fashion in these patients, AF-MSCs are the ideal candidate cell type for perinatal cell-based regenerative medicine therapies in CHD newborns. The purpose of this study was to derive human transgene-free cardiomyocytes with potential for therapy from cells obtained by routine amniocentesis. Human AF-MSCs (n=2) were isolated, expanded, and reprogrammed into induced pluripotent stem cells using non-integrating Sendai viral (SeV) vectors. The cells were then differentiated into functional cardiomyocytes using a modified small molecule protocol for up to 90 days. After six weeks, quantitative gene expression revealed a mixed population of differentiated atrial, ventricular, and nodal AF-CMs as demonstrated by upregulation of multiple cardiac markers, including MYH6, MYL7, TNNT2, TTN, and HCN4, which were comparable to levels expressed by neonatal dermal fibroblast-derived cardiomyocyte controls. AF-CMs had a normal karyotype and demonstrated loss of NANOG, OCT4, and the SeV transgene. Functional characterization of SIRPA$^+$ AF-CMs showed a higher spontaneous beat frequency compared to dermal fibroblast controls but revealed normal calcium transients and appropriate chronotrophic responses after b-adrenergic agonist stimulation. These data suggest that AF-CMs generated using a transgene-free, small molecule approach may be useful for disease modeling and clinical therapeutic applications in prenatally diagnosed CHD.

MUSCLE CELLS

F1084

HEMATOPOIETIC STEM CELL CO-TRANSPLANT INDUCES TOLERANCE AND ROBUST ENGRAFTMENT OF ALLOGENEIC MUSCLE STEM CELL GRAFTS IN MICE WITH MUSCULAR DYSTROPHY

Burnett, BA, Cassandra E, Chhabra, Akanksha, Kwon, Hye-Sook, Goldstone, Andrew B, Woo, Y. Joseph and Shizuru, Judith A, Stanford School of Medicine, Stanford, CA, U.S.

Muscular dystrophy is a heterogeneous group of degenerative muscle disorders characterized by severe, progressive muscle wasting. Syngeneic transplantation of skeletal muscle precursor cells (CD45-, Sca-1-, Mac-1-, CXCR4+, β1-Integrin+) reverses the dystrophic phenotype in a murine muscular dystrophy model (DMD^{MDX}). However, the immunologic barrier of allogeneic transplantation must be overcome to permit effective clinical translation. Co-transplantation of donor matched hematopoietic cells has been shown to induce immune tolerance to solid organs in animals, and more recently in clinical studies of kidney allografts. Here we tested in a haploidentical allogeneic model system if establishment of a chimeric hematopoietic system using purified hematopoietic stem cells (HSC) from the same muscle stem cell donor would allow long-term allogeneic SMP engraftment without immunosuppression. DMD^{mdx} recipients received either an autologous purified hematopoietic stem cell transplant (HSCT) (5 mice), an allogeneic haploidentical HSCT (9 mice), or no HSCT (9 mice). FACS analysis of peripheral blood demonstrated durable long-term multilineage chimerism at 5 weeks (71.0%±8.7) and 28 weeks (90.5%±3.3); no incident acute or chronic graft vs. host disease was observed. Six weeks after HSCT, 1000 allogeneic GFP+, luciferase+ SMP cells were transplanted into the left tibialis anterior. With in vivo bioluminescent imaging, robust engraftment, early proliferation, and long-term 24-week maintenance of donor cells was exclusively observed in 100% of mice that had previously received an allogeneic HSCT. Autologous HSC transplant recipients and non-HSCT recipients routinely rejected the SMP graft (80% and 100% rejection, respectively). Furthermore, at 12 and 24 week timepoints histologic analysis revealed GFP+ myofibers and restoration of dystrophin expression, thereby confirming successful reversal of the dystrophic phenotype. Thus, a purified allogeneic HSC transplantation safely and effectively induces tolerance to allogeneic stem cells. This novel approach precludes immunosuppression and permits stem cell-mediated cure of degenerative disorders such as muscular dystrophy.

F1086

MITOCHONDRIAL COCHAPERONE PROTEIN Tid1 IS REQUIRED FOR ENERGY HOMEOSTASIS DURING SKELETAL MYOGENESIS

Lo, Jeng-Fan, National Yang-Ming University, Institute of Oral Biology, Taipei, Taiwan, Cheng, Li-Hao, National Yang-Ming University, Taipei, Taiwan and Huang, Tung-Fu, Taipei Veterans General Hospital, Taipei, Taiwan

Tid1 is a mitochondrial cochaperone protein, and Tid1 transcripts are abundantly expressed in the adult skeletal muscle tissues. However, the physiological function of Tid1 during skeletal myogenesis remains unclear. In vitro mouse myoblast C2C12 cells induced differentiation was applied to understand the role of Tid1 during myogenesis. Additionally, transgenic mice (HSA-$Tid1^{f/f}$) with muscle specific (HSA-Cre) Tid1 deficiency were established and further examined to characterize the physiological function of Tid1 during skeletal muscle development in vivo. We observed that expression of Tid1 protein was up-regulated in the differentiated C2C12 cells. Notably, compared to the control mice the HSA-$Tid1^{f/f}$ mice displayed more severe muscular dystrophic phenotype in vivo. Next, the expression profile of muscular development markers in both the HSA-$Tid1^{f/f}$ and control mice at postnatal day (P) 5 and P8 was examined, respectively. We observed that expression of myosine heavy chain (MyHC) proteins within muscular tissues was reduced in HSA-$Tid1^{f/f}$ mice. In addition, the protein level of ATP sensor (AMPK/P-AMPK) and mitochondrial biogenesis protein (PGC-1α) was also significantly reduced in HSA-$Tid1^{f/f}$ mice in comparison with that in the control mice at P8. Moreover, Tid1 deficiency induced apoptosis marker Caspase-3 in muscle tissues from HSA-$Tid1^{f/f}$ mice at P5 and P8, respectively. Next, we observed that down-regulation of Tid1 abolished the differentiation ability of C2C12 cells via impairing the mitochondria activity, in vitro. Together, our results suggest that Tid1 deficiency reduces the PGC-1α expression and mitochondria activity resulting in energy imbalance and promoting apoptosis of muscle cells during myogenesis. The significance of this study warrants the importance of Tid1 in muscular development. It will be of importance to understand the function of Tid1during human muscular dystrophy in the future.

F1088

CELL THERAPY FOR MUSCULAR DYSTROPHY BY HUMAN IPS CELL-DERIVED MUSCLE STEM CELLS

Sakurai, Hidetoshi[1], Takayama, Satoru[1], Ikeya, Makoto[2], Hotta, Akitsu[3], Zhao, Mingming[1], Takenaka-Ninagawa, Nana[1], Nakasa, Masanori[1], Tazumi, Atsutoshi[1] and Sato, Takahiko[4], [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]Department of Life Science Frontier, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [4]Kyoto Prefectural University of Medicine, Kyoto, Japan

Cell therapy is one of desired method for treating intractable muscular diseases, such as Duchenne muscular dystrophy (DMD). Skeletal muscle contains a stem cell, called satellite cell, which has remarkable muscle regeneration potential and is considered as a good source of cell therapy. However, the clinical trials of cell therapy using adult satellite cells have never been succeeded mainly due to the difficulty of expansion of satellite cells with maintaining their regeneration potentials. Instead of adult satellite cells, generating satellite cells from induced pluripotent stem cells (iPSCs) would have advantage for application of cell therapy, because of their unlimited proliferation potentials. Here, we demonstrated the effective stepwise differentiation method from human iPSCs to engraftable muscle stem cells without transgene induction. In the first step, we induced the dermomyotome-like population that is identified as Pax3 positive and neural crest marker negative cells over 90% efficiency. In the second step, we induced myotome-like population that is identified as Myf5 positive cells, which showed highly myogenic differentiation potential in vitro. Gene expression profile of purified Myf5+ cells demonstrated that the expression of Pax7, a marker of satellite cells, was significantly increased in Myf5+ cells at the late stage of differentiation. To assess the regeneration potential, we transplanted the Myf5+ cells at the late stage of differentiation into immunodeficient DMD-model mice. The Myf5+ cells could be engrafted in more than one hundred of host myofibers and regenerate the diseased muscles with producing dystrophin. Moreover, a part of the engrafted cells settled as a satellite cells in vivo with expressing Pax7. Finally, we confirmed the functional recovery of cell transplanted DMD gastrocnemius by the assessment of the planterflex torque with electric stimulation. Taken together, we demonstrate that the transplantation of the human iPSC-derived muscle stem cells with step-wise differentiation can be effective for DMD with amelioration of muscle function. Our results facilitate to establish the cell therapy of muscular diseases using iPS cell-derived muscle stem cell.

PANCREATIC, LIVER, LUNG, OR INTESTINAL/GUT CELLS

F1092

HUMAN iPSC-DERIVED FUNCTIONAL BETA-CELLS FOR USE IN DRUG SCREENING AND OTHER IN VITRO APPLICATIONS

Andersson, Christian X, Takara Bio Europe AB, Gothenburg, Sweden

Diabetes mellitus is a condition where the pancreatic beta-cells fail to produce enough insulin required to regulate the circulating blood glucose level in the body, thus resulting in hyperglycemia. This can further lead to severe long-term complications, such as renal failure, neuropathy, increased risk of cardiovascular diseases, and retinopathy that may cause blindness. Type I diabetes is an autoimmune variant of this disease where the immune system attacks and destroys the pancreatic beta-cells. Typically, Type I diabetes patients are treated by external administration of insulin, but it has lately been shown that the disease can also be treated by transplanting islets from deceased donors; illustrating proof-of-concept that cell therapy can be used to restore euglycemia. Besides using beta-cells for clinical therapies, they can also be utilized in in vitro applications for studying beta-cell function (e.g. insulin secretion mechanism, calcium influx, pancreatitis, etc.) or as a screening tool to identify potential drugs to regulate insulin secretion. Here we describe a differentiation protocol that has been developed to generate insulin-producing beta-cells from hiPS cells for use in in vitro applications. In the final differentiation/maturation step of the protocol, the cells are cryopreserved as single cells so that they can easily be thawed and seeded into a 2D monolayer, thus enabling different analyses, such as Glucose-Stimulated-Insulin-Secretion (GSIS). Our hiPS-derived beta-cells express markers characteristic of human primary beta-cells, including MAFA, along with displaying GSIS functionality comparable to primary islets. Results presented here show that hiPS-derived beta-cells, a nearly inexhaustible source of functional human beta-cells, could replace the use of primary pancreatic islet cells in in vitro studies, thus reducing donor dependence and individual variability.

F1094

IMMUNE-TOLERABLE BETAβ-LIKE CELLS GENERATED FROM DIRECT HEPATOCYTE REPROGRAMMING AMELIORATE AUTOIMMUNE DIABETES

Chang, Fang-pei[1], Chien, Chiao-Yun[1], Shen, Chia-Rui[2], Sytwu, Huey-Kang[3] and Shen, Chia-Ning[1], [1]Academia Sinica, Taipei, Taiwan, [2]Chang-Gung University, Taipei, Taiwan, [3]National Defense Medical Center, Taipei, Taiwan

Type 1 diabetes is characterized by complete loss of β-cells due to T-cell mediated autoimmune attack leading to a deficiency of insulin. Transplantation of islets has proved to be an effective treatment for patients with type 1 diabetes. However, transplantation is severely limited by the lack of islet donors. Moreover, patients received the allogeneic islet transplantation were still suffering from side effects of the immunosuppressive medications. Hence, the possibility of producing immune-tolerable β-cells would be a key challenge for developing cell-based therapeutics for type 1 diabetic patients. We and others had previously demonstrated hepatocytes can be transdifferentiated to insulin-producing β-cells after introducing Pdx1. However, it is unclear whether these transdifferentiated β-cells are glucose responsive and immune-tolerable in autoimmune diabetic status. Liver not only has remarkable capacities to regenerate after injury but also an immune privileged organ. We hypothesize β-cells derived from hepatocyte reprograming may possess characteristics for avoiding attack from auto-reactive immune cells. In current work, we demonstrated simultaneously expressing Pdx1, Ngn3, and PDGFRα could induce direct reprogramming of hepatocytes from non-obese diabetic (NOD) mice, which spontaneously develop autoimmune diabetes, to insulin-producing cells display characteristics of pancreatic β-cells including expression of mafa, nkx2.2, rfx6, kir6.2, glut2 and proprotein convertase 1/3 and possessing the capability to secrete insulin responding to stimulatory levels of glucose. Addition of PDGF-AA could induce Rb phosphorylation and trigger hepatocyte-derived β-like cells to expand. Autologous transplantation of hepatocyte-derived β-like cells to diabetic NOD mice significantly improved hyperglycemia without needs of tolerogenic treatments. Further characterization demonstrated β-like cells derived from hepatocyte reprogramming displayed reduced levels of MHC-Class-I molecules and autoantigens and expressed PD-L. The results explain why hepatocyte-derived β-like cells were immune-tolerable. The findings from the present work raises the possibility of developing cell therapeutic strategy for patients with type 1 diabetes via autologous hepatocyte reprogramming.

F1096

GLOBAL GENE EXPRESSION PROFILING CONFIRMS REPRODUCIBLE HEPATIC DIFFERENTIATION OF HPSCS AND REVEALS DIFFERENCES BETWEEN IN VITRO DERIVED HEPATOCYTES AND HUMAN LIVER TISSUE

Ghosheh, Nidal[1,2], Küppers-Munther, Barbara[3], Asplund, Annika[2], Edsbagge, Josefina[3], Andersson, Tommy B.[4,5], Björquist, Petter[6], Andersson, Christian X.[3], Carén, Helena[1], Simonsson, Stina[7], Sartipy, Peter[2,4] and Synnergren, Jane Marie[2], [1]The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden, [2]The University of Skövde, Skövde, Sweden, [3]Takara Bio Europe AB, Gothenburg, Sweden, [4]AstraZeneca R&D, Mölndal, Sweden, [5]Karolinska Institutet, Stockholm, Sweden, [6]NovaHep AB, Gothenburg, Sweden, [7]The Sahlgrenska Academe at University of Gothenburg, Gothenburg, Sweden

Numerous differentiation protocols have been reported to generate hepatocytes from human pluripotent stem cells (hPSCs) in vitro. However, fully functional, drug metabolizing, hepatocytes derived in such models has yet to be achieved. We recently showed high synchronicity in the expression of some key lineage specific genes across several hPSC-lines during in vitro hepatic differentiation, applying a standardized protocol. In the present study we investigated, on the global level, differentially expressed genes of definitive endoderm, hepatoblast, fetal hepatocyte, and adult-like hepatocyte stages during differentiation of six hPSC lines (including three hESCs and three human induced stem cells (hiPSCs)) using the Affymetrix Human Transcriptome Array 2.0. Human liver tissue and undifferentiated hPSCs were used as reference samples. The results show not only very low variation between repeated experiments, but also between the different hPSC lines, indicating high robustness of the culturing system and a high reproducibility of the differentiation process, which also was confirmed by ANOVA and gene clustering analysis. Moreover, confirmed that neither hESC lines nor hiPSC lines tend to cluster together. In addition, more differentially expressed genes (3740 genes) were detected between in vitro generated adult-like hepatocytes and liver tissue, while fewer (268 genes) were detected between in vitro fetal hepatocytes and the adult-like hepatocytes achieved in this study. Notably, biological processes that were enriched among the differentially expressed genes between in vitro generated hepatocytes and liver tissue included for example drug metabolic and catabolic processes, and P450 pathways. Taken together, these findings shed further light on the similarity of the fetal- and adult-like hepatocyte stages, as well as the differences between the in vitro derived hepatocyte-like cells and liver tissue. Data presented describe present status of the hPSC-derived hepatocyte research and serve as a basis

for improvement of the differentiation process to achieve fully functional hepatocytes with adult characteristics, to be used in down-stream applications, such as drug discovery and regenerative medicine.

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F1098

A MICROFLUIDIC FORSKOLIN-INDUCED SWELLING ASSAY ON LGR5+ SMALL INTESTINAL ORGANOIDS

Kurek, Dorota[1], Trietsch, Sebastiaan J.[2], van de Wetering, Marc[3], van Duinen, Vincent[4], Wilschut, Karlijn[2], Vries, Rob[3,5], Clevers, Hans[3,5], Joore, Jos[2] and Vulto, Paul[2], [1]Mimetas B.V., Leiden, Netherlands, [2]Mimetas BV, Leiden, Netherlands, [3]Hubrecht Institute, Utrecht, Netherlands, [4]LACDR, Leiden University, Leiden, Netherlands, [5]Foundation Hubrecht Organoid Technology, Utrecht, Netherlands

The culture of human organ stem cells in organoids offers novel opportunities for regenerative and personalized medicine. Recently a the forskolin-induced swelling (FIS) assay for primary intestinal organoids was proposed for drug testing in cystic fibrosis. The assay delivers a visual readout of the CFTR function, a protein mutated in cystic fibrosis patients. In this work we use a high throughput microfluidic platform, called OrganoPlate for implementation of the assay in an automated setting. The OrganoPlate a designed around the microtiter plate standard and is therefore fully compatible with any standard laboratory equipment. A forskolin assay was implemented on mouse lgr5+ small intestinal organoids upon 3-4 days in culture. Since the organoids were grown in a confined microfluidic space, a swollen organoid occupied the complete height of a 120mm high channel and expanded further in lateral direction. Automated readout of the swelling assay was realized on a MolDev ImageXpress High Content Imager, followed by image analysis and quantification of the swelling process. The implementation of this assay in a microtiter plate based microfluidic platform yields a robust assay that can be processed in a high throughput manner towards personalized drug response studies in patients.

F1100

E2f4 CONTROLS A NUCLEOCYTOPLASMIC PROGRAM CRUCIAL FOR MULTICILIOGENESIS IN LUNG EPITHELIAL PROGENITORS

Mori, Munemasa, Columbia University Medical Center, New York, NY, U.S.

Multiciliated cells are crucial for fluid and ion transport in epithelia of a variety of organs and their impaired development and function are seen in human diseases affecting the brain, respiratory, and reproductive tracts. Multiciliogenesis requires activation of a specialized transcription program coupled to complex cytoplasmic events that lead to large-scale centriole amplification to generate multicilia. Yet, it remains unclear how these events are coordinated to initiate multiciliogenesis in epithelial progenitors. Here we identify an unsuspected mechanism orchestrated by the transcription factor E2f4 essential to integrate these processes. We show that after inducing a transcriptional program of centriole biogenesis, E2f4 translocates to the cytoplasm to become a core component of structures classically identified as fibrous granules (FG), acting as organizing centers for deuterosome assembly and centriole amplification. Remarkably, loss of cytoplasmic E2f4 prevents FG aggregation, deuterosome assembly and multicilia formation even when E2f4's transcriptional function is preserved. Moreover, in E2f4-deficient cells multiciliogenesis is rescued only if both nuclear and cytoplasmic E2f4 activities are restored. Thus, E2f4 integrates previously unrelated nuclear and cytoplasmic events of the multiciliated cell program.

F1102

β-CATENIN DIRECTS THE PHENOTYPE OF MYELOID LINEAGE CELLS DURING LIVER FIBROSIS IN MICE.

Sadri, Ali-Reza[1,2], Amini-Nik, Saeid[1,2], Diao, Michael[1,2], Belo, Cassandra[2] and Jeschke, Marc G.[1,2], [1]University of Toronto, Toronto, ON, Canada, [2]Sunnybrook Research Institute, Toronto, ON, Canada

Severe burn injury results in local and systemic responses including profound hepatic alterations. While accumulating data suggests that macrophages contribute to the transformation of mesenchymal hepatic stellate cells into myofibroblasts, contributing to fibrosis, their mechanism of action and spatiotemporal role is not clear. Through the use of Cre-transgenic mice that specifically marks myeloid cells and burn as a model of systemic injury, we seek to ascertain the role of myeloid lineage cells in liver fibrosis and evaluate the mechanism of their action. We show that thermal injury in mice (30% Total Body Surface Area) promotes an influx of myeloid cells to the liver with a peak in fibrosis 1-2 weeks post injury, which is localized predominantly around portal

venules, whereas myeloid cells are enriched throughout the liver. When we ablate macrophages using liposomal clodronate, less fibrosis is observed. An essential role for β-catenin, the key molecule of Wnt pathway, has been shown during fibrosis in other organs, particularly in myeloid lineage cells. As such, we evaluated the role of Wnt/β-catenin signalling in myeloid lineage cells during liver fibrosis. Our data show that in mice lacking β-catenin in their myeloid cells, there was an impaired migratory capacity to the wound site accompanied with lower fibrosis post-thermal injury. To unravel characteristics of myeloid cells that are lacking β-catenin during liver fibrosis, we used the carbon-tetrachloride (CCL_4) model, an established model of liver fibrosis. Differential Ly-6C expression has been widely used to identify functionally distinct populations of circulating murine monocytes and macrophage populations in pathology. Our data show that knocking down the Ctnnb1 gene in myeloid lineage cells impairs recruitment of pro-fibrotic myeloid cells (EYFP+ve, Ly-6C^{Hi}, CD11b^{Hi}, F480^{Low}) to the liver while there is an accumulation of pro-resolution myeloid cells (EYFP+ve, Ly-6C^{low}, CD11b^{low}, F480^{Hi}) in the liver. β-catenin in myeloid lineage cells directs the phenotype of these cells during liver fibrosis. Targeting Wnt/β-catenin signalling may be an effective method of modulating the phenotype of macrophages and their interaction with mesenchymal progenitor cells in favor of a more anti-fibrotic rather than pro-fibrotic phenotype in the liver.

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F1104

GENERATION OF HUMAN IPS CELL-DERIVED HEPATOCYTE-LIKE CELLS UNDER CHEMICALLY DEFINED CONDITION FOR CELL TRANSPLANTATION

Takayama, Kazuo[1], Hagihara, Yasuko[2], Sekiguchi, Kiyotoshi[3], Morio, Tomohiro[4], Ohara, Osamu[5], Tachibana, Masashi[1], Sakurai, Fuminori[1] and Mizuguchi, Hiroyuki[1], [1]Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan, [2]National Institute of Biomedical Innovation, Health and Nutrition, Osaka, Japan, [3]Institute for Protein Research, Osaka University, Osaka, Japan, [4]Tokyo Medical and Dental University, Tokyo, Japan, [5]Kazusa DNA Research Institute, Chiba, Japan

It is known that more than 20,000 patients with severe liver failure are dying every year because of shortage of donor livers and hepatocytes. Recently, human iPS cell-derived hepatocyte-like cells (human iPS-HLCs) are expected to be utilized as alternative donor sources. To realize the clinical trials of hepatocyte-like cell human iPS-HLC transplantation, it is necessary to establish a method to generate human iPS-HLCs, which have various liver functions and have no risk of teratoma formation and tumorigenicity, under chemically defined condition. In this study, integration-free human iPS cells (1383D6) were cultured on E8 fragments of laminin 511 (LN511-E8) with AK03 medium (chemically defined medium for human iPS cells). Interestingly, the gene expression levels of collagen receptors (integrin α1, α2, α10, and α11) were decreased by using LN511-E8. Consistently, we confirmed that laminin-rich extracellular matrix was suitable for hepatocyte differentiation of human iPS cells which were cultured on LN511-E8 as compared with collagen-rich extracellular matrix. In hepatocyte differentiation, human iPS cells were sequentially treated with Activin A, BMP4, FGF4, HGF, and OsM. To examine whether human iPS cells differentiate into hepatocyte-like cells, the expression levels of albumin (ALB) were examined. The percentage of ALB-positive cells was more than 87%, and the ALB secretion amount was approximately 4,300 ng/24hr/ml. In addition, the gene expression levels of α-1-antitrypsin and cytochrome P450 3A4 in human iPS-HLCs were approximately 50% of primary human hepatocyte. Because it is known that teratoma could be generated from residual undifferentiated cells, we measured the percentage of undifferentiated cells in human iPS-HLCs. The percentage of undifferentiated cells, which could form alkaline phosphatase positive colonies, was lower than 0.003%. Furthermore, the genomic mutations of cancer-related genes in human iPS-HLCs were examined by RNA-seq analysis to examine the risk of tumorigenicity. There was no significant genomic mutation in cancer-related genes. In conclusion, we could generate human iPS-HLCs, which have various liver functions and have low risk of teratoma formation and tumorigenicity, under chemically defined condition.

ENDOTHELIAL CELLS/ HEMANGIOBLASTS

F1110

ANALYSIS OF THE ROLE OF VEGFR2 IN THE GENERATION OF HEMATOPOIETIC STEM CELLS DURING MOUSE EMBRIONIC DEVELOPMENT

Binagui-Casas, Anahi[1], Souilhol, Celine[2] and Medvinsky, Alexander[2], [1]University of Edinburgh, Edinburgh, U.K., [2]MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, U.K.

In the mouse embryo the first definitive hematopoietic stem cells (HSCs), capable of repopulating an adult irradiated mice, emerge at mid-gestation by embryonic day E11. At this stage, the aorta-gonad-mesonephros (AGM) region is able to initiate and expand HSCs. Recently, it has been shown that the development of HSC in the

AGM region results from the maturation of hematopoietic precursors called pre-HSCs. Many evidences point at an endothelial origin for these cells, the hematogenic endothelium. Analysis of mutants for VEGF signaling, a critical pathway for endothelial development, suggested that it also plays a role during early hematopoiesis. Knock-out mutants for the main receptor, VEGFR2 *(*also known as Flk-1*),* showed a decrease of intra-embryonic hematopoietic progenitors. Although VEGFR2 has been identified as an essential gene for HSC emergence, its exact point of action in HSC development remains unresolved. Here we aim to define precise stages and cell types during HSC development in which VEGF signaling is critically involved. By using a reporter line, we first demonstrated that VEGFR2 is expressed in the pre-HSCs/HSC lineage. Germ-line VEGFR2 knockout results in embryonic lethality at around E9.5, before HSC emergence. Therefore, to determine the role of the pathway in HSC development, we used a conditional inducible mutagenesis approach that allowed us to delete VEGFR2 receptor specifically when pre-HSCs mature into HSCs in the E10.5 and E11.5 AGM region. Our data indicates that VEGFR2 deletion at these stages affects both endothelial and hematopoietic progenitors, as well as HSC development. This is the first report showing that the VEGF pathway is not only involved in early stages of hematopoietic development, as previously suggested, but it is also essential for maturation of HSCs at later stages.

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F1112

VASCULOGENIC CONDITIONED PERIPHERAL BLOOD MONONUCLEAR CELLS RESCUE THE RADIATION-INDUCED SALIVARY GLAND HYPOFUNCTION

I, Takashi[1], Sumita, Yoshinori[2], Masuda, Haruchika[3], Kuroshima, Shinichiro[2], Tran, Simon D.[4], Asahara, Takayuki[3] and Asahina, Izumi[2], [1]Nagasaki university, Nagasaki, Japan, [2]Nagasaki University Graduate School of Biomedical Sciences, Nagasaki-shi, Japan, [3]Tokai University, Isehara, Japan, [4]McGill Univ, Montreal, QC, Canada

To develop the cell therapy for radiogenic xerostomia, we have focused on peripheral blood-derived mononuclear cells (PBMNCs), purified by recently improved quality and quantity (QQ) culture system of endothelial progenitor cells (EPCs). QQ cultured-PBMNCs (QQ-PBMNCs) that contained EPC population abundantly can be obtained after only 5-days of culture, and be expected to display the enhanced-vasculogenic and anti-inflammatory effects. In this study, we investigated how exogenous QQ-PBMNCs rescue the radiation-induced SG hypofunction. We have shown the therapeutic potential of QQ-PBMNCs with increasing saliva production at last annual meeting. However, the detailed mechanisms of gland regeneration by QQ-PBMNCs remains unclear. Methods; Firstly, we analyzed the characteristics of QQ-PBMNCs by colony-forming unit and flow-cytometory *in vitro.* Then, 5x10^{4}QQ-PBMNCs after labeling with PKH26 were transplanted to submandibular glands directly in mice after 3-days of head and neck-irradiation (IR). After 1, 2, 4, 8 and 12 weeks of IR, saliva outputs were measured, and then the gland tissues were harvested for the histological and gene expression analyses. Non-cell transplanted mice after IR were employed as an experimental control. Result; After 5-days of QQ-culture, populations of CD206+/CD11b+ cells (M2 macrophages) and Sca-1+/c-Kit+/Lin- cells (EPCs) were significantly increased in PBMNCs. Then, in vivo, Salivary outputs in mice that transplanted QQ-PBMNCs were increased 2- to 4-fold sequentially for 4-12 weeks after IR compared with that in control mice. In vivosamples, we firstly found PKH26-positive cells migrated to the perivascular-area in damaged glands by 1-2 weeks after IR. Then, after 4 weeks, activations of stem cell markers (Sca-1/c-Kit) in ductal-area and cell proliferation in acinar-area were observed in QQ-PBMNCs-treated SGs, while mRNA expressions of IL-1β, INF-γ or TNF-α were downregulated. After that, we could recognize the sequential vasculogenesis in QQ-PBMNCs-treated SGs, and finally abundant acinar cells with the less fibrosis were observed at 12 weeks. QQ-PBMNCs can work on the rescue of damaged SGs as angiovasculogenic and anti-inflammatory agents.

F1114

CCL5 DERIVED FROM ENDOTHELIAL COLONY-FORMING CELLS (ECFCs) MEDIATES RECRUITMENT OF SMOOTH MUSCLE PROGENITOR CELLS (SPCS) TOWARD VASCULAR LOCATIONS IN HUMAN MOYAMOYA DISEASE

Moon, Youn joo, Seoul National University Hospital, Seoul, Korea, South

The etiology and pathogenesis of moyamoya disease (MMD) are still obscure. Previous studies indicated that angiogenic chemokines may play an important role in the pathogenesis of the disease. Recently, it was discovered that peripheral blood-derived endothelial colony-forming cells (ECFCs) and smooth muscle progenitor cells (SPCs) have defective functions in MMD patients. Therefore, the interactions of ECFCs and SPCs, the precursors of two crucial cellular components of vascular walls, with some paracrine molecules is an intriguing subject.

Co-culture of ECFCs and SPCs from MMD patients and healthy normal subjects revealed that MMD ECFCs, not SPCs, are responsible for the defective functions of both ECFCs and SPCs. Enhanced migration of SPCs toward MMD ECFCs supported the role for some chemokines

secreted by MMD ECFCs. Expression arrays of MMD and normal ECFCs suggested that several candidate cytokines differentially produced by MMD ECFCs. We selected chemokine (C-X-C motif) ligand 6 (CXCR6), interleukin-8 (IL8), chemokine (C-C motif) ligand 2 (CCL2), and CCL5 for study, based on the relatively higher expression of these ligands in MMD ECFCs and their cognate receptors in MMD SPCs. Migration assays showed that only CCL5 significantly augmented the migration activities of SPCs toward ECFCs. Treatment with siRNA for the CCL5 receptor (CCR5) abrogated the effect, confirming that CCL5 is responsible for the interaction of MMD ECFCs and SPCs.

These data indicate that ECFCs, not SPCs, are the major players in MMD pathogenesis and that the chemokine CCL5 mediates the interactions. It can be hypothesized that in MMD patients, defective ECFCs direct aberrant SPC recruitment to critical vascular locations through the action of CCL5.

EPITHELIAL CELLS (NOT SKIN)

F1116

TELOMERASE REACTIVATION IN THE Lgr5+ CELLS RESCUES STEM CELL DEPLETION AND ENHANCES LIFE-SPAN THROUGH THE SUPPRESSION OF THE ER/UPR STRESS PATHWAY

Chakravarti, Deepavali, Hu, Baoli, Wang, Alan, Dunner, Kenneth and Depinho, Ronald A., MD Anderson, Houston, TX, U.S.

Telomere shortening has been correlated with several aging related pathogenesis of the intestine like Crohn's disease and ulcerative colitis. GWA studies have revealed ER stress related proteins like spliced Xbp1 plays important role in the initiation of such inflammatory bowel diseases. Late generation (G4) telomere dysfunctional mice exhibit both high rate of intestinal crypt loss and surprisingly, inflammatory tumors (DALMS) of both the small and the large intestine by 6 months of age compared to the early generation counterpart (G0). In order to study the role of telomerase loss followed by activation in the intestinal compartment we crossed the late generation telomerase deficient animals to the tamoxifen inducible Lgr5-EGFP Cre model. In this model we could reactivate telomerase at desired time specifically in the intestinal stem cell compartment and differentiate the effects of telomerase reactivation in the hematopoietic compartment versus the intestinal compartment. With the help of immunohistochemistry, beta galactosidase lineage tracing experiments and BrDU incorporation assay we determined the proliferation rate and the cell turn over rates in these animals. We also performed electron microscopy on the crypts. RNA-seq revealed a differential expression of the unfolded protein response pathway and immune pathways. Closer examination revealed an increase in ER stress protein expressions in the stem cell compartment leading to premature differentiation of the stem cells to a more progenitor like population. Telomerase reactivation reverses the phenotype and extends the lifespan of these mice. In conclusion telomerase reactivation preserves stem cells by reducing ER stress.

F1118

OPTIMISATION OF A METHOD TO ISOLATE AND EXPAND BASAL CELLS FROM HUMAN RESPIRATORY EPITHELIUM

Gowers, Kate H. C., Hynds, Robert E., Butler, Colin R., Thakrar, Ricky and Janes, Sam M., Lungs for Living Research Centre, London, U.K.

Basal cells are stem cells in the upper airways and are important in the maintenance of a healthy respiratory epithelium. Perturbations in airway homeostasis are thought to drive pathogenesis in airway diseases such as asthma, fibrosis and lung cancer. Autologous airway epithelial cells have been used in tissue-engineered airway transplantations to regenerate the mucosa. For these tissue engineering applications, large numbers of airway epithelial cells must be rapidly expanded in culture. We sought to compare the cell number and composition of two methods of tissue acquisition, endobronchial brushing and endobronchial biopsy. We investigated the benefit of expanding airway epithelial cells from human endobronchial biopsy samples in co-culture with mitotically inactivated 3T3-J2 fibroblasts and ROCK inhibitor, which has recently been reported to improve expansion compared with traditional techniques. Furthermore, we investigated whether expansion from endobronchial biopsy samples was comparable in 3T3-J2 fibroblast-conditioned medium. We found that creating a single cell suspension prior to culture, either from an endobronchial brushing or by digesting endobronchial biopsies, generated increased numbers of epithelial cells during outgrowth. Furthermore, co-culture of biopsy samples with 3T3-J2 fibroblasts was superior to 3T3-J2 fibroblast-conditioned medium in terms of generating high numbers of cells and maintaining basal cell growth potential. These data show that expansion of endobronchial brushings or digested biopsies in co-culture with 3T3-J2 fibroblasts and ROCK inhibitor selectively expands basal cells and could reduce the time required to grow adequate numbers of cells for tissue-engineered airway procedures.

F1120

MYELOID CELL REGULATION OF ADULT MURINE LUNG ALVEOLOGENESIS POST-PNEUMONECTOMY

Lechner, Andrew John and Rock, Jason, University of California, San Francisco, San Francisco, CA, U.S.

The only current treatment for end stage lung diseases is a full lung transplant. This procedure is limited by poor survival and an inadequate supply of donor lungs. An alternative is to promote regeneration of normal lung tissue from endogenous progenitor cells. Partial pneumonectomy (PNX), the surgical removal of one or more lobes, stimulates compensatory lung growth in the remaining lobes. In this animal model, the regenerative response is mediated by alveolar epithelial type 2 cells (AEC2s), which are distal lung epithelial stem cells. Significant questions remain regarding the regenerative potential of human lungs, the identities of human lung epithelial stem cells, and the cellular and molecular signals that control their activation. Recently macrophages have been implicated in tissue repair and regeneration, but little is know about their role in lung regeneration. We are using fluorescent reporters and genetic gain- and loss-of function in mice to identify populations of immune cells that modulate epithelial stem cell behaviors in adult lung regeneration. We have found that CD115+ myeloid cells infiltrate regenerating alveolar lung tissue post-PNX. Arginase-1, a marker of M2-polarized macrophages that have previously been shown to promote wound healing in other injury models, co-localizes with a subset of CD115+ cells post-PNX. These cells show some local proliferation by EdU incorporation. In addition, we have found that CCR2+ monocytes increase post-PNX and the chemokine CCL2 is upregulated in lung epithelium and mesenchyme post-PNX. This CCL2-CCR2 axis may explain increased macrophages numbers through the recruitment of circulating monocytes into alveolar tissue post-PNX. A genetic loss of function allele in mice for CCR2 diminished myeloid cell numbers, regenerated lung mass, and AEC2 proliferation post-PNX. Furthermore, adoptive transfer of CCR2+ bone marrow cells into CCR2-deficient mice restored lung regeneration. Our data suggest that CCR2+ monocytes and CD115+, Arg1+ M2-polarized macrophages are critical immune components of the regenerative AEC2 stem cell niche. We hope to identify molecular targets that mediate these effects that might be exploited to stimulate lung regeneration and may be developed into novel therapies for patients with end-stage lung disease.

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F1122

BRM LOSS PROMOTES TUMOR PROGRESSION THROUGH EXTRACELLULAR MATRIX REMODELING AND ELEVATED MAMMARY EPITHELIAL STEM/PROGENITOR ACTIVITY

Northey, Jason, University of California, San Francisco, San Francisco, CA, U.S., Damiano, Laura, UCSF, San Francisco, CA, U.S. and Weaver, Valerie, Department of Anatomy and Department of Bioengineering and Therapeutic Sciences, Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research and Helen Diller Family Comprehensive Cancer Center, UCSF, San Francisco, CA, U.S.

BRM and BRG1 are necessary but mutually exclusive subunits of the SWI/SNF ATP-dependent chromatin remodeling complex. We have shown that oncogenic downregulation of BRM promotes malignancy of mammary epithelial cells (MECs) and loss of BRM expression in breast cancer is predictive of poor prognosis. To explore the physiological role of BRM in the normal mammary gland, we studied BRM germline knockout mice (BRM KO) and observed a 40% increase in animal size compared to wild type littermates. Developing BRM KO mammary glands revealed enhanced ductal branching, a 60% increase in numbers of terminal end buds and elevated proliferation of BRM KO MECs. Picrosirius red staining of mammary glands further demonstrated an accumulation of collagen around epithelial ducts in BRM KO mice that correlated with an upregulation of genes encoding the extracellular matrix (ECM) proteins Collagen-I and Fibronectin as well as collagen remodeling enzymes. Subsequent flow cytometry analysis of mammary glands demonstrated a preferential expansion of the basal lineage in BRM KO mice compared to controls. Basal MECs are in direct contact with the ECM and mammary stem cells are proposed to exist within this population. To investigate the possibility that BRM KO augments mammary stemness, we performed colony formation and limiting dilution transplantation assays, which served to confirm that the expanded basal population was indeed associated with elevated MEC stem/progenitor activity. To gain more mechanistic insight, we sorted MEC populations for qPCR analysis and found that BRM KO MECs produce more abundant gene transcripts associated with stemness. Our preliminary data now suggest a compensatory upregulation of BRG1 in BRM KO MECs and elevated expression of several known YAP/TAZ target genes involved in the control of cell proliferation. Finally, to describe a role for BRM in tumor progression, we examined the outcome of BRM KO in MMTV-NEU mice and observed more rapid tumor growth with a trend to higher tumor incidence and metastasis in mice lacking BRM. Taken together, our data indicate that SWI/SNF chromatin remodeling regulates YAP/TAZ control over MEC growth and a loss of BRM expression leads to BRG1-directed ECM

remodeling, MEC proliferation and elevated mammary stemness, resulting in heightened mammary tumor aggression.

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F1126

IN VITRO GENERATION OF ENTEROENDOCRINE CELLS FROM INTESTINAL STEM CELLS

Yin, Xiaolei[1,2], Langer, Robert[2] and Karp, Jeffrey M.[1], [1]Brigham and Women's Hospital, Cambridge, MA, U.S., [2]Massachusetts Institute of Technology, Cambridge, MA, U.S.

Enteroendocrine cells (EECs) form the largest endocrine system in the body. The key function of EECs is to sense luminal contents, particularly nutrients, and to respond by the secretion of a diversity of hormones (e.g. GLP-1) which modulate food intake, energy homeostasis and glucose tolerance. It's also suggested that EECs play a key role in gastric bypass surgery by secreting hormones such as GLP-1, PYY and GLP-2. These properties suggest that EEC may be an important therapeutic target in diabetes and obesity. However, the knowledge of signals controlling the differentiation and function of EEC are largely unknown. Direct in vitro study of EECs has not been possible as they are terminally differentiated cells which do not divide, and due to their dispersed distribution and scarcity (1%) in the gut epithelium, it has been difficult to study the function and regulation of EECs in situ. Furthermore, high-throughput screening hasn't been possible using EECs. We have established an in vitro culture system for high purity culture of intestinal stem cells (ISCs) and elucidated the mechanisms for controlled differentiation of ISCs to obtain high purity cultures of enterocytes, Goblet cells, and Paneth cells. By harnessing essential signaling pathways controlling the self-renewal and differentiation of intestinal stem cells (e.g. Wnt and Notch pathways), we further developed a system to generate enteroendocrine cells with high efficiency (>80%). These cells contain multiple EECs subtypes such as cells expressing Gip (K-Cell), GLP-1 (L-cell), 5HT (EC-Cell), CCK (I-Cell) and Sct (S-Cell). This EEC differentiation culture platform has the potential to identify novel interventions for gastrointestinal and metabolic diseases through the gut-brain-pancreas axis.

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EPIDERMAL CELLS

F2002

KLF4/TGF-BETA1 SIGNALING INTERPLAY REGULATES IMMATURITY AND SELF-RENEWAL OF HUMAN EPIDERMIS PRECURSOR CELLS: PERSPECTIVES FOR REGENERATIVE MEDICINE

Fortunel, Nicolas O[1], Chadli, Loubna[1], Auvré, Frédéric[1], Tost, Jörg[2] and Martin, Michèle T[1], [1]CEA-LGRK, DRF/iRCM, INSERM/UMR967, Evry, France, [2]CEA-CNG, DRF/IG, Evry, France

In cutaneous cell therapy, skin substitute bio-engineering requires *ex vivo* expansion of keratinocytes from donors, during which the preservation of stem and progenitor cell properties is critical for the long-term outcome of grafts. In this context, defining new gate-keepers of stem cell self-renewal constitutes a pre-requisite to reinvestigate original therapeutic perspectives. We have investigated the function of the transcription factor *KLF4* in human keratinocyte precursor biology and skin regeneration. Holoclone keratinocytes were used to study *KLF4* functions, as they are representative of an immature status. A stable lentiviral-based *KLF4* knock-down (KD) approach was developed and used as a proof-of-principle to study [$KLF4^{WT}$] and [$KLF4^{KD}$] keratinocyte properties. *KLF4* down-modulation promoted immaturity, long-term growth and clonogenic potential of keratinocyte precursors, as well as *in vivo* regenerative capacity in a reconstructed skin xenograft model, including through iterative grafting. Decreased *KLF4* expression increased immaturity and cell-cycling, thus promoting self-renewal. By integrating data from cellular, genomic (RNA-seq), and epigenomic analysis (DNA methylation mapping), we show that self-renewal was driven by cross-antagonism towards the anti-proliferative and differentiative effects mediated by TGF-beta1. Notably, marked down-regulation of a large set of transcripts belonging to the TGF-beta1 network was found to occur through both epigenetic and transcriptional regulations, leading to promotion of cell immaturity and inhibition of differentiation. We finally tested the feasibility of mimicking anti-*KLF4* shRNA action without inducing any stable modification. Basal keratinocytes transiently transfected with anti-*KLF4* siRNA exhibited an increased expression of the immaturity marker integrin alpha-6, thus validating our working hypothesis. Altogether, these results pinpoint *KLF4* as a major molecular target to improve *ex vivo* expansion of human keratinocytes fully functional for epidermis regeneration. Our contribution opens direct perspectives for the development of clinically relevant molecules that can modulate either *KLF4* or its downstream pathway in keratinocytes to improve skin repair and regeneration.

F2004

A ROLE OF PROMYELOCYTIC LEUKEMIA PROTEIN IN SKIN DEVELOPMENT

Anna Połeć[1], Alexander Rowe[1], Pernille Blicher[1], Rajikala Suganthan[2], Magnar Bjoras[2] and Stig Ove Boe[1], [1]Department of Clinical Biochemistry, Oslo, Norway, [2]Oslo University Hospital, Oslo, Norway

The promyelocytic leukemia (Pml) protein is a tumor suppressor involved in several cellular processes, including growth control, senescence, apoptosis and differentiation. A unique feature of this protein is its ability to organize distinct nuclear compartments called PML bodies. Previous studies have suggested that Pml and Pml bodies regulate gene expression at a subset of loci (such as the major histocompatibility (MHC) gene cluster) at the level of chromatin remodeling. In the present study we have investigated the role of Pml in mouse embryonic skin development. We observed a significant increase in Pml expression in basal keratinocyte stem cells concomitant with the onset of skin stratification at embryonic day (E) 15.5. In agreement with this, Pml depleted mice exhibit several defects in embryonic skin and hair follicle development. Differential gene expression analysis of skin cells in $Pml^{+/+}$ and $Pml^{-/-}$ mice at different developmental stages suggests a key role of Pml in promoting a gene expression program involved in embryonic skin stratification. Notably, several Pml regulated genes, including a subset of genes within the epidermal differential complex, appeared as clusters within the genome. The role of Pml and Pml bodies in global gene expression regulation during embryonic skin development will be discussed at the meeting.

F2006

MACROPHAGES INDUCE AKT/β-CATENIN-DEPENDENT Lgr5+ STEM CELL ACTIVATION AND HAIR FOLLICLE REGENERATION THROUGH TNF-α

Wang, Xusheng and Wu, Yaojiong, Tsinghua University, Shenzhen, China

The skin harbors stem cells with the potential to regenerate epidermal appendages, but hair follicles (HF) lost to injury are barely regenerated, suggesting complex regulations of the stem cells in HF regeneration. Here we show that macrophages in wounds activate epidermal stem cells, leading to telogen-anagen transition (TAT) in around wound areas and de novo HF regeneration in wounds in mice, largely through TNF-a. Loss of TNFA attenuates wounding-induced TAT and decreases HF neogenesis. TNF-a induces AKT phosphorylation, whose level is elevated in epidermal stem cells after wounding and in the re-epithelized epidermis. TNF-a could also induce the accumulation of beta-catenin in epidermal stem cells, which requires the activation of AKT pathway. Inhibition of PI3K/AKT abolishes injury-induced TAT. Conversely, inducible Pten loss in $Lgr-5^{+}$ HF stem cells led to AKT phosphorylation and the proliferation of the cells, resulting in HF TAT independent of injury and increased HF neogenesis after wounding. Thus our results suggest that TNF-a mediated AKT/beta-catenin signaling plays a crucial role in HF activation and de novo regeneration.

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EYE OR RETINAL CELLS

F2010

ASSESSING VISUAL FUNCTION FOLLOWING TRANSPLANTATION OF MOUSE EMBRYONIC STEM CELL-DERIVED ROD PHOTORECEPTOR PRECURSORS

Goh, Debbie[1], Gonzalez-Cordero, A.[2], Duran, Y.[2], Robinson, M.R.[2], Powell, K.[2], Waldron, P.[2], Naeem, A.[2], Blackford, S.J.[2], Kloc, M.[2], Sampson, R.[2], Decembrini, S.[3], West, E.L.[2], Pearson, R.a.[2], Arsenijevic, Y.[3] and Ali, R.R.[2], [1]UCL, London, U.K., [2]UCL Institute of Ophthalmology, London, U.K., [3]University of Lausanne, Jules-Gonin Eye Hospital, FAA, Unit of Gene Therapy and Stem Cell Biology, Lausanne, Switzerland

Transplantation of photoreceptor precursors is a promising strategy for treating blindness caused by photoreceptor loss. To this end, embryonic stem cells (ESCs) represent a renewable cell source. We have previously demonstrated that rod precursors derived from suspension cultures of mouse ESCs can integrate and mature within degenerate adult mice retinae following transplantation. However, the integration levels of transplanted mESC-derived rod precursors into the degenerate retina was not sufficient to establish if transplanted cells could restore visual function. Therefore, we sought to improve integration efficiency to probe for the restoration of visual function following the transplantation of mESC-derived photoreceptors. Using a Crx.GFP mESC line, we first investigated the optimal stage in culture to transplant in vitro-derived Crx.GFP-positive photoreceptors. We then assessed the number of integrated mESC-derived photoreceptor precursors following dual subretinal transplantation into $Gnat1^{-/-}$ recipient mice. The effect of cyclosporine A-mediated immunosuppression on integrated mESC-derived photoreceptors following transplantation was also assessed. Finally, we established a multi-electrode array (MEA) experimental setup that allows us to

record retinal ganglion cell (RGC) responses in control and transplanted recipient retinas using Gnat1$^{-/-}$ and OGC (Opn4$^{-/-}$, Gnat1$^{-/-}$, Cnga3$^{-/-}$) mouse models of retinal degeneration. Preliminary results suggest an improvement in integration efficiency of optimally-staged Crx.GFP-positive photoreceptors with immunosuppression of recipient mice. Initial MEA recordings suggest the restoration of visual response in the OGC total knockout mouse model. Further characterisation of this visual response, along with behavioural testing, is essential to understanding and evaluating the re-establishment of retinal circuitry following transplantation.

F2012

SCLERAL STEM CELLS PREVENT MYOPIA DEVELOPMENT

Juo, Suh-Hang Hank[1], Yeh, Ching-Hua[1], Hsi, Edward[1] and Liang, Chung-Ling[2], [1]kaohsiung medical university, kaohsiung, Taiwan, [2]Bright-Eyes Clinic, kaohsiung, Taiwan

Myopia has become a pandemic disease, however, the underlying mechanism for myopia development is poorly understood. During the development of myopia, the sclera has dynamic changes and remodeling leading to an elongation of eyeball. We previously reported that over-expression of microRNA-328 can increase a risk for myopia. Recent studies identified the existence of rodent scleral stem cells (SSCs) that highly express CD44. Since microRNA-328 can directly target CD44, we explored the role of SSCs in myopia development. Myopia is induced by the following method: the right eye was covered to induce form deprivation myopia (FDM) in a mouse of 23 days old, while the fellow eye (i.e. left eye) was not covered. The normal control mice were not treated in both eyes. The axial length (AXL) was measured and difference of AXL (right AXL minus left AXL) of the same animal is a surrogate of myopia. SSCs were isolated from the sclera and ciliary body of the right eyes from either experimental or control mice. We measured the number of SSCs and examined their ability of differentiation to various cell types. The correlation test was performed for the relationship between stem cell abundance and myopia severity. In addition, microRNA-328's effects on CD44 expression and stem cell functions were assessed. We showed that most SSCs reside in the ciliary body, and myopic eyes had fewer SSCs than the normal eyes ($p< 0.0025$). The correlation between the difference of AXL and stem cell abundance is -0.6 ($p< 0.05$) in the experimental mice, which means more SSCs less myopic. In addition, induction of myopia was failed in some mice and SSC percentage in the fail-to-induced eyes was not statistically different from that in normal eyes. These results indicate sufficient SSCs are important to prevent myopia development. Since microRNA-328 can target CD44 mRNA, a reduction of microRNA-328 level by its anti-sense could increase the SSC proliferation rate ($p<0.0001$), elevate ALP expression ($p=0.03$) and ALP activities ($p=0.0003$). In addition, a decrease of microRNA-328 could substantially enhance the stem cells ability of differentiation to osteocytes, chondrocytes and adipocytes. In conclusion, we demonstrated the relationship among microRNA-328, SSCs and myopia development, which implies a potential application of stem cell treatment in myopia.

F2014

MESENCHYMAL STEM CELLS PROMOTE VASCULAR REPAIR IN A MODEL OF OXYGEN-INDUCED RETINOPATHY

Noueihed, Baraa[1,2], Rivera, Jose Carlos[2] and Chemtob, Sylvain[1,2], [1]McGill University, Montreal, QC, Canada, [2]Maisonneuve-Rosemont Hospital Research Center, Montreal, QC, Canada

Retinopathy of prematurity (ROP) is a leading cause of visual impairment and blindness in infants. Premature babies exposed to the hyperoxic extrauterine environment leads to vaso-obliteration (VO), followed by ischemia, and subsequent pathological intravitreal neovascularization (NV) in the immature retina. Current ROP treatments target only aberrant intravitreal vessel growth without repopulating the avascular regions of the retina. Thus there is a dire need of new therapies that arrest pathological NV and promote normal retinal revascularization. Mesenchymal stem cells (MSCs) have shown the ability to migrate to the damaged tissue in different animal models and enhance revascularization. We, therefore, investigated whether MSCs can promote vascular repair in a mouse model of ROP. The oxygen-induced retinopathy (OIR) model consists of 2 phases: exposure of postnatal day 7 (P7) mice to 75% O_2 until P12 to induce VO followed by 5 days of room air leading to NV. Compact bone-derived MSCs were isolated from adult C57BL/6 mice and injected intravitreally either at the onset of VO (P7) or NV (P12) to assess prevention and repair of vascular damage, respectively. Retinal migration of MSCs was traced following injection. Similarly, conditioned media of MSCs cultured in hypoxia (5% O_2) or normoxia (21% O_2) was injected at P12 OIR mice. Gene expression analysis by quantitative PCR was performed on OIR retinas injected with MSCs to determine possible factor(s) involved in revascularization. Our findings show that MSCs significantly ($p<0.001$) reduced VO areas and inhibited formation of neovascular tufts in OIR retinas. MSCs migrated to the avascular ischemic regions of the retina and were localized adjacent to the vessels. Hypoxic MSC conditioned media significantly ($p<0.01$) decreased NV areas in comparison to the normoxic counterpart. Interestingly, MSC-injected OIR retinas showed an augmented expression of IGF-1, VEGF, Netrin-1, and Sema3E, and decreased expression of IL-1β and Sema3A. In this study, we demonstrated that MSCs promote healthy vessel growth in OIR retinas, possibly in

a paracrine fashion by regulating expression of angiogenic factors.

Funding Source: N/A

F2016

FUNCTIONAL VALIDATION OF INDUCED PLURIPOTENT STEM CELLS DERIVED RETINAL PIGMENT EPITHELIUM "PATCH" USING GLP COMPLAINT DIFFERENTIATION PROTOCOL FOR AUTOLOGOUS CELL BASED THERAPY.

Sharma, Ruchi[1], Khristov, Vladimir[2], Jha, Balendu Shekhar[1], Wan, Qin[3], Patel, Dishita[1], Lotfi, Mostafa[1], Hotaling, Nathan[1], Hua, Fang[2], Miller, Sheldon S.[1] and Bharti, Kapil[4], [1]National Institutes of Health, Bethesda, MD, U.S., [2]National Eye Institute, National Institutes of Health, Bethesda, MD, U.S., [3]NEI/NIH, Bethesda, MD, U.S., [4]NEI, NIH, Bethesda, MD, U.S.

Age-related Macular Degeneration (AMD) is an age-associated disease and the leading cause of blindness in elderly population in the US. AMD has two main advanced stages, the "dry" and the "wet" form. Currently, wet AMD or Choroidal Neo Vascularization (CNV) is treated using anti-VEGF antibodies, but the dry form of AMD is not yet treatable. Dry AMD is caused by atrophy of retinal pigment epithelium (RPE), a monolayer of cells located adjacent to photoreceptors, are responsible for maintaining health and functioning of photoreceptors. Induced Pluripotent Stem Cells (iPSCs) derived RPE provides an autologous source of cells to replace lost RPE in AMD patients. Developing a transplantable RPE patch for cell therapy requires viral free iPSC generation, a xeno-free RPE patch manufacturing process, and a thorough-testing of RPE patch for safety and efficacy. Karyotyping and exome sequencing of patient-derived GLP-grade iPSCs confirmed their stable genome and the lack of any tumorigenic mutations. Xenofree differentiation methods were used to differentiate iPSCs to RPE monolayer. Differentiating cells are free of pluripotent markers (TRA1-81 and OCT4) and more than 95% of cells were positive for RPE markers BEST1, CRALBP. RPE patch grown on bio-degradable PLGA (poly (lactic-co-glycolic acid) scaffold is fully-polarized as confirmed by the presence of RPE markers ZO-1 (tight junction), EZRIN (apical processes), and RPE65 (visual cycle protein), and the presence of extensive apical processes, apically located melanosomes, and basal infoldings. The electrophysiological measurements of iPSC-derived RPE patch confirmed its electrical intactness. iPSC-RPE monolayer secretes cytokines VEGF and PEDF in a polarized fashion with higher VEGF basally and higher PEDF apically. In conclusion, using molecular, structural, and functional assays we have authenticated the functionality of GLP-grade iPSC-RPE patches. These patches are currently being tested in preclinical animal models.

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F2018

NOVEL IN VITRO ASSAY SYSTEM FOR INCIPIENT FUNCTIONAL IMPAIRMENT OF RETINAL TOXICITY USING RETINA EPITHELIAL PIGMENT CELLS

Xie, Yucheng, Kim, Mee-Hae and Kino-oka, Masahiro, Osaka University, Suita, Japan

Retinal pigment epithelial (RPE) is a major ocular tissue protecting the retina and is well known for its critical role in the pathogenesis of age-related macular degeneration, one of the leading causes of blindness in the elderly. RPE cells are known to be highly metabolically active and vulnerable to oxidative stress, requiring the establishment of toxicity assays to understand the RPE impairment. In this study, we demonstrated a novel system using RPE cells derived from embryonic stem cells and quantitative fluorescent imaging approaches for chemical toxicology. The RPE cells at the confluent state were obtained after 7-day culture in the limited area inside of removable ring, and after ring removal, toxic chemicals of arecoline and chloroquine were exposed to confluent RPE cells for 24 h. In further culture, ZO-1 and nuclei of cells were stained, which determined the cell density, X_T and ZO-1 positive cell density, X_P, respectively along with radius direction from center of the culture to the outer, describing the dose-dependency as well as spatial-dependency of the chemical influences on cell death and tight-junction decay. The exposure to arecoline shows the same dose-dependent manners of cell death (X_T) and tight-junction decay (X_P) with IC_{50} values of 1.2 and 1.0 mM, respectively. The exposure to chloroquine induced different dose-dependent manners in X_T and X_P with IC_{50} values of 84.5 and 8.6 μM, respectively, implying that tight junction is more sensitive to the chloroquine than cell survival. Thus, our proposed culture system can distinguish toxicities between tight-junction decay and cell survival, and the chloroquine has the preferential toxicity to the tight junction, suggesting that our system is a useful tool for evaluation in incipient functional impairment of retinal toxicity.

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NEURAL CELLS

F2020

IN VITRO ISOLATION AND DIFFERENTIATION OF NEURAL STEM CELLS FROM TRANSGENIC PIGS WITH A CREERT2 SYSTEM CAPABLE OF CONTROLLING THE EXPRESSION OF EGFP GENES

Kim, Eunhye, Kim, Mirae and Hyun, Sang-Hwan, College of Veterinary Medicine, Chungbuk National University, Cheongju, Korea, South

Despite the increasing importance of the pig as a large animal model, which has gyrencephalic brain with similar gray and white matter composition and size more comparable to humans than rodents, little is known about porcine neural precursor cells. Here, we isolated the neural stem cells (CreERT2-NSCs) from the transgenic pigs with CreERT2 system (CreERT2 pigs) which is using a Cre recombinase fused to a mutated ligand-binding domain of the human estrogen receptor inducible system. The forebrain was sampled from deeply anaesthetized CreERT2 pigs and dissociated in the appropriate medium. In the presence of epidermal growth factor and basic fibroblast growth factor, the CreERT2-NSCs expanded into either neurospheres or single cells with or without poly-L-lysine treatment showing representative NSC markers such as *NESTIN* and *VIMENTIN*. These cell lines have been maintained for more than two months (15 passages) without losing their proliferative potential having normal 36+XY karyotype with all analyzed metaphases free of any discernable cytogenetic abnormalities. Although further studies will be needed, the CreERT2-NSCs would be considered a promising source of cells for the treatment of central nervous system diseases.

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F2022

PREDICTING THE ELECTROPHYSIOLOGICAL MATURITY OF HUMAN NEURONS WITH SINGLE-CELL TRANSCRIPTOMICS

Bardy, Cedric[1], van den Hurk, Mark[1,2], Kakaradov, Boyko[3], Erwin, Jennifer[1], Jaeger, Baptiste[1], Hernandez, Ruben V[1], Eames, Tameji[1], Paucar, Andres[1], Gorris, Mark[1], Marchand, Cynthia[1], Jappelli, Roberto[1], Barron, Jerika[1], Bryant, Alex[1], Kellogg, Mariko[1], Lasken, Roger S.[4], Steinbusch, Harry W.M.[2], Yeo, Gene W.[3] and Gage, Fred H.[1], [1]Salk Institute - Sanford Consortium for Regenerative Medicine, La Jolla, CA, U.S., [2]Maastricht University, Maastricht, Netherlands, [3]University of California, San Diego, La Jolla, CA, U.S., [4]J Craig Venter Inst, San Diego, CA, U.S.

Human neural progenitors develop into electrophysiologically active neurons at variable rates, providing a major challenge to in vitro studies of neurological disorders. Using unbiased computational analyses, we found that two statistics of action potentials can explain most of the variance of ~300 human neurons in culture, defining a continuum of electrophysiological maturation stages. While whole-cell electrophysiology is the gold-standard for functional evaluation, it is not as high-throughput as transcriptome measurements. To bridge the gap between the transcriptome and neurophysiology, we combined single-cell measurements of electrophysiology, morphology and the transcriptome. The strong correlations between action potentials, synaptic activity, dendritic complexity and gene expression highlight the importance of methods for isolating functionally comparable neurons. Here we demonstrate that machine-learning classification can be used to predict the physiological profile of a neuron based on its single-cell transcriptome. As further proof of concept, we developed an electrophysiological marker for highly functional human neurons.

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F2024

SYNAPTIC INTEGRATION OF INTRASTRIATAL VERSUS INTRANIGRAL GRAFTS OF HUMAN EMBRYONIC STEM CELL DERIVED NEURONS IN THE ADULT RAT BRAIN

Cardoso, Tiago Bento, Heuer, Andreas, Kirkeby, Agnete, Nolbrant, Sara, Grealish, Shane and Parmar, Malin, Lund University, Lund, Sweden

Human embryonic stem cell (hESC)-derived neurons have been shown to survive long-term, release dopamine and to extensively innervate correct host structures after transplantation into adult rat brain. Using the monosynaptic

tracing technique, we have recently shown that hESC-derived neurons integrate into host circuitry by establishing both host-to-graft and graft-to-host synaptic connectivity. Here we use the same tracing methodology to investigate connectivity of midbrain (MB) and forebrain (FB) patterned hESCs-derived neurons transplanted in striatum or substantia nigra. 6-OHDA lesioned rats received intrastriatal or intranigral transplants of MB-patterned and FB-patterned neural progenitors. To assess for synaptic connectivity, animals were injected with rabies vector either 5 or 17 weeks after transplantation and perfused one week later for histological analysis. We show that 6 weeks post-transplantation, host neurons from local or distant afferent structures are able to establish synaptic connections with both intrastriatal and intranigral grafts. The pattern of connectivity varied depending on the location of transplantation as intranigral grafts received more inputs from hypothalamus and midbrain nuclei while intrastriatal grafts revealed preference to thalamus and prefrontal cortex. This pattern of connectivity was maintained 18 weeks post-transplantation. The neuronal subtype of the graft did not seem to influence synaptic integration, as both MB-patterned and FB-patterned transplanted neural progenitors received inputs from similar host structures. Overall, we show that both intrastriatal and intranigral grafts of hESC-derived neurons can integrate into host circuitry and that the pattern of connectivity seems to be dependent on the location of transplantation rather than the subtype of neurons.

F2026

A COMPARISON OF IPSC DERIVED ASTROCYTES AND THE ASTROCYTOMA CELL LINE CCF-STTG1 AS MODELS OF ASTROCYTE BIOLOGY

Delsing, Louise[1,2], Meuller, Johan[2] and Ramne, Anna[2], [1]Skovde University, Skövde, Sweden, [2]AstraZeneca, Mölndal, Sweden

The number of individuals affected by Alzheimer's Disease (AD) is increasing as the population ages. Today, effective treatments for the disease are lacking and there is great unmet need for drugs to treat AD. Astrocytes are the major producer of apolipoprotein E (apoE) in the CNS and since apoE genotype have been strongly linked to Alzheimer's disease development and progression the regulation of apoE expression and secretion pathways is of high interest. A large part of drug discovery relies on screening of small molecules. When characterizing and designing in-vitro models for drug screening it is important that the model is biologically relevant and that results from it translate well to events in the actual tissue. We have compared induced pluripotent stem cell (iPSC) derived astrocytes to an astrocytoma cell line CCF-STTG1 as in-vitro models of astrocyte biology. iPSC derived astrocytes and the astrocytoma cell line show some differences in expression of astrocyte specific proteins, most notably the astrocytoma cell line show much lower expression of glial fibrillary acid protein. However they show similar functionality in glutamate uptake, despite some difference in expression of glutamate transporters. To provide insight in a disease relevant setting the cell types were compared in a phenotypic screening designed to identify compounds that increase secretion of apoE. Results were analyzed qualitatively where active compounds were distinguished from non-active compounds and novel targets for increasing the secretion of apoE were identified. Several compounds were active in both cell types and some were only active in one of them. The liver X receptor LXR is of particular interest as it governs the expression of many genes linked to lipid metabolism and one of its target genes is apoE. LXR were identified as one of the targets that is differently affected in iPSC derived astrocytes and astrocytoma cells, this could be an effect of differences in expression levels of LXR. In conclusion, this data provides a fist analysis of some of the differences between cell lines and iPSC derived cells as in-vitro models of astrocyte biology.

F2028

SIMULATED MICROGRAVITY ENHANCES OLIGODENDROCYTE MITOCHONDRIAL FUNCTION AND SUPPORTS CENTRAL NERVOUS SYSTEM MYELINATION

Espinosa-Jeffrey, Araceli, Konicki, Athena, Nguyen, Kevin, Green, Joshua, Vergnes, Laurent and de Vellis, Jean, University of California, Los Angeles, CA, U.S.

The need to understand the effects of weightlessness on cells from the central nervous system (CNS) for long-term space travel has brought us to study the effects of simulated microgravity (sim-μG) on CNS cells in culture. Cellular metabolism is essential to provide the necessary energy for the synthesis of all the elements involved in cell structure and function. Normal cells use oxidative phosphorylation in the presence of oxygen to generate adenosine triphosphate as a cellular fuel. We previously showed that neural stem cells and oligodendrocytes (OLs) the myelin forming cells in the CNS, proliferate more in sim-μG cells kept in 1G. Here we sought to determine the impact that sim-μG on energy metabolism of human (Hu) HuOLs at a postmitotic stage. We examined the extracellular oxygen consumption and acidification rates as measures of mitochondrial respiration and glycolysis, respectively. OLs were maintained 48h in 1G and then one plate remained in earth gravity and another one in sim-μG for 24h. We found that OLs maintained in sim-μG possess higher OXPHOS activity compared with OLs maintained in 1G while anaerobic glycolysis remained unchanged. These results indicate that mitochondrial function is enhanced in OLs by sim-μG. In order to ascertain if OLs exposed to sim-μG remain functional, we next co-cultured

HuOLs at a post-mitotic stage with neonate mouse brain sliced (diced) tissue in our proprietary co-culture, chemically-defined medium for myelination. We found that OLs matured in sim-μG expressing myelin basic protein and proteolipid protein and appear to coat mouse axons. In conclusion, OL maturation and myelination appear to take place in sim-μG. Our co-culture system is an in vitro model to study normal CNS myelination, mechanisms of myelin loss and as a novel platform to test compounds aimed at promoting re-myelination. Mitochondrial dysfunction in white and grey matter lesions is recognized in multiple sclerosis and could be a determinant of axonal dysfunction/degeneration. In addition, Parkinson's and Alzheimer's diseases are also associated with mitochondria defects. Could simulated microgravity hold the key to the cure for neurodegeneration and myelin deficient spectrum disorders? ***Innovation:*** To our knowledge, this is the first report on CNS myelination in microgravity. Supported by CG-NIH #0461.

F2030

FUNCTIONAL IMPLICATIONS OF MIR-19 IN MIGRATION OF NEWBORN NEURONS IN THE ADULT BRAIN

Han, Jinju[1], Kim, Hyung Joon[2] and Gage, Fred H.[2], [1]Salk Institute, La Jolla, CA, U.S., [2]Salk Institute for Biological Studies, La Jolla, CA, U.S.

While microRNAs have been implicated in the differentiation of cells, the *in vivo* functions of individual microRNAs in adult neural progenitor cells (NPCs) remain largely unknown. Here we report biological functions for miR-19, which is encoded in polycistrons, during adult neurogenesis. We have determined that miR-19 is enriched in NPCs and downregulated during neurogenesis in the adult hippocampus. With manipulation of miR-19 in NPCs for gain- and loss-of function studies, we discovered that miR-19 regulates cell migration by directly targeting Rapgef2. Dysregulation of miR-19 in NPCs alters the positioning of newborn neurons in the adult brain. Furthermore, we discovered abnormal expression of miR-19 in human NPCs generated from schizophrenic patient-derived induced pluripotent stem cells that have displayed aberrant migration. Our study demonstrates the significance of miR-19 in preventing the irregular migration of newborn neurons in the adult brain that may contribute to the etiology of schizophrenia.

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F2032

GLIAL HETEROGENEITY AND MYELINATION CAPACITY OF MODULATED RAT AND MOUSE ADULT NEURAL STEM CELLS AFTER TRANSPLANTATION INTO THE RODENT CENTRAL NERVOUS SYSTEM

Jadasz, Janusz Joachim, Beyer, Felix and Küry, Patrick, Heinrich-Heine-University, Neurology, Duesseldorf, Duesseldorf, Germany

The generation of new oligodendrocytes and the repair of myelin sheaths represent processes that are essential for functional restoration after injury in the central nervous system (CNS). Two resident cell populations within the CNS serve as a glial cell pool for myelin repair and functional restoration after injury. The first population consists of oligodendroglial precursor cells (OPCs) while the second population involves adult neural stem cells (aNSCs). Our research focuses on glial cell differentiation mechanisms and regulatory aspects of myelination and we identified the multifunctional $p57^{kip2}$ protein as a negative regulator of myelinating glial cell differentiation and as an important intrinsic switch for glial fate decision in aNSCs. Moreover, suppression of $p57^{kip2}$ was found to neutralize bone morphogenetic protein signalling and to promote OPC generation at the expense of astrocytic differentiation in vitro as well as in vivo. This raises the question, if generated oligodendroglial cells from $p57^{kip2}$-suppressed adult neural stem cells can give rise to mature oligodendrocytes and to what degree these stem cells can therefore contribute to glial heterogeneity upon engraftment into the adult brain. Furthermore we want to know if such cells can also myelinate axons after injury. Therefore we investigate whether aNSCs descendants can equally mature in white- and grey matter structures and to what extent genetically modulated adult neural stem cells can induce myelination and axonal regeneration in the injured spinal cord. In order to study functionality, we transplanted control stem cells as well as $p57^{kip2}$ suppressed stem cells into white and grey matter structures of the adult brain as well as into the hemisectioned spinal cord injury (SCI) model. First grafting experiments showed that $p57^{kip2}$ suppressed aNSCs expressed the OPC marker GST-pi while we detected reduced astrocytic GFAP protein levels, as opposed to control transfected stem cells after 14 days post transplantation into the CNS. Our results therefore indicate that $p57^{kip2}$ plays a crucial role in the glial fate decision process of aNSC-derived OPCs, which is of interest regarding the promotion of remyelination activities and the prevention of gliosis in the context of damage or injury in the CNS.

F2034

Nurr1 FINE-TUNES HUMAN TH EXPRESSION BY INTERACTING TFII-I SPLICED ISOFORMS IN DOPAMINERGIC NEUROGENESIS

Kausar, Rukhsana, Ajou university school of medicine, Suwon, Korea, South

Nurr1 plays a vital role in development and maintenance of midbrain dopaminergic (DA) neurons. Our previous study showed that Nurr1 actively represses human tyrosine hydroxylase (hTH) transcription in human neural stem cells (hNSCs), while it activates hTH expression via NBRE-A site in DA neuronal cells. To identify the interacting proteins with Nurr1 to regulate hTH expression, we performed DNA pulldown assay and identified TFII-I, a multifunctional transcription factor having four spliced isoforms. PCR analysis of VMB of embronic mice from E9.5 to E13.5 showed that TFII-I expression shifted from TFII-I I to TFII-I II isoform, neuron-specific form. In hNSC, TFII-I I repressed hTH promoter activity while TFII-I II had no effect. TFII-I I and II majorly localize in nucleus and cytoplasm of hNSCs, respectively. In addition, TFII-I I preferentially interact with Nurr1 and occupies hTH promoter in hNSCs. Our data showed that Nurr1 interacts with TFII-I spliced forms to fine-tune hTH expression during midbrain dopaminergic neurogenesis.

Funding Source:

F2036

AGE- AND BRAIN REGION-DEPENDENT DYSREGULATION OF OLIGODENDROCYTE PRECURSOR CELL POPULATION DYNAMICS IN A MOUSE MODEL OF NEUROFIBROMATOSIS TYPE I.

Lennon, James, Stanford University, Stanford, CA, U.S., Gutmann, David, Washington University in St. Louis, St. Louis, MO, U.S. and Monje, Michelle, Stanford Univ, Stanford, CA, U.S.

Neurofibromatosis type I (NF1) is a common genetic syndrome that affects 1 in 2,500 individuals. This condition is characterized by germline mutations of the NF1 tumor suppressor gene, resulting in numerous neurological complications, including low-grade gliomas, autism, attention deficit and cognitive impairment. While the mechanisms underlying these cognitive impairments are unknown, studies have shown that children with NF1 have enlargement of the corpus callosum (CC), and this is correlated with lowered IQ in these individuals. Since the CC is a major white matter tract involved in higher-order functions, such as problem solving, this region may be an important contributor to the cognitive pathology in children with NF1. To understand the cellular activity underlying these CC changes, we analyzed oligodendrocyte precursor cells (OPCs) in wild-type (WT) and mice harboring a germline inactivating Nf1 gene mutation (Nf1+/- mice) at various developmental time points. At postnatal day 35 (PN35), Nf1+/- mice exhibit a 62% increase in PDGFRa$^+$ OPC density in the corpus callosum ($5.5x10^{-5}$ vs. $3.4x10^{-5}$ cells/um^2; $p<0.05$) relative to control animals. This increase in OPC density remains significant at PN53 but normalizes to WT levels by P84. Interestingly, this OPC increase is region dependent, as OPC density is not altered in the cortical grey matter at any of these time points. Extending our analysis to other white matter areas, we demonstrated that OPC density within the optic nerve is also elevated at PN35 in Nf1+/- relative to WT mice ($11.2x10^{-5}$ vs. $8.5x10^{-5}$, respectively; $p<0.05$). In the optic nerve, a structure that completes developmental myelination at an earlier age than the CC, OPC levels normalize to control levels by PN53. Collectively these data indicate that the effects of Nf1 heterozygosity on the OPC population is both age- and brain-region dependent, with the greatest changes occurring within the white matter of younger mice. Given that OPCs are the progenitors that give rise to myelin-forming cells, and therefore contribute to the maturation of cortical circuits, it is conceivable that alterations in this population may have significant effects on circuit-level function, including behavior and learning.

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F2038

MODELING DRUG RESPONSES OF NEURONS FROM ASD PATIENTS

Marchetto, Maria Carolina, Santos, Renata, Mandes, Ana, Kim, Yeni, Linker, Sara and Gage, Fred H., Salk Institute for Biological Studies, La Jolla, CA, U.S.

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 major impediment to testing hypotheses and potential therapeutic interventions for autism is the lack of relevant animal and cell models. The direct study of live brain tissue from ASD patients is not feasible, and no suitable animal models can adequately reproduce the complicated structure, proper wiring and function of the human brain. 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 for complex neurogenetic disorders such as ASD. Here we use a new platform (Multielectrode Arrays-MEA) to perform functional field potential analysis of neuronal populations from 8 ASD individuals and 6 controls during development and after treatment with drugs that are currently in clinical trials for ASD. Our preliminary results indicate that ASD neurons

respond to drug treatment by increasing neuronal spiking and neuronal bursts. Additionally, we performed expression profile analysis on developing ASD neurons and neurotypical controls after drug treatment to uncover which pathways are potentially targeted and are potentially involved in the recovery of the neuronal activity. Studying biological basis of ASD and cellular drug responsiveness would likely lead to the development of clinically useful biomarkers of risk for this disorder, which may lead to the development of novel therapies.

F2040

PROLIFERATION OF NEURAL STEM/ PROGENITOR CELLS ON POLY (ACRYLIC ACID)-GRAFTED POLYAMIDE FIBERS

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

Fabrics processed into a mesh form have the desired mechanical and biological properties as the biomaterials to culture neural cells in three dimensions. Because the micro-scale interspaces between oriented fibers provide the passage for cell migration and regularly-oriented fibers become axonal guidance for neural cells. We investigated the adhesiveness and growth of neural stem/progenitor cells (NSPCs) on the poly (acrylic acid)-grafted polyamide fibers processed into a woven mesh.

We prepared the poly (acrylic acid)-grafted polyamide 66 mesh under gamma-irradiation. Matrigel, gelatin or albumin were covalently immobilized on the mesh by chemical conjugation using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxyscuccinimide (NHS). NSPCs 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). The cells were applied to test the ability of poly (acrylic acid)-grafted polyamide mesh conjugated with Matrigel, gelatin or albumin as the cell culture substrate. NSPCs were incubated on each polyamide mesh at 37°C, 5%CO_2 for 7days, and then the cell numbers were counted. Additionally, the cell populations in the NSPC culture were examined using immunocytochemistry and quantitative RT-PCR analysis. The NSPCs adhered and proliferated on each polyamide mesh. On the Matrigel-immobilized PAA-grafted polyamide 66 fibers, the cells showed migration and elongation obviously. On the other hand, the cells adhered on gelatin-immobilized PAA-grafted polyamide 66 fibers showed neurosphere-like aggregate formation. The growth rate of the cells on the Matrigel-immobilized PAA-grafted polyamide fibers was the highest in all samples. However, the neural stem cell markers, nestin-positive cells were detected abundantly in all samples. The Matrigel-immobilized PAA-grafted polyamide 66 mesh showed a suitable property as substrate for adherent culture of mouse NSPCs keeping their undifferentiated state.

F2042

HOW AN EMBRYO KNOWS ITS BOUNDARIES: USING INDUCED PLURIPOTENT STEM CELLS TO MODEL THE CONGENITAL DISEASE CRANIOFRONTONASAL SYNDROME

Niethamer, Terren Kathryn, Larson, Andrew R., O'Neill, Audrey K., Bershteyn, Marina, Hsiao, Edward, Klein, Ophir, Pomerantz, Jason and Bush, Jeffrey O., University of California, San Francisco, San Francisco, CA, U.S.

Normal development requires self-organization of tissues by cellular signaling that both establishes and maintains boundaries. This often involves a phenomenon known as cell sorting, in which cells with differing properties segregate from each other. Eph/ephrin signaling is essential for tissue organization and boundary formation in the developing embryo. Preliminary data from our laboratory indicate that the EPHRIN-B1 signaling molecule is a potent regulator of cell segregation in the embryonic neuroepithelium, and that aberrant cell sorting in this cell type leads to congenital human disease. Mutations in EPHRIN-B1 cause craniofrontonasal syndrome (CFNS), an X-linked neurocristopathy characterized by craniofacial and skeletal anomalies. Unlike most X-linked conditions, females heterozygous for loss of *EFNB1* are more severely affected than hemizygous males. This unique inheritance pattern is due to mosaicism for *EFNB1* expression after random X inactivation in heterozygous females. In *Efnb1*$^{+/-}$ mice, mosaicism for ephrin-B1 expression leads to active cell sorting between ephrin-B1 expressing and non-expressing cells in the neural plate neuroepithelium, but cell sorting has not been demonstrated in a human model for CFNS, and it is unknown whether or not cell sorting contributes significantly to disease pathophysiology. To determine whether Eph/ephrin-mediated cell sorting occurs in human cells, and to study the underlying cellular and molecular etiologies of CFNS in a human system, our lab has created human induced pluripotent stem cells (hiPSCs) from a CFNS patient, mother, and father and differentiated them into neuroepithelial cells. We demonstrated that mosaicism for EPHRIN-B1 expression in our human neuroepithelial cells results in cell sorting between EPHRIN-B1 expressing and non-expressing cells, indicating that this aberrant cell sorting behavior in CFNS patients may contribute to the disease. This the first use of hiPSCs to model a congenital craniofacial anomaly, and we are further using this model to study the cellular signaling and cell behaviors underlying cell sorting in human neuroepithelial cells.

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F2044

EVALUATION OF THE SIGNIFICANCE OF HLA MATCHING IN ALLOGENEIC HUMAN IPS CELL-DERIVED NEURAL STEM/PROGENITOR CELL TRANSPLANTATION USING MIXED LYMPHOCYTE REACTION

Ozaki, Masahiro[1,2], Iwanami, Akio[1], Kohyama, Jun[2], Itakura, Go[2], Iwai, Hiroki[1], Matsumoto, Morio[1], Okano, Hideyuki[2] and Nakamura, Masaya[1], [1]Department of Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Japan, [2]Department of Physiology, Keio University School of Medicine, Tokyo, Japan

Aiming for a first-in-human trial of human iPS cell-derived neural stem/progenitor cells (hiPSC-NS/PCs) transplantation therapy for spinal cord injury patients, it would be realistic to produce allogeneic three human leukocyte antigen loci (HLA-A, -B, -DRB1) -matched hiPSC-NS/PCs from the hiPS cell stock. Mixed lymphocyte reaction (MLR) has been used as an *in vitro* model for assessing immune rejection in the fields of organ transplantation. The purpose of this study is to evaluate the feasibility of MLR in hiPSC-NS/PCs transplantation and determine the clinical significance of HLA matching. Peripheral blood mononuclear cells (PBMCs) from volunteers were co-cultured with irradiated hiPSC-NS/PCs and the proliferative activities of PBMCs were quantitatively measured by incorporation of ^{3}H-thymidine represented as counts per minute (CPM). HLA types of hiPSC-NS/PCs and PBMCs were genotyped prior to mixed culture. Allogeneic HLA-matched response was compared to HLA-mismatched response using hiPSC-NS/PCs from three HLA loci homozygous donors. Furthermore, autologous response was compared to allogeneic response. Stimulation index (SI) was calculated as the CPM in each MLR divided by the CPM in unstimulated PBMCs. Cut off value for immune rejection was defined as SI = 2.5. Allogeneic HLA-matched response was equivalent to HLA-mismatched response. All of MLR experiments represented SI < 2.5. In particular, low responses (SI < 1) were observed in two of four HLA-matched MLR experiments and one of four HLA-mismatched MLR experiments. Autologous response was also similar to allogeneic response with SI < 2.5. Two of six allogeneic MLR experiments exhibited low response (SI < 1). Immunological examination revealed no significant difference in immune response between HLA-matched and -mismatched group. Stimulation indexes in both groups were less than 2.5, suggesting that the immure response in hiPSC-NS/PCs transplantation would be unexpectedly low. Since stimulation indexes in several samples were less than 1 and lower compared to those of unstimulated PBMCs, there might be some detection limits of immune rejection by MLR under these particular conditions. Although feasible, it is crucial to further verify whether MLR is appropriate as a detection procedure of immune rejection in hiPSC-NS/PCs transplantation.

F2046

ROLE OF ID4 IN DEVELOPMENT AND REGULATION OF THE MOUSE SUBVENTRICULAR ZONE

Rocamonde-Esteve, Brenda[1], Herranz-Pérez, Vicente[2], Lepannetier, Sophie[1], García-Verdugo, José Manuel[2] and Huillard, Emmanuelle[1], [1]Institut du Cerveau et de la Moelle Epinière, Paris, France, [2]Instituto Cavanilles de Biodiversidad y Biología Evolutiva, Universidad de Valencia, Valencia, Spain

Neurogenesis in the subventricular zone (SVZ) is tightly regulated by several intrinsic and extrinsic factors. Among extrinsic factors, the bone morphogenic protein (BMP) signalling pathway has been shown to positively regulate neurogenesis. However, the downstream transcriptional factors mediating the effects of BMPs on adult neurogenesis are not well characterized. Inhibitor of DNA binding (Id) proteins are downstream effectors of BMP signalling pathway. Id proteins regulate numerous cellular processes such as differentiation, proliferation and apoptosis, by dimerizing with basic helix-loop-helix transcription factors, preventing them from binding DNA. Id4 protein –the last described member of the family– has been shown to be involved in the development of the central nervous system. However, Id4 function in adult neurogenesis is not well understood. In the present work, we sought to understand the function of Id4 in the regulation of NSC niche. We first determined the expression of Id4 protein in the adult SVZ by immunohistochemistry. We show that Id4 is expressed in the majority of stem cells, progenitor cells and neuroblasts. In order to understand the function we analysed the Id4 knockout mouse. Histological and ultra-structural analysis showed a cellular disorganization of the SVZ as well as an enlargement of the ventricles in the absence of Id4.. In addition, we found decreased numbers of proliferating cells in the SVZ and migrating neuroblasts. Consequently, a decrease in the proportion of newborn neurons was observed in the olfactory bulb. Taken together, our results suggest a role of Id4 in development and regulation of the mouse SVZ.

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F2048

HUMAN IPSC-DERIVED NSC GRAFTING AFTER STATUS EPILEPTICUS MODERATES CHRONIC EPILEPSY DEVELOPMENT AND PRESERVES COGNITIVE, MEMORY AND MOOD FUNCTION

Zanirati, Gabriele[1], Hattiangady, Bharathi[1,2], Shetty, Geetha[1], Bates, Adrian[1,2], Upadhya, Dinesh[1,2], Attaluri, Sahithi[1], Shuai, Bing[1,2], Kodali, Maheedhar[1,2] and **Shetty, Ashok K.**[2,3], [1]Institute for Regenerative Medicine, Texas A&M HSC College of Medicine, Temple, TX, U.S., [2]Olin E. Teague Veterans' Medical Center, CTVHCS, Temple, TX, U.S., [3]Institute for Regenerative Med, TAMHSC College of Medicine at Scott and White, Temple, TX, U.S.

Multiple epileptogenic changes and incessant inflammation ensue after status epilepticus (SE) induced brain injury, which in due course result in chronic temporal lobe epilepsy typified by spontaneous recurrent seizures (SRS) and impairments in cognition, memory and mood. We tested whether early intervention after SE with grafting of hiPSC-derived neural stem cells (hNSCs) into hippocampi is efficacious for curbing SRS and thwarting cognitive, memory and mood dysfunction. Young male F344 rats first underwent kainic acid induced SE for two hours and then received grafts of hNSCs into hippocampi at 7 days post-SE (3 grafts/side, ~100,000 cells/graft). Other cohorts of rats underwent similar SE but received either sham-grafting surgery or maintained as epilepsy-only controls. Sixty-four hour observations in the 3rd month after SE revealed diminished SRS (46-60% reduction) in grafted animals, in comparison to animals in sham-grafted or epilepsy only groups. Seven days of continuous video-EEG recordings in the 4th month after SE corroborated these findings. Furthermore, grafted animals displayed competence for discerning minor changes in the environment (pattern separation function), ability for forming recognition and location memories in novel object recognition, object location and object in place tests, and no anhedonia-like behavior in sucrose preference test. Contrastingly, epileptic rats in both sham-grafted and epilepsy-only groups showed pattern separation, recognition and location memory impairments and anhedonia. Histological analyses performed 4 months after grafting revealed robust yield and mostly (~87%) neuronal differentiation of hNSC graft-derived cells. Some GABA-ergic neurons (~12%), astrocytes (11-16%) and Ki-67+ proliferating cells (~1%) were also seen among graft-derived cells. Yet, grafts did not contain Oct-4+ PSCs. In addition, hNSC grafting maintained normal levels of hippocampal neurogenesis with reduced aberrant migration of newly born neurons, modulated the hypertrophy of astrocytes and activation of microglia and rescued host neuropeptide Y and parvalbumin positive interneurons. Thus, hiPSC-derived NSC grafting into the hippocampus early after SE considerably mitigates SE-induced chronic epilepsy and preserves cognitive, memory and mood function.

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F2050

DIRECT INDUCTION OF FUNCTIONAL DOPAMINERGIC NEURONS FROM HUMAN PLURIPOTENT STEM CELLS IN SHORT TERM CULTURE.

Tran, Hoang-Dai[1,2], Jo, Junghyun[1], Tan, Zi Ying[1,2], Sun, Xuyang Alfred[1] and Ng, Huck-Hui[1,2], [1]Genome Institute of Singapore, Singapore, Singapore, [2]National University of Singapore, Singapore, Singapore

Parkinsons' disease (PD) is one of the most common neurodegenerative movement disorders. The pathological hallmark is the lost of midbrain dopaminergic (DA) neurons in the substantia nigra pars compacta. Studies of PD have been hindered by the lack of access to the affected human DA; in vitro modeling represents a trackable means to investigate the neuropathological features of PD. Current differentiation protocols to generate DA neurons from human pluripotent stem cells (hPSCs) are laborious and time-consuming which require at least 1.5 to 3 months in order to generate functional DA neurons. Recent findings show that neurons can be induced directly from postnatal somatic cells by overexpression of defined combinations of genes. Following the same strategy, in this study we are optimizing our protocol to specifically induce DA neurons from human neural progenitor cells within a short time frame by introducing a cocktail of transcription factors and microRNA. We demonstrate that within only 3 weeks post-induction of these factors, approximately 70% induced neurons expressed Tyrosine Hydroxylase which is a specific marker of DA neurons. Moreover, the neurons also expressed a subset of functional DA markers, and they were electrophysiologically active. Moving forward, we are going to use this robust differentiation protocol to generate large quantity of functional DA neurons for in vitro disease modeling using CRISPR/Cas9-mediated engineering cell lines. The induced DA neurons could also be useful for chemical screening purposes, or for future applications in regenerative medicine.

F2052

3D NETWORKS OF IPSC-DERIVED NEURONS AND GLIA FOR HIGH-THROUGHPUT NEUROTOXICITY SCREENING

Wevers, Nienke, **Wilschut, Karlijn**, van Vught, Remko, Lanz, Henriëtte, Trietsch, Sebastiaan J., Joore, Jos and Vulto, Paul, Mimetas BV, Leiden, Netherlands

The assessment of neurotoxicity remains a major scientific challenge due to the complexity of the central nervous system. Current strategies to evaluate toxicity of drugs and chemicals are predominantly based on ex vivo or in vivo animal studies. These models have limited predictability for neurotoxicity in humans and are not amenable to high-throughput testing. In order to overcome these limitations we are developing a neurotoxicity model based on iPSC-derived neurons in OrganoPlates™. This microfluidic platform enables high-throughput screening of miniaturized organ models. A mixed population of human iPSC-derived neurons consisting of GABAergic and glutamatergic neurons with supporting astrocytes was cultured in 3D, closely representing the physiology of the human brain. As a part of the validation, proper network formation was observed by neuron-specific immunostainings and neuronal electrophysiology was analyzed by a calcium sensitive dye indicating spontaneous neuronal firing. Additionally, we investigated the dose-response neurotoxic effects of methylmercury and endosulfan on neuronal viability. The OrganoPlate™ platform enables real time analysis of neurotoxic effects of compounds in high-throughput. This iPSC-derived neuronal model can be used to refine animal experiments and has the potential to better predict adverse effect in humans and hence to improve clinical development success.

F2054

MODELING SHANKOPATHIES IN HUMAN NEURONS BY CONDITIONAL DELETION OF SHANK3

Yi, Fei, Danko, Tamas, Wernig, Marius and Sudhof, Thomas, Stanford University, Stanford, CA, U.S.

The clinical and genetic landscapes of human neurological diseases are often highly heterogeneous thereby molecular mechanisms behind them remain largely elusive. Shank3 is an ubiquitously expressed scaffolding protein enriched in the postsynaptic density of excitatory neurons where it scaffolds an intricate protein complex. Heterozygous SHANK3 mutations are associated with idiopathic autism and Phelan-McDermid syndrome. Enormous previous efforts have been spent to study the SHANK3 deficiency in neuronal diseases using mouse genetic models; however, major neuronal or behavior manifestations observed were complex. Besides, innate limitations of mouse models in studying human neurological disorders and the lack of direct proof of Shank3's function in human neurons represent major obstacles in the field. In this regard, here we report the generation of conditional SHANK3 knockout human neurons by gene editing in human embryonic stem cells (ESC). Induced human neurons differentiated from human ESC with either heterozygous or homozygous SHANK3 deletion are closely compared to their conditional control counterparts. Our model revealed major defects of neuronal morphology and synaptic transmission in SHANK3 deficient human neurons which recapitulate previous findings in Shank3 mouse models. Moreover, unprecedented non-synaptic defects caused by SHANK3 deletion were also observed in human neurons and were confirmed in mouse genetic model. Therefore, our new discovery reveals a novel mechanism behind Shank3 haploinsufficiency which may be amenable to pharmacological intervention. Overall, our model represents the first conditional approach in studying SHANK3 function in human neurons which renders us significant advantages over previous models in investigating the authentic function of SHANK3 in human neurological diseases.

Funding Source: National Institute of Mental Health

F2056

AMYLOIDβ-INDUCED MITOCHONDRIAL FISSION PLAYS AN IMPORTANT ROLE IN NEURONAL APOPTOSIS THROUGH AKT ACTIVATION

Han, Ho Jae, Kim, Dah Ihm, Lee, Ki Hoon, Lim, Hyeon Su, Lee, Sei-Jung, Kim, Jun Sung, Oh, Ji Young, Song, Eun Ju and Onphachanh, Xaykham, Seoul National University, Seoul, Korea, South

Accumulation of evidence revealed that mitochondria became a significant controller affecting the cell fates in aging related diseases such as Alzheimer's disease (AD). Mitochondria are dynamic organelles changing their morphology which is tightly related to mitochondrial function. Mitochondrial dysfunction is one of causative factor inducing neuronal cell death. Here, we investigated the effect of Amyloid β (Aβ) on mitochondrial dynamics which regulating the cellular function. In this report, Aβ induced neuronal apoptosis in a dose-dependent manner, which was accompanied with increase in caspase-9 and -3 activities. Consistently, Aβ treatment induced mitochondrial fragmentation, which suggests that the mitochondrial dymamic are highly dependent on the concentration of Aβ. Aβ-induced calcium influx up-regulated Akt activation through CaMKII leading Drp1 phosphorylation level in a time-dependent manner. Translocation of Drp1 from cytosol to mitochondria inducing mitochondrial fission was blocked by Akt inhibitor. These results strongly suggest that Aβ induces mitochondrial fission through Akt signaling cascade. Recruitment of Drp1 on the mitochondria leads to mitochondrial fission generating ROS. Oxidative stress through excessive fission induce mito-

chondrial dysfunction such as loss of membrane potential and ATP production. Furthermore, treatment with mdivi-1, selective Drp1 inhibitor, attenuated ROS generation and mitochondrial dysfunction by Aβ. As another role of Akt signaling is suppressing autophagy, we investigated the relationship between Aβ-induced Akt activation and mitochondrial dynamics. Sustained Akt activity inhibited autohpagy through activating mTOR signaling. Inhibition of autophagic clearance by Aβ accelerate ROS level and worsens mitochondrial defect which was blocked by Rapamycin (an mTOR inhibitor). Together, Aβ-induced Akt activation leads to Drp1-mediated mitochondrial fission which induce neuronal apoptosis. Thus, targeting Akt as a regulator of mitochondrial fission would protect neuronal apoptosis against Aβ toxicity.

F2058

GENOME EDITING TECHNOLOGIES ENHANCE THERAPEUTIC POTENTIAL OF HUMAN NEURAL STEM CELLS

Dever, Daniel P[1], Bak, Rasmus O[1], Kildebeck, Eric J[1], Clark, Joseph T[1], Tsukamoto, Ann[2], Uchida, Nobuko[2] and Porteus, Matthew H.[1], [1]Stanford University, Stanford, CA, U.S., [2]StemCells, Inc., Newark, CA, U.S.

Neural stem cells (NSC) have therapeutic potential for unmet medical needs for neurological disorders with one-time intervention with a life-long impact. HuCNS-SC®, developed by StemCells Inc, have expected biological NSC activities with multiple mechanism of actions, providing neuroprotection, myelination and retinal preservation via site-appropriate global migration. HuCNS-SCs have been tested in four Phase I/II clinical trials with promising outcomes for safety, donor cell survival and/or preliminary efficacy. The recent advances in genome editing technologies, namely the TALEN and CRISPR/Cas9 platforms, have accelerated opportunities for producing gene-modified (GM) cells for cell therapy, which will ultimately broaden the therapeutic potential of HuCNS-SC; for example, by serving as mini-factories of neuroprotective proteins to overcome the blood brain barrier. We targeted the NSC "safe harbor loci", CCR5 and IL2RG, for homologous recombination (HR) of a GFP cassette by inducing site-specific double strand breaks (DSBs), along with homologous plasmid donor templates. GM-HuCNS-SC display robust long-term GFP expression, and importantly, genomic analysis by in-out PCR confirmed on-target integration. Truncated CD19 enabled us to purify GM-HuCNS-SC population >90%. Furthermore, delivering the CRISPR platform as an "all RNA" system with chemically modified sgRNAs, significantly improved the frequency of DSBs, which subsequently improved HR targeting rates two fold over plasmid delivery of CRISPR. Most importantly, transplantation of GM-HuCNS-SC into oligodendrocyte mutant shiver mice showed that GM-HuCNS-SC migrate and differentiate into myelin producing oligodendrocytes, comparable to non-genetically modified Hu-CNS-SCs, suggesting GM-HuCNS-SCs retain their NSC characteristics. The self-renewal and global migration properties of the HuCNS-SC suggest that GM-HuCNS-SC could serve as mini-factories for the continuous delivery of proteins to broaden treatment options since these proteins cannot cross the blood brain-barrier. In conclusion, with the success of these proof-of-concept studies, we would like to further develop transplantable GM-HuCNS-SC for the treatment of a battery of neurodegenerative disorders and injuries for brain, spinal cord and eye.

REPROGRAMMING

F2060

FACTORS THAT ENHANCE SENDAI VIRUS MEDIATED SOMATIC REPROGRAMMING

Zarrabi, Aryan[1], Avagyan, Samvel[1,2], **MacArthur, Chad C.**[1] and Lakshmipathy, Uma[1], [1]Thermo Fischer Scientific, Carlsbad, CA, U.S., [2]Rensselaer Polytechnic Institute, Troy, NY, U.S.

Improvements in induced pluripotent stem cell (iPSC) reprogramming technologies have led to the generation of patient-derived stem cells from various genetic backgrounds, creating valuable tools in drug discovery and future cell therapies. As clinical applications become a larger reality there will be an increased demand for iPSC's generated from diseased cells and from various somatic cell sources. One of the difficulties of patient derived iPSC's is that of intrinsic resistance to reprogramming. In such cases, factors that overcome reprogramming barriers are necessary to enhance its efficiency.

Sendai viruses are non-integrating RNA viruses that enable a safe, efficient and consistent way to generate iPSC from a wide variety of cell types. Using Sendai virus mediated reprogramming, we explored the effect of three different classes of enhancers that are known to influence reprogramming efficiency. Small molecules such as epigenetic modulators, pathway inhibitors and hypoxia mimics were examined for their effect on the efficiency of fibroblast mediated reprogramming. The class of molecules that had a consistent effect on both feeder-dependent and feeder-free reprogramming was HDAC inhibitors such as sodium butyrate. Additionally, evaluation of different matrices for feeder-free reprogramming indicated Ln521 as a robust component that enabled more efficient reprogramming. Finally, reprogramming in the presence of hypoxia was also shown to be beneficial for higher reprogramming rates. These factors, alone or in combination, can be used to enhance efficiencies of hard to reprogram somatic cell types, enable iPSC generation from diverse somatic cell sources, and potentially in other species.

F2062

IN VIVO REPROGRAMMING: REVERSING GLIAL SCAR FOR BRAIN REPAIR

Chen, Gong, Pennsylvania State Univ, University Park, PA, U.S.

Glial scar is widely associated with brain and spinal cord injury, stroke, glioma, and neurodegenerative disorders such as Alzheimer's disease. Reactive glia initially exert neuroprotective role but later form glial scar to inhibit neuronal growth. Currently, there is no effective way to reverse glial scar back to neural tissue. We have recently developed an innovative in vivo reprogramming technology to directly convert reactive glial cells into functional neurons inside the mouse brain (Guo et al., Cell Stem Cell, BEST of 2014 article). This is achieved through in vivo expression of a single neural transcription factor NeuroD1 in the reactive astrocytes in injured mouse brain or Alzheimer's disease mouse model. Our in vivo cell conversion technology makes use of internal glial cells to regenerate new neurons, making it possible for the first time in history to reverse glial scar back to neural tissue. Such internal cell conversion method will avoid transplantation of external cells and its associated immune rejection. We have further discovered a cocktail of small molecules that can directly convert cultured human astrocytes into functional neurons (Zhang et al., Cell Stem Cell, 2015), paving the way for a potential drug therapy for brain repair in human patients.

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F2064

REMOVING EPIGENETIC AND CYTOSKELETAL ROADBLOCKS TO REPROGRAMMING

Galloway, Kate[1], Babos, Kimberley[2], Quintino, Brooke[3], Kissler, Kassandra[2], Zitting, Madison[2], Li, Yichen[2], Shi, Yingxiao[2], Zlokovic, Berislav[2] and Ichida, Justin[2], [1]University of Southern California, CA, U.S., [2]University of Southern California, Los Angeles, CA, U.S., [3]California State University Fullerton, Fullerton, CA, U.S.

The central hypothesis of transcription factor-mediated reprogramming relies on optimal cocktails of factors to redirect transcriptional networks and, thus, cellular identity. However epigenetic roadblocks may impede transcription factor-mediated changes by inhibiting access to various loci and prohibiting the activation of critical subnetworks of genes. Additionally, competition between established and newly induced networks to define central cellular properties (e.g. the actin-cytoskeleton or the ensemble of ECM proteins excreted) may limit the full adoption of the alternative identity during reprogramming, resulting in a spectrum of conversion.

Using morphometric and single-cell transcriptional analysis, we exploited the observed heterogeneity of reprogramming from fibroblasts to induced motor neurons (iMNs) to identify subpopulations of differentially converted cells. Further, we have characterized the heterogeneity of expression for key MN markers as well as the residual expression of the fibroblast gene regulatory network (GRN). We observe that morphometric maturity correlates with increases in MN markers and diminution of the fibroblast GRN. By modulating fibroblast cell cycle rate using several genetic and chemical methods, we uncovered a positive correlation between the cell cycle rate and generation and maturation of iMNs. Inhibition of p53 during conversion not only increased cell cycle rate but increased accessibility at a host of neuronal loci and induced rapid realignment of the transcriptional profile leading to more robust, efficient generation of morphologically mature iMNs.

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F2066

GLYCINE DECARBOXYLASE IS IMPORTANT FOR GLYCOLYTIC METABOLIC SHIFT DURING SOMATIC CELL REPROGRAMMING INTO INDUCED PLURIPOTENT STEM CELLS

Kang, Phil Jun and You, Seungkwon, Korea University, Seoul, Korea, South

Metabolic shift is one of the most important step in somatic cell reprogramming to change oxidative phosphorylation to aerobic glycolytic pathway. Similar to cancer development, it has been reported that the metabolic shift during somatic cell reprogramming has clearly importance for somatic cells to gain pluripotency. We found that glycolytic shift occurs very early stage during somatic cell reprogramming and regulated by well-known reprogramming factors OCT4, SOX2, KLF4 and c-MYC in transcriptional level. Each factor has specific role to upregulate core glycolytic modulators and these results might lead to metabolic shift during somatic cell reprogramming. Moreover, this metabolic conversion is accompanied by other related metabolic pathways such as glycine cleavage system. In this study, we found that expression of glycine decarboxylase (GLDC) gene which is one of the important enzymes in glycine cleavage system is upregulated in early stage during somatic cell reprogramming. We also found that embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have distinct expression levels of GLDC compared to other somatic cell types. Knock-down of GLDC decreased glycolysis-related genes expression and inhibits reprogramming process, however, overexpression of GLDC enhanced the reprogram-

ming efficiency and could replace KLF4 during somatic cell reprogramming. Furthermore, inhibition of its metabolic pathway using small molecules completely block the generation of iPSCs. These results provide new approach that upregulation of GLDC is important step for somatic cell reprogramming related to glycolytic metabolic shift. In conclusion, our study provides a new insight into roles of reprogramming factors and metabolic regulation with induced pluripotency of somatic cells

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F2068

EFFICIENT AND REPRODUCIBLE RNA-BASED REPROGRAMMING OF DISEASE-SPECIFIC HUMAN PRIMARY FIBROBLASTS

Kogut, Igor, Ortega, Sandra M., Pavlova, Maryna V., Astling, David P., Chen, Xiaomi, Jones, Kenneth L., Roop, Dennis R. and Bilousova, Ganna, University of Colorado, Aurora, CO, U.S.

Recent advances in reprogramming somatic cells into induced Pluripotent Stem Cells (iPSCs) offer a possibility of developing new stem cell-based approaches for the treatment of a variety of human diseases and for tissue bioengineering. Despite the almost limitless therapeutic potential of iPSCs for tissue repair, several obstacles must still be overcome before iPSCs can be applied in the clinic. One of these obstacles is the low efficiency and inconsistency of clinically relevant, integration-free approaches for the reprogramming of patient's somatic cells into iPSCs. Here, we show that the reprogramming of patient's fibroblasts into iPSCs can be significantly enhanced via the synergistic activity of reprogramming modified mRNAs and mature miRNA mimics in combination with optimized culturing conditions. Our novel, integration- and feeder-free reprogramming approach generates clinically relevant iPSCs from human primary fibroblasts with unprecedented efficiency, reaching 90% on a single cell level, and produces iPSCs from a variety of disease-specific adult fibroblasts with a 100% success rate. Using this methodology, we have successfully generated iPSCs from individuals with inherited skin blistering diseases, Down syndrome, and Danon disease. All iPSC lines 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 human somatic cells with an efficiency and kinetics that surpass all previously published studies and addresses the inconsistency of previously reported RNA-based approaches. The method 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.

F2070

THE ROLE OF CHROMATIN ASSOCIATED PROTEIN HMGB2 IN SETTING UP PERMISSIVE CHROMATIN STATES FOR DIRECT GLIA TO NEURON CONVERSION

Lepko, Tjasa[1,2], Hauck, Stefanie[3], Buettner, Maren[4], Theis, Fabian[4], Goetz, Magdalena[1,5] and Ninkovic, Jovica[1,5], [1]Institute of Stem Cell Research, Helmholtz Zentrum Muenchen, Neuherberg, Germany, [2]Helmholtz Zentrum Muenchen, Muenchen, Germany, [3]Research Unit Protein Science, Helmholtz Zentrum Muenchen, Neuherberg, Germany, [4]Institute of Computational Biology, Helmholtz Zentrum Muenchen, Neuherberg, Germany, [5]Physiological Genomics, Biomedical Center, University of Munich, Munich, Germany

Functional replacement of degenerated neurons appears to be one of the major challenges for regenerative therapies. A novel approach to induce neurogenesis in reactive glial cells generating glial scar has a potential to reduce the scar formation and provides new source of neurons at the injury site. Overexpression of neurogenic fate determinants efficiently converts astroglial cells into neurons in vitro. However, recent studies identified a number of roadblocks including the metabolic obstacles that interfere with efficient glia to neuron conversion in vivo. Therefore, it became clear that we still need better understanding of mechanisms maintaining the glial fate even after the overexpression of potent neurogenic fate determinants such as Ngn2 in the in vivo environment. Towards this end, we adjusted the growth factors composition in vitro in order to better recapitulate environment generated at the injury site. We cultured postnatal astrocytes in the medium lacking EGF, as its levels rapidly decrease after injury. Interestingly, the absence of EGF in the medium rendered the astroglia into the cell types resistant to the reprogramming similar to reactive astrocytes in vivo. We next compared proteome of astrocytes prone and resistant to reprogramming withaim to identify barriers for efficient lineage reprogramming in vivo. Our analysis suggests that the changes in the chromatin could maintain the astrocytes in the glial lineage even after neurogenic factor overexpression. To test this hypothesis, we overexpressed the most regulated chromatin related protein, Hmgb2, together with Ngn2 and analyzed reprogramming efficiency. Indeed, the overexpression of Hmgb2 and Ngn2 in the astrocytes resistant to reprogramming significantly increased direct reprogramming in vitro suggesting the role of chromatin associated pro-

teins in bypassing the lineage roadblocks. To delineate these roadblocks, we compared the chromatin compaction of astrocytes resistant to reprogramming with the chromatin of astrocytes prone to reprogram. This analysis revealed an enrichment of opened enhancer regions of bone fide neuronal genes in the astrocytes prone to reprogram. Taken together, our data identify chromatin states in the glia cells permissive for lineage conversion and possible role of Hmgb2 in regulation of these states.

F2072

REPROGRAMMING OF INACTIVATED HUMAN HAIR FOLLICLE DERMAL PAPILLA CELLS INTO HAIR-INDUCING DERMAL PAPILLA CELLS BY TRANSCRIPTION FACTORS

PARK, Junghyun, Korea university, Seoul, Korea, South and You, Seungkwon, Korea University, Seoul, Korea, South

Alopecia is a common disease in contemporary society and demands appropriate treatment. Hair loss can be caused by numerous conditions. One of the most effective reason is the death of hair follicle dermal papilla cells(DPCs) which have a pivotal role in hair regeneration cycle. One of the effective therapeutic approaches of alopecia is the hair transplantation surgery. DPCs are good source of hair implantation because of its trichogenic ability, however, the drawback of DPCs is the loss of their trichogenic capacity when they are maintained in vitro. In this study, we hypothesize that overexpression of transcription factors which are related with stemness of hair follicle DPCs can re-activate the trichogenic ability of DPCs. In this study, we demonstrate that induction of transcription factors in vitro maintained DPCs can reduce the cell size, ROS level and increase proliferation of DPCs as well as enhance trichogenic markers of DPCs (ALP, LEF, Versican…). Contraction ability, tubulogenic process which represent the ability of hair-inducible DPCs were performed to analyze the trichogenic characteristic of reprogrammed DPCs in vitro and in vivo. These results suggest that overexpression of transcription factor can re-activate the inactivated DPCs and their application to hair regeneration with hair follicle DPCs.

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F2074

GENERATION OF HUMAN URINE-DERIVED STEROIDOGENIC CELLS THROUGH LINEAGE CONVERSION: A NEW TECHNOLOGY TO STUDY THE ADRENAL GLAND.

Ruiz-Babot, Gerard[1], Hadjidemetriou, Irene[1], Jane Ajodha, Sharon[1], Ghataore, Lea[2], Taylor, David[2], Taylor, Norman[2] and Guasti, Leonardo[1], [1]Queen Mary University of London, London, U.K., [2]King's College Hospital, London, U.K.

The adrenal cortex is the primary site of steroid synthesis, producing glucocorticoids under the control of the hypothalamic-pituitary axis and mineralocorticoids under the control of the renin-angiotensin system. Adrenal insufficiency, which can be life threatening, is caused by a number of adrenal disorders, and lifelong management of these patients with exogenous steroids can be challenging. Our long-term goal is to develop novel personalized and curative treatments that use stem cells to treat the many progressive and debilitating conditions affecting the adrenal cortex. Steroidogenic Factor-1 (SF1) is a transcription factor essential for both adrenal and gonadal development. SF1 not only binds to responsive elements in the promoter region of steroidogenic genes to positively regulate their transcription, but can be considered a true effector of cell fate as it starts a genetic program driving embryonic mesenchymal cells towards a steroidogenic lineage. The capacity to impose an SF1-dependent steroidogenic-like gene expression program in a variety of murine cells has been verified by several groups. We have discovered that cells derived from urine (urine-derived stem cells, USCs) can be reprogrammed to a steroidogenic phenotype with a highly reproducible phenotype, in terms of gene expression profile and hormonal output, and with higher efficiency compared to other cell sources such as fibroblasts from skin or endothelial progenitor cells from blood (L-EPCs). Urine is the perfect cell source reservoir as its harvest is the least invasive. By forcing the expression of SF1, but not of other transcription factors involved in adrenal development, and together with an in house-developed and stage-dependent cocktail of growth factors and specific culture conditions, we demonstrate the ability of USCs, L-EPCs and fibroblasts to lineage convert to steroidogenic-like cells, as assessed by changes in cell morphology, gene expression, activation of adrenal-specific signaling pathways and hormonal output. To test the functionality of reprogrammed USCs, in vivo cell transplantation experiments in mice and in vitro assessment of hormonal profile of affected versus CRISPR-Cas9 gene corrected cells derived from familial glucocorticoid deficiency (FGD) patients are being tested in our laboratory.

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F2076

MESOANGIOBLASTS -VESSEL ASSOCIATED PROGENITOR CELLS- ENGRAFT EPITHELIAL TISSUES AND EXPRESS CFTR CHANNEL: PROSPECTS AND PROMISE FOR A CELL THERAPY FOR CYSTIC FIBROSIS

Vezzali, Chiara[1], Antonini, Stefania[1], Barone, Christina[2], De Stefano, Daniela[3], Maiuri, Luigi[3], Egan, Marie[2], Bruscia, Emanuela[2] and Messina, Graziella[1], [1]University of Milan, Milan, Italy, [2]Yale University, New Haven, CT, U.S., [3]Ospedale San Raffaele, Milan, Italy

Cystic Fibrosis (CF) is caused by mutations in the gene that encodes for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which functions mainly as a cAMP-dependent chloride channel and is primarily expressed in the apical membrane of secretory epithelia. Lung disease, characterized by airway obstruction, inflammation and bacterial infection is the leading cause of death. At variance with some pharmacological approaches, no efficacious gene and cell therapy have been proved to date. We are developing a cell therapy based on transplantation of mouse mesoangioblasts (mMABs) in mouse models of CF. MABs are vessels-associated progenitor cells that are able to cross the vessel wall upon intra-arterial injection, undergo skeletal muscle differentiation and rescue skeletal muscle dystrophy in mice and dogs. Our results unexpectedly show that mMABs, upon a systemic delivery, engraft lung, tracheal and intestinal epithelium up to 2 months in *wt* mice and up to 4/6 months in the CF mouse models *KOCftr*^{tm1UN} and *F508del CFTR,* respectively. Importantly, this engraftment results in both rescue of CFTR-dependent chloride transport and inflammation. By a biological point of view, we are now characterizing mMAB plasticity to differentiate in epithelial cells, aspect never explored so far that would enormously increase the potential of these cells in a cell therapy approach for CF. We demonstrated that donor engrafted mMABs, re-isolated from *F508del CFTR* transplanted mice, express the epithelial markers E-cadherin and Cytokeratin 6 and, surprisingly, the characteristic marker of Club Cells, CC10, but it seems they do not differentiate in SPC+ alveolar epithelial type II cells or Acetylated a-Tubulin+ ciliated cells. Moreover, culturing mMABs with a specific epithelial medium in vitro, leads to the increase of the expression of epithelial markers (e.g. E-cadherin, Adam10) and to the decrease of mesenchymal and myogenic ones (e.g. Fibronectin, Vimentin, MyoD, Myogenin), followed also by the decrease of the expression of epithelial to mesenchymal transition markers (e.g. Snail, N-cadherin). In the end, we suppose that mMABs might be recruited by the epithelia and contribute to their homeostasis and stem cell niches, making mMABs would be promising for the development of an efficacious cell therapy for CF.

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F2078

MLL1 INHIBITION REPROGRAMS EPIBLAST STEM CELLS TO NAïVE PLURIPOTENCY

Zhang, Hui, University of Michigan, Ann Arbor, MI, U.S.

Embryonic stem cells (ESCs) and epiblast stem cells (EpiSCs) represent two distinct pluripotent states that are interconvertible in vitro. Conversions between the primed-naïve states are accompanied by dramatic epigenetic reorganization (e.g. H3K4me defined enhancer landscape). However, the causal link between a discrete epigenetic modification and acquisition of pluripotency has not been established. Recently, we have developed a small molecule inhibitor MM-401 that specifically inhibits MLL1 methyltransferase activity but not other MLL HMTs (MLL2-4, SET1a and SET1b) and functionally mimics the Mll1 gene deletion. Here, we use MM-401 to study the potential function of MLL1-mediated H3K4me in pluripotent cells. Surprisingly, we find that blocking MLL1 function is sufficient to reprogram EpiSCs to developmentally competent naïve ESCs. The reversion is independent of extrinsic signaling factors (i.e. LIF and bFGF) and occurs with high efficiency with ~50% cells expressing PECAM1/REX1 after a 3-day MM-401 treatment. Complete EpiSC reprogramming is also confirmed by Xi-chromosome reactivation in female EpiSCs. Mechanistic studies show that MM-401 disrupts MLL1 chromatin binding at a significant subset of MLL1 targets in EpiSCs and blocks MLL1-mediated-H3K4me1 at regulatory enhancers. These changes directly reduce expression of lineage specification factors as well as EpiSC markers, resulting in a rapid change towards the naïve pluripotent state. Our study causally links down-regulation of H3K4me to EpiSC reprogramming and highlights MLL1-mediated H3K4me as an intrinsic epigenetic determinant that regulates acquisition of differentiated pluripotent identities. Our study strongly supports that erasure of key epigenetic marks is associated with restoration of the naïve pluripotent state. It also reveals a previously uncharacterized role of MLL1 in pluripotent stem cells and demonstrates that perturbation of a discrete histone mark is sufficient/causal to reverse developmental commitments.

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IPS CELLS

F2082

INDUCED PLURIPOTENT STEM CELL-DERIVED REJUVENATED T-CELL THERAPY WITH SUICIDE GENE-BASED SAFEGUARD SYSTEM

Ando, Miki[1], Nishimura, Toshinobu[2], Yamazaki, Satoshi[1], Yamaguchi, Tomoyuki[1], Brenner, Malcolm[3] and Nakauchi, Hiromitsu[1,2], [1]The University of Tokyo, The Institute of Medical Science, Tokyo, Japan, [2]Stanford University, Stanford, CA, U.S., [3]Baylor College of Medicine, Houston, TX, U.S.

Adoptive T-cell therapy, the transfer of ex vivo expanded activated antigen-specific cytotoxic T lymphocytes (CTLs) can induce durable remissions in patients with selected tumors such as melanomas. However, for the majority of cancers, the clinical utility is often limited, because CTLs continuously exposed to viral or tumor antigens, with long-term expansion, may become exhausted. The discovery of iPS cells (iPSCs) has created promising new avenues for therapies in regenerative medicine. We have shown that antigen specific CTLs can be generated from iPSCs and these rejuvenated CTLs (rejCTLs) have higher proliferative capacity and longer telomeres than the original CTLs. Whereas, the tumorigenic potential of undifferentiated iPSCs and the malignant transformation of differentiated iPSCs are major safety concerns for clinical translation. In preparation for a clinical trial, we introduced inducible a caspase-9 (iC9)-based safeguard system into iPSCs to address these issues. First, the effectiveness of iC9 system was tested using iC9-iPSC lines. CID (the dimerization drug) could induce >95% apoptosis in four independent iPSC lines tested. To examine the effect of CID treatment on established tumor, iC9-iPSCs were injected into testis of NOD-Scid mice. After the tumor was fully enlarged, CID was administered to the host mice. Dramatic reduction of tumor size was observed only in mice that had tumors from iC9-iPSCs. Next, the iC9 system was implemented to iPSC lines derived from EBV- or HIV-specific CTLs. iC9-rejCTLs maintained antigen specificity and showed cytotoxicity against antigen presented cells. The antitumor effect of iC9-rejCTLs for EBV-induced tumors was higher than that of the original CTLs when compared in vivo. Moreover, iC9-rejCTLs provide a survival advantage in the group treated with iC9-rejCTLs compared to the group treated with original EBV-CTLs. Finally, the system efficiently induced apoptosis in these rejCTLs in vivo. iPSC-derived EBV-CTLs have strong anti-tumor effect against EBV-infected tumors in vivo and the iC9 suicide gene system provides a reliable safeguard for iPSC-derived rejuvenated T-cell therapy. Now we are starting the preclinical studies to proceed with the powerful and novel immunotherapy for virus associated lymphoma.

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F2084

SOURCES AND DRIVERS OF TRANSCRIPTIONAL VARIABILITY IN A MULTICLONAL LARGE-SCALE HUMAN iPSC LIBRARY

Carcamo-Orive, Ivan[1], Hoffman, Gabriel E.[2], Cundiff, Paige[3], Beckmann, Noam D.[2], D'Souza, Sunita[3], Knowles, Joshua W.[1], Papatsenko, Dimitri[3], Patel, Achchhe[3], Abassi, Fahim[1], Reaven, Gerald M[1], Whalen, Sean[4], Shahbazi, Mohammad[5], Henrion, Marc[2], Zhu, Kuixi[2], Roussos, Panagoitis[2], Schadt, Eric[2], Pandey, Gaurav[2], Chang, Rui[2], Quertermous, Thomas[1] and Lemischka, Ihor[3], [1]Stanford School of Medicine, Cardiovascular Institute, Stanford, CA, U.S., [2]Institute of Genomics and Multiscale Biology, Icahn School of Medicine at Mount Sinai, New York, CA, U.S., [3]Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY, U.S., [4]Gladstone Institutes, University of California, San Francisco, CA, U.S., [5]Stanford university, Stanford , CA, U.S.

Variability in induced pluripotent stem cells (iPSCs) remains a major roadblock in their applicability in regenerative medicine and disease modeling. Moreover, the variability in gene expression levels across or within individuals may be generated and sustained by different mechanisms. To study iPSC transcriptional variability, we have generated a multiclonal large-scale human iPSC library coupled with RNA sequencing. Through the use of linear mixed models, Bayesian networks and key driver analyses, we have deconstructed the different sources and identified drivers of gene expression variability in 317 human iPSC lines from 101 individuals. Based on gene expression levels and allelic specific expression, we show that variability in iPSC lines derived from different subjects (across individual variability) is substantially greater than in lines derived from the same patient (within individual variability). Nearly 50% of the contribution to overall transcriptional variability is due to genetic background variation across individuals. We have defined a set of expression quantitative trait loci (eQTLs) associated to 4,150 genes, at 5% false discovery rate, that drive transcriptional variability across individuals. Integration with the Epigenomics Roadmap indicates that the most significant eQTL variants for each gene are enriched in enhancers and promoters active in iPSCs and ESCs. On the other hand, within individual variability is associated with polycomb targets and key driver analysis points to a subset of Hox family genes as well as other early developmental genes as drivers of within individual

variability. Our results demonstrate that human iPSCs retain a patient-specific gene expression pattern and open new avenues to reduce the variability associated with the reprogramming process and improve disease modeling.

F2086

MECHANISM OF IMPAIRED OSTEOGENESIS IN CFC SYNDROME PATIENT-SPECIFIC INDUCED PLURIPOTENT STEM CELLS

Choi, Jung-Yun[1], Han, Kyu-Min[1], Kim, Dongkyu[2], Lee, Beom-Hee[3], Yoo, Han-Wook[3] and Han, Yong-Mahn[1], [1]Korea Advanced Institute of Science & Technology (KAIST), Daejeon, Korea, South, [2]Institute for Basic Science, Daejeon, Korea, [3]University of Ulsan College of Medicine, Seoul, Korea

Cardio-facio-cutaneous (CFC) syndrome is a genetic disorder caused by mutations in RAS/MAPK signaling pathway. CFC syndrome is characterized by various symptoms including short stature, heart defects, craniofacial deformities, and mental retardation. Although RAS/MAPK pathway plays important roles in development, little is known about the relationship between ERK activation and the skeletal abnormalities of CFC syndrome. Here, induced pluripotent stem cells (iPSCs) were generated from dermal fibroblasts of a patient carrying c.770A>G mutation on BRAF gene (CFC-iPSCs). CFC-iPSCs were normally differentiated into mesenchymal stem cells (CFC-MSCs) that were positive for CD44, CD73, CD90, and CD105. However, CFC-MSCs failed to develop into osteoblasts showing aberrant phenotypes in ALP activity, Alizarin red S staining, and Von kossa staining. In addition, transcriptional expression of osteogenic genes was lower in CFC-MSCs than wild type MSCs. These results demonstrate that abnormal bone formation in CFC patients may be responsible for defective mineralization in CFC-MSCs during osteogenesis.

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F2088

STABLE MANUFACTURING OF CARDIOMYOCYTES AND ENDOTHELIAL CELLS DERIVED FROM INDUCED PLURIPOTENT STEM CELLS

Enoki, Tatsuji[1], Nishie, Toshikazu[1], Yamamoto, Yuki[1], Tosaka, Yasuhiro[1], Hatsuyama, Asako[1], Yamashita, Jun[2] and Mineno, Junichi[1], [1]Takara Bio Inc., Kusatsu, Japan, [2]Kyoto University, Kyoto, Japan

Induced pluripotent stem (iPS) cells are promising to use for regenerative medicine field as well as drug development study. Although iPS cells can be differentiated to each desired cell type, stable manufacturing is still difficult due to the complicated differentiation process, use of uncontrollable materials and so on. Further, iPS cell condition as a raw material is another important factor for the differentiation efficiency. Thus, stable iPS cell culture should lead to stable differentiation. In some differentiation methods, iPS cells cultured on feeder cells are transferred to extracellular matrix-coated plate in conditioned medium. Feeder cells and condition medium are uncontrollable materials, and they may cause unstable manufacturing. For this reason, we used a feeder-free culture system by commercial available DEF-CS culture medium to construct their differentiation procedures for cardiomyocytes and endothelial cells. The differentiation could be started within 1 – 2 weeks after cell thawing because iPS cells cultured by DEF-CS medium could be cryopreserved and then directly seeded to feeder-free condition after thawing. Such simplification of the pre-culture period of iPS cells probably contributes to the stable differentiation. For cardiomyocytes production, we tested eight iPS cell lines and confirmed all cell lines were differentiated to cardiomyocytes with high purity (cTnT$^+$: >90%). Further, those cardiomyocytes responded to various reference drugs such as E-4031, chromanol 293B, mexiletine, and verapamil. In our method, any drug selections and virus infections were not conducted to purify cardiomyocyte population, thereby eliminating their influences on such as cardiotoxicity evaluation. For endothelial cell production, seven iPS cell lines were tested for their abilities on the differentiation. As a result, all cell lines showed high differentiation efficiency (CD31$^+$: >80%) similarly to the cardiomyocyte production. In conclusion, we successfully constructed stable manufacturing processes for cardiomyocytes and endothelial cells from iPS cells and our robust iPSC culture system would be widely applied to other differentiation methods.

F2090

BUILDING ORIG3N's IPSC BIOBANK FOR MODELING HUMAN DISEASES

Glicksman, Marcie A., Orig3n, Inc, Boston, MA, U.S.

A great deal of phenotypic and genotypic diversity can be found among both disease and non-disease populations. This is being captured in a program at ORIG3N that is called LifeCapsule™. LifeCapsule is a crowd-sourced banking service with tens of thousands of samples from people who have provided uniform consent. By using the clinically diverse population of inherited and idiopathic disease patients represented in LifeCapsule, we will address differences in disease phenotypes in patient populations. PBMCs are isolated from whole blood and reprogrammed to iPSCs as a route to access neural, hepatic and cardiac cells from specific genetic backgrounds. This presentation will focus on our work with cardiac and neural diseases.

F2092

HEREDITARY SPASTIC PARAPLEGIA TYPE 5: "PATHOPHYSIOLOGY AND STEM CELL MODELS"

Hauser, Stefan[1], Höflinger, Philip[1], Theurer, Yvonne[1], Rattay, Tim W.[1], Björkhem, Ingemar[2], Schüle, Rebecca[3] and Schöls, Ludger[1], [1]German Center for Neurodegenerative Diseases (DZNE), Tübingen, Germany, [2]Karolinska Institutet, Stockholm, Sweden, [3]Hertie Institute for Clinical Brain Research, Tübingen, Germany

Hereditary spastic paraplegia (HSP) is a rare monogenetic neurodegenerative disorder characterized by progressive lower limb spasticity and weakness due to axonal degeneration of the corticospinal tract. Spastic paraplegia gene type 5 (SPG5) is an autosomal recessive subtype of HSP caused by mutations in CYP7B1, a gene encoding for the cytochrome P-450 oxysterol 7-α-hydroxylase, essential for the liver-specific alternative pathway in bile acid synthesis. Mutations within CYP7B1 lead to a decreased enzyme activity and consecutively to an accumulation of oxysterol substrates (e.g. 27-hydroxycholesterol) in plasma and cerebrospinal fluid (CSF) of patients. Research into molecular pathogenesis of HSP is limited by the restricted access to primary cell material from patients. Derivation of disease-specific induced pluripotent stem cells (iPSCs) provide an unlimited cell population which can give rise to any somatic cell type. Therefore, we reprogrammed primary fibroblasts of five SPG5-patients using non-integrative episomal plasmids. Differentiation into iPSC-derived neurons and hepatocyte-like cells could be established, leading to a disease-specific cell model. In cultures of iPSC-derived neurons a neurotoxic effect of 27-hydroxycholesterol could be demonstrated and supports the hypothesis that the accumulation of oxysterols leads to progressive axonal degeneration. We aim to use this cell model to study further molecular mechanisms of SPG5 via lipidomic analysis of supernatant and cell pellets of differentiated hepatocytes and neurons. These studies will improve our insight in pathogenesis of SPG5 and may help to develop new therapeutic approaches for the treatment of HSP.

F2094

MODELING LYMPHANGIOLEIOMYOMATOSIS (LAM) USING A TSC2-DEFICIENT PATIENT-DERIVED iPSC REPROGRAMMING APPROACH

Julian, Lisa Marie[1], Delaney, Sean[1,2], Wang, Ying[1], Goldberg, Alex[3], Tam, Roger[1,4], Dore, Carole[1], Yockell-Lelievre, Julien[1], Henske, Elizabeth[5], Kwiatkowski, David[6], Darling, Thomas[7], Moss, Joel[8], Shoichet, Molly S.[9], Kristof, Arnold[3] and Stanford, William[2], [1]Ottawa Hospital Research Institute, Ottawa, ON, Canada, [2]University of Ottawa, Ottawa, ON, Canada, [3]McGill University Health Centre, Montreal, QC, Canada, [4]Univeristy of Toronto Donnelly Centre for Cellular & Biomolecular Research, Toronto, ON, Canada, [5]Division of Pulmonary and Critical Care Medicine, Boston, MA, U.S., [6]Division of Translational Medicine, Boston, MA, U.S., [7]Uniformed Services University of Health Sciences, Bethesda, MD, U.S., [8]National Institutes of Health, Bethesda, MD, U.S., [9]University of Toronto, Toronto, ON, Canada

Lymphangioleiomyomatosis (LAM) is a progressive neoplasm of the lung affecting at least 6 per million women, and is the most clinically severe manifestation of the multi-system tumour disorder Tuberous Sclerosis Complex (TSC). LAM is characterized by the growth of immature smooth muscle-like cells (SMCs) expressing neural crest (NC) markers in the lungs, leading to cystic tissue destruction and eventual respiratory failure. LAM and TSC are characterized genetically by inactivating *TSC1* or *TSC2* mutations. This drives hyper-activation of the mTOR signaling pathway, a central regulator of cell growth, proliferation, differentiation, metabolism and survival. Current treatment options for LAM are limited to lung transplant and the mTOR inhibitor Rapamycin, which provides some clinical benefit but is not curative. Our objective is to accelerate the development of improved therapeutic options for LAM by generating humanized LAM models, which are currently entirely lacking. Toward this goal, we have generated *TSC2+/-* induced pluripotent stem cell (iPSC) lines from TSC/LAM patient fibroblasts. Subsequently, we undertook an *in vivo* differentiation approach to allow *TSC2+/-* iPSCs to differentiate into postulated LAM cell lineages. To test the hypothesis that LAM cells exist as smooth muscle-like cells, teratoma explants were cultured under SMC growth conditions. Using this approach, we have successfully established *TSC2*-deficient SMC lines that can be maintained in culture and, importantly, reflect characteristic LAM phenotypes. These include: expression of NC and SMC markers, mTOR dysregulation, increased cell size, expression of LAM biomarkers, and a shift toward glycolytic metabolism. Thus, through our iPSC reprogramming and *in vivo* differentiation and selection approach, we have established the first humanized cellular model for LAM. We show that these cells can

be selectively sensitized to death compared to wild-type control SMC lines and a pre-existing *TSC2*-deficient mammalian cell line in the presence of chemical regulators of glycolysis and autophagy. As LAM cells are thought to be dependent on autophagy and glycolysis for their survival, these findings demonstrate the high validity and potential of our patient-derived SMC lines to model LAM and aid in identification of novel therapeutics.

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F2096

HUNTINGTON'S DISEASE PATHOLOGY MANIFESTATION IN HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED NEURONS

Kiselev, Sergey[1,2], Lagarkova, Maria[1,2], Vigont, Vladimir[3], Suldina, Lubov[4], Kiseleva, Elena[4], Vassina, Ekaterina[1], Bobrovsky, Pavel[2], Lebedeva, Olga[2], Chestkov, Ilya[1], Bogomazova, Alexandra[1], Illarioshkin, Sergei[5], Kaznacheyeva, Elena[3] and Nekrasov, Evgueny[1], [1]Vavilov Institute of General Genetics, Moscow, Russia, [2]Research Center for Physical-Chemical Medicine, Moscow, Russian Federation, [3]Institute of Cytology, RAS, St.-Petersburg, Russian Federation, [4]Federal Research Center Institute of Cytology and Genetics SB RAS, Novosibirsk, Russian Federation, [5]Research Center of Neurology, Moscow, Russian Federation

Huntington's disease (HD) is an incurable hereditary neurodegenerative disorder, which manifests itself as a loss of GABAergic medium spiny (MS) neurons in the striatum and caused by an expansion of the CAG repeat in exon 1 of the huntingtin gene. There is no cure for HD, existing pharmaceutical can only relieve its symptoms. HD pathology is linked to the deregulation of multiple cellular processes including autophagy, calcium homeostasis, and mitochondrial functions, but the critical factors behind HD advance are still unknown. Various challenges complicate the deciphering of HD molecular mechanisms, including a limited access to human neurons, the complexity of the molecular mechanisms underlying HD pathology, and the lack of adequate animal models. The discovery of somatic cell reprogramming technology engendered new disease models based on iPSCs derived from the somatic cells of patients with particular disease. IPSCs were established from patients with low CAG repeat expansion in the huntingtin gene, and were then efficiently differentiated into GABA MS-like neurons under defined culture conditions. The generated HD neurons recapitulated disease pathology in vitro, as evidenced by mutant huntingtin protein aggregation, nuclear indentations, and enhanced neuronal death during cell aging. Moreover, store-operated channel (SOC) currents were detected in the differentiated neurons, and enhanced calcium entry was reproducibly demonstrated in all HD genotypes. Additionally, the quinazoline derivative, EVP4593, reduced lysomal content and SOC activity in HD neurons and exerted neuroprotective effects in aging cells. Our data demonstrates the direct link of nuclear morphology and SOC calcium deregulation to mutant huntingtin protein expression in iPSCs-derived neurons with disease-mimetic hallmarks, providing a valuable tool for identification of candidate anti-HD drugs.

F2098

MECHANISTIC STUDY OF SKM REPROGRAMMING INDICATES UNIQUE ROLES OF SOX2 IN INITIATING PLURIPOTENCY ESTABLISHMENT

Liu, Peng, Gladstone Institutes, San Francisco, CA, U.S., Zheng, Jiashun, University of California, San Francisco, San Francisco, CA, U.S. and Ding, Sheng, Gladstone Institutes of Cardiovascular Disease, San Francisco, CA, U.S.

Pluripotency holds great promise for the understanding of cell fate commitment and regenerative medicine. Reprogramming differentiated cells to induced pluripotent stem cells (iPSCs) by overexpressing a combination of 4 transcription factors (i.e. Oct4, Sox2, Klf4, and c-Myc) provides a unique angle for studying the establishment of pluripotency. Although *Sox2* is not specific to pluripotent cells, previous studies support that its activation is essential for pluripotency establishment. Our results also showed that, of the 7 pluripotent factors tested individually in combination with polycistronic Klf4 and c-Myc, Sox2 has the highest efficiency in the generation of Oct4-GFP positive colonies, even higher than Oct4. iPSC lines can be successfully established from these Sox2/Klf4/c-Myc (SKM) colonies, and they meet a panel of criteria for pluripotency. To understand how pluripotency is initiated by SKM, we probed a spectrum of mechanisms represented by over 80 small molecules. Compared to Oct4/Klf4/c-Myc (OKM), SKM reprogramming responded differentially to several epigenetic and signaling small molecules, indicating distinct mechanisms in Sox2-driven reprogramming. To further understand how these mechanisms are correlated with the Sox2 initiation of global chromatic switch, we examined Sox2 binding sites across the genome at an early stage. Not surprisingly, our primary results showed that Sox2 binding at the promoter and genic regions are overrepresented. However, when compared to Sox2 binding in mature pluripotent stem cells, Sox2 prefers the intron regions rather than the coding exons in early SKM reprogramming (Coding exon: 0.4% vs 1.1%; Intron: 44.5% vs 38.6%). These observations may indicate that Sox2's function in initiating reprogramming relies on

direct gene activation by targeting promoters as well as chromatin re-organization by targeting the non-coding regions.

F2100

BET PROTEIN ANTAGONIST IS POTENT IN ELIMINATING RESIDUAL UNDIFFERENTIATED CELLS FROM HUMAN IPS-DERIVED CARDIOMYOCYTES IN SYNERGY WITH CDK INHIBITORS

Masuda, Shigeo, Miyagawa, Shigeru, Fukushima, Satsuki, Okimoto, Kaori, Tada, Chika, Ueda, Yumi, Kawaguchi, Kohei, Saito, Atsuhiro and Sawa, Yoshiki, Osaka University Graduate School of Medicine, Osaka, Japan

Clinical application of iPS-derived cardiomyocytes (iPS-CMs) is considered to be one of the most promising approach to regenerative treatment for severe heart failure. However, its success would largely depend on safety, including prevention of tumor formation (Masuda S, et al. Nature Rev Cardiol. 2014;11:553-4). Here, we demonstrate that BET protein bromodomain antagonist is efficacious in removing residual undifferentiated cells in vitro that express pluripotent markers such as Lin28 or TRA-1-60, while leaving human iPS-CMs intact. In this context, it was revealed that BET protein antagonist functions as a Nanog or Oct4 inhibitor within human iPS cells, leading to specific targeting of undifferentiated cells. Furthermore, co-treatment with BET protein antagonist and CDK inhibitors (CDK9 or CDK1 inhibitor) synergistically eliminated residual undifferentiated cells among human iPS-CMs. Although BET protein BRD4 is known to be involved in positive transcription elongation factor b (P-TEFb) complex consisting of CDK9, CYCLIN T, and BRD4, CDK9 inhibitor was shown not to function as a Nanog nor Oct4 inhibitor, suggesting that other mechanisms might be involved in this synergism. BET protein antagonist and CDK inhibitors used here are now investigated under Phase II trials for various cancers, and these findings imply that combination treatment in vitro with these drugs contributes to molecular-targeted treatment on "human iPS cells".

F2102

APPLICATION OF THE UNIQUE MICROFABRIC VESSELS EZSPHERE FOR EFFICIENT GENERATION, EXPANSION AND DIFFERENTIATION OF HUMAN IPS CELL AGGREGATES

Miwa, Tatsuaki, Sato, Hiroki, Alimujiang, Yidiresi and Kumagai, Hiromichi, ASAHI GLASS CO., LTD., Yokohama, Japan

For the early realization of regenerative medicine using human induced pluripotent stem cells (hiPSCs), it is necessary to develop large-scale, reproducible and low-cost stem cell culture techniques. In such direction, suspension culture of iPSCs as aggregates or embryoid bodies (EBs) has been known to be a effective method for expansion and differentiation processes. So far, we verified that the unique microfabric vessels "EZSPHERE", in which a large number of micro-wells are solely created on the plastic dishes or plates by laser beams and followed by coating with low-cell-attachment reagents, permit high-throughput generation of uniformly-sized EBs in high density. The diameter and depth of each micro-wells on the microfabric vessels can be easily and flexibly altered respectively around 200-1,400 μm and 100-400 μm by tuning exposure time or intensity of the laser beam. When hiPSCs dissociated to single cells were inoculated into a 35 mm dish-type EZSPHERE, on which approximately 2,400 micro-wells were created, over 2,000 uniformly-sized EBs were generated within only 3-6 hours. In addition, the size of EBs could be controlled by changing the inoculated cell number and/or size of the micro-wells. It was identified by culturing the EBs on the EZSPHERE with maintenance medium for several days that the size of each EB and the total cells number were increased significantly with keeping their undifferentiation state. Furthermore, to confirm pluripotency of the generated and expanded EBs on the EZSPHERE, a cardiomyocyte differentiation test was performed on them and as a result over 85% cTnT positive cardiomyocyte was obtained. In addition, it was also demonstrated by a neural differentiation assay that the EBs have a very high differentiation capacity to dopaminergic neuron on the same EZSPHERE that continuously used throughout from proliferation to differentiation by only changing medium. These results indicated that high-throughput generation and culture of EBs by using the novel microfabric vessels EZSPHERE have a high potential of application for not only basic or clinical research, but also for regenerative medicine. This study was performed as a part of the AMED (Japan Agency for Medical Research and Development) project "Research Center Network for Realization of Regenerative medicine.

F2104

ESTABLISHMENT OF COMPOUND SCREENING SYSTEM FOR TREATMENT OF DOWN SYNDROME-RELATED TRANSIENT ABNORMAL MYELOPOIESIS

Nishinaka-Arai, Yoko, Niwa, Akira, Osawa, Mitsujiro, Nakahata, Tatsutoshi and Saito, Megumu K., Center for iPS cell Research and application, Kyoto University, Kyoto, Japan

Transient abnormal myelopoiesis (TAM) is a transient status of the hematopoietic abnormality specific to newborns of Down syndrome (DS). Although TAM patients usually go into spontaneous remission, 20 to 30% of them subsequently develop acute megakaryoblastic leukemia

(AMKL). The mutation in GATA1 is found in all TAM blasts, while other genetic mutations were scarcely detected according to the previous genome wide screening. These data urged us to hypothesize that epigenetics may be involved in the TAM-pathogenesis and epigenetic modification could alleviate the abnormal hematopoiesis of TAM. To address this issue, we established strictly controlled induced pluripotent stem cell (iPSC) lines derived from a TAM patient. First, for the purpose of identifying affected hematopoietic lineages in vitro differentiation, we differentiated TAM-iPSCs into hematopoietic lineages in a step-wise manner and identified the abnormal phenotype in vitro; the frequency of the erythroid-committed CD71+CD235a+ cells from GATA1-mutated iPSCs were extremely lower than that from controls. Next, in order to find candidate compounds that can alleviate this abnormal phenotype in vitro, we established high-throughput hematopoietic differentiation system (HTS), in which percentage of erythroid-commited differentiation under multiple conditions can be determined at the same time in small scales (0.33+/-0.26% vs 74.0+/-7.1%, $p<0.01$). The coefficient of variation (CV) and Z'-factor in this HTS were 9.5% and 0.7, respectively, and these values satisfied the well-known criteria for the drug screening (CV ≦ 10% and Z'-factor > 0.5). As the first screening with this HTS, we applied 95 compounds known as epigenetic modifiers and identified 2 compounds (2.1% of total) that increased the frequency of erythroid-committed cells. We are now evaluating whether these compounds work in a dose-dependent manner or megakaryo-committed cells as the validation study. In conclusion, we specified in vitro phenotype of TAM and established the HTS to find out the compounds that can normalize this abnormal phenotype in differentiation. Considering the observation that some small-molecule epigenetic modifiers were positive in the screening so far, controlling the epigenetic status may be a promising strategy to treat TAM and subsequent AMKL.

F2106

MAKING MULTIPLE THERAPEUTIC CELL PRODUCTS FROM A CLINICALLY COMPLIANT iPSC LINE

Zeng, Xianmin[1], **Pei, Ying**[2], Cifuentes, Helen[1], Zhu, Jie[1], Ahmadian Baghbaderani, Behnam[3], Shi, Yichen[4], Lamba, Deepak[1] and Rao, Mahendra[5], [1]Buck Institute for Research on Aging, Novato, CA, U.S., [2]XCell Science Inc, Novato, CA, U.S., [3]Lonza, Walkersville, MD, U.S., [4]Axol Bioscience Ltd, Cambridge, U.K., [5]NxCell Inc, Novato, CA, U.S.

Induced pluripotent stem cell (iPSC) can differentiate into multiple phenotypes, and indeed multiple protocols for such differentiation have been established. Although the efficiency of different protocols is variable, most protocols work with most lines with some tweaking. To confirm this hypothesis and to make a clinically compliant line available for independent evaluation, we have taken an iPSC line developed with a clinically compliant protocol, and used a seed bank stock of the line to make ectodermal, mesodermal and endodermal materials from the same batch using standardized protocols. We have repeated this process with a reporter subclone of this line to show that such a subclone could be used to perform preclinical studies with a reasonable prediction of the behavior of the parental line, and lines made in a similar fashion. We suggest that this package of lines will be an invaluable resource for developing customized protocols for future use and will serve as a ready comparative material in evaluating competing protocols.

F2108

NOVEL CELL SURFACE MARKER FOR PURIFICATION OF HUMAN ESC/IPSC-DERIVED MIDBRAIN DOPAMINERGIC PROGENITORS

Samata, Bumpei, Doi, Daisuke and Takahashi, Jun, CiRA, Kyoto University, Kyoto, Japan

Human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) represent a promising source of midbrain dopaminergic (mDA) neurons to treat Parkinson's disease (PD). Current method using dual SMAD inhibition and GSK3B inhibition has enabled us to induce mDA neurons from human ESCs/iPSCs, but the differentiated cells are heterogeneous and may contain residual undifferentiated stem cells or proliferating neural progenitor cells, which may cause tumor formation. For a safe and efficient transplantation, mDA neurons derived from ventral midbrain (VM) need to be purified as donor cells. Therefore, this study is aimed to identify a specific cell surface marker to sort VM-derived mDA progenitors. In order to identify the VM cells, we first generated LMX1A::GFP, a midbrain marker, knock-in mouse ESC line and then performed cell sorting with antibody against CORIN, a floor plate marker. After maturation, CORIN$^+$LMX1A::GFP$^+$ cells expressed 10-fold more TH$^+$ DA neurons than unsorted the one. To find a novel cell surface marker of CORIN$^+$ LMX1A::GFP$^+$ cells, we next performed microarray analyses to compare gene expression profiles between the following cell populations: mouse ESC-derived CORIN$^+$LMX1A::GFP$^+$ cells vs. CORIN$^-$LMX1A::GFP$^+$ cells and CORIN$^+$ cells vs. CORIN$^-$ cells in the mouse fetal VM. We selected 5 candidate genes that coded a cell surface antigen and commonly up-regulated in ESC-derived CORIN$^+$LMX1A::GFP$^+$ cells and CORIN$^+$ cells in the fetal mouse VM. Finally, we validated the efficacy of the candidate genes using human ESCs/iPSCs. When we performed immunostaining at 14 days after sorting, hiPSC-derived the sorted cells expressed 4-fold more TH$^+$FOXA2$^+$ DA neurons than unsorted the one. After transplantation into PD rat models, human iPSC-derived the sorted cells survived and differentiated into mature DA neurons, resulting in significant improvement in motor behavior without tumor formation.

Thus, a novel cell surface marker for VM-derived mDA progenitors can provide a powerful tool for efficient and safe cell therapy for PD.

F2110

CRISPR/CAS9-MEDIATED GENE EDITING IN CELLS FROM ARGINASE-1 DEFICIENT PATIENTS

Sin, Yuan Yan, Richmond, Christopher, McCracken, Crystal and Funk, Colin, Queen's University, Kingston, ON, Canada

Arginase-1 deficiency is a rare autosomal recessive inborn error of metabolism that affects the liver-based urea cycle, leading to impaired ureagenesis. This disorder is caused by mutation(s) in the gene encoding liver-type arginase-1 (ARG1), resulting in partial or complete loss of enzyme function. ARG-1 deficient patients exhibit hyperargininemia with progressive neurological and intellectual impairments, persistent growth retardation, and sometimes with sporadic life-threatening hyperammonemic crises. Current treatments are primarily limited to pharmacologic agents and/or lifelong dietary regimen. No cure is currently available. Here, we utilize a gene-based strategy for ARG1 deficiency with the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs)/Cas9 system to target and modify specific DNA sequences at the disease-associated locus in combination with patient-derived induced pluripotent stem cells (iPSCs). Dermal fibroblasts obtained from three ARG-1 deficient patients were reprogrammed into iPSCs as a model to exploit CRISPR/Cas9-mediated gene editing. Through a selection-independent targeting approach, a short single-stranded oligodeoxynucleotide (ssODN) was introduced, along with CRISPR/Cas9 to initiate homology-directed repair (HDR)-mediated genetic alterations in patients' cells. The gene-edited cells were enriched through repeated rounds of clonal sib selection using digital droplet PCR for the detection of on-target HDR events. Our results show that CRISPR/Cas9 efficiently cleaves chromosomal DNA at the precise location (indel frequency ≈20%) and correction of a patient-specific mutation can be achieved by using ssODN as a donor template to initiate HDR (edited allelic frequency ≈10%). We have differentiated iPSCs to ASGPR-1^{+} immature hepatocyte-like cells and an optimized protocol to generate mature functional hepatocytes is being followed to confirm functional corrections. Collectively, we provide proof-of-concept for precision gene-editing in an isogenic iPSC-based model system for ARG1 deficiency that could facilitate the development of new therapeutic approaches, including but not limited to disorders amenable to hepatocyte targeting.

F2112

INVESTIGATION OF MOLECULAR MECHANISM UNDERLYING ENHANCED CHONDROGENESIS IN NEONATAL-ONSET MULTISYSTEM INFLAMMATORY DISEASE USING PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELLS

Tamaki, Sakura[1], Watanabe, Makoto[2,3], Yamamoto, Rie[2,4], Yoshitomi, Hiroyuki[1,4], Nishikomori, Ryuta[5] and Toguchida, Junya[1,4], [1]Department of Tissue Regeneration, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan, [2]Life Science Research Center, Technology Research Laboratory, Shimadzu Corporation, Kyoto, Japan, [3]Shimadzu Corporation, Kyoto, Japan, [4]Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [5]Department of Pediatrics, Graduate School of Medicine, Kyoto University, Kyoto, Japan, Kyoto, Japan

Neonatal-onset multisystem inflammatory disease (NOMID) is a dominantly inherited autoinflammatory disease caused by NLRP3 mutations and characterized by systemic inflammation and arthropathy. Mutant NLRP3 triggers the formation of the NLRP3 inflammasome irrespective of exogenous stimuli, which causes dysregulated interleukin-1β (IL-1β) secretion and uncontrolled multisystem inflammation. However, clinical and pathologic findings suggested that the arthropathy of NOMID patients is not inflammatory arthritis, but the abnormal growth of bone. In accordance with these findings, NOMID arthropathy is resistant to anti-IL1 therapy and may therefore occur via the NLRP3 inflammasome-independent mechanism. In this study, we investigated the effect of mutant NLRP3 on chondrocytes using patient-derived induced pluripotent stem cells (iPSCs). We established isogenic iPSCs with wild-type or mutant NLRP3 from NOMID patients with NLRP3 somatic mosaicism and the iPSCs were then differentiated into chondrocytes in vitro. Mutant iPSCs produced larger chondrocyte masses with extracellular matrix overproduction than wild-type iPSCs, which correlated with increased expression of the chondrocyte-related genes. The enhanced chondrogenesis was independent of caspase 1 and IL-1, and thus the NLRP3 inflammasome. To further investigate the molecular mechanism, we focused on the *SOX9* gene, which is a master regulator of chondrocyte proliferation and differentiation. Reporter assay revealed that the CREB/ATF-binding site was critical for *SOX9* overexpression in mutant pre-chondrocytes. This was supported by increased levels of cAMP and phosphorylated CREB in mutant clones. In summary, we successfully recapitulated arthropathy of NOMID patients in vitro using patient-derived iPSCs and showed that the enhanced chondrogenesis of mutant iPSCs oc-

curs via caspase1-independent cAMP/PKA/CREB pathway. We have been investigating the mechanism in detail, especially direct target of the mutant NLRP3, and will discuss about this issue.

F2114

MONOAMINERGIC REGULATION OF THE ACTIVITY OF HUMAN PLURIPOTENT STEM CELL-DERIVED NEURONS IN VITRO

Vadodaria, Krishna C., Fredlender, Callie, Dave, Sonia, Fung, Lianna, Li, Xinyi and Gage, Fred H., Salk Institute for Biological Studies, La Jolla, CA, U.S.

Monoamine neurotransmitters play important roles during neuronal maturation in vivo, and regulate a variety of neuronal processes in the brain. Dysfunction in monoamine neurotransmission has been proposed to play a role in neuropsychiatric disorders such as schizophrenia and major depression. Patient induced pluripotent stem cells (iPS)-derived neurons offer distinct ways for investigating the pathophysiology of such neurological and neuropsychiatric disorders in vitro. One aspect of establishing cellular model systems to study such disorders in vitro involves studying monoaminergic neurotransmission in human iPS-derived neurons in vitro. Utilizing calcium responsive dyes, we examine calcium transients and activity responses of pluripotent stem cell-derived human neurons in vitro. Here, we study the basal activity patterns and activity responses of human neurons to key monoamine neurotransmitters, such as serotonin and norepinephrine in vitro.

Funding Source: This work is supported by the Outgoing postdoctoral fellowship of the Swiss National Science Foundation, and Lynn and Edward Streim.

F2116

METABOLOMIC ANALYSIS-BASED IDENTIFICATION OF PREDICTIVE MARKERS FOR DIFFERENTIATION OF HUMAN IPS CELLS INTO CARTILAGE CELLS

Watanabe, Makoto[1], Yamamoto, Rie[1,2], Tamaki, Sakura[2,3], Ikeya, Makoto[4], Yoshitomi, Hiroyuki[2,3], Sato, Taka-Aki[1] and Toguchida, Junya[5,6], [1]Life Science Research Center, Technology Research Laboratory, Shimadzu Corporation, Kyoto, Japan, [2]Department of Cell Growth and Differentiation, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [3]Department of Tissue Regeneration, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan, [4]Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan, [5]DeInstitute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan, [6]Inst for Frontier Medical Sciences Kyoto Univ, Kyoto, Japan

Induced pluripotent stem cells (iPSCs) are expected to contribute greatly to regenerative medicine and disease treatment. There are, however, several issues to be resolved, one of which is their heterogeneity, in particular of differentiation potential. In previous our studies for chondrogenic differentiation through the neural crest cell (NCC) lineage, we used CD271^{high+} as a maker for ectomesenchymal cells, which showed long-term chondrogenic differentiation property. Therefore induction of CD271 $^{high+}$ population is a crucial step for chondrogenic differentiation. However, it is currently impossible to predict which iPSCs can be efficiently differentiated into CD271^{high+} NCCs before the induction. Biomarkers to predict such differentaiton property can be used to select proper iPSC clones for NCC induction. Among possible biomarkers, we focused on metabolome in this study. At first we investigated the induction efficiency of five human iPSC clones and found that three out of 5 clones showed efficient induction property (more than 40%, good clones), while the efficiency was below 15% in the remaining two clones (poor clones). Then we obtained metabolomic profiles for both cell extracts (CE) and their culture medium (CM) of these clones. Approximately 180 and 210 metabolites were observed in CE and CM, respectively. Among them, approximately 80 metabolites were commonly detected in both CE and CM, and multivariate analyses identified a set of metabolites, of which the amount was significantly different between good and poor clones. There are intra- or intercellular metabolites that were associated with the induction of CD271^{high+} NCC from iPSCs. As a conclusion, these data suggested that the properties of iPSCs for the chondrogenic differentiation through NCC can be predicated by metabolome. In particular, the metabolites detected in CM may be excellent candidates for non-invasive biomarkers. In the future,

we will apply these data to investigate whether we can prospectively predict the differentiation property of other iPSC clones and furthermore to identify key molecules involved in differentiation toward the chondrocyte lineage.

F2118

PRACTICAL METHOD FOR THE GENERATION OF IPS CELLS FROM HUMAN PERIPHERAL BLOOD

Yang, Han-Mo[1], Kim, Ju-Young[2], Lee, Joo-Eun[2], Kim, Joon Oh[2], Cho, Hyun-Jai[3], Kwon, Yoo-Wook[4], Hur, Jin[5], Park, Young-Bae[5] and Kim, Hyo-Soo[3], [1]Seoul National University Hospital, Seoul, Korea, South, [2]Seoul National University Hospital, Biomedical Research Institute, Seoul, Korea, South, [3]Seoul National University Hospital, Seoul, Korea, South, [4]Seoul National University Hospital Seoul, Korea, South, [5]Seoul National University Hospital Division of Cardiology Department of Internal Medicine, Seoul, Korea, South

Among methods for the generation of induced pluripotent stem(iPS) cells, the most important issue for clinical practice is cell source. Human peripheral blood is one of the easy-obtainable cell sources. However, the generation of iPS cells from fresh peripheral blood has shown low gene transfection efficiency and inconveniences requiring specific methods to isolate. Here, we investigate a novel population of peripheral blood-derived stem cells, which can be easily reprogrammable to iPS cells. We isolated and cultured peripheral mononuclear cells (PBMC) from human peripheral blood on the non-coated plates. We observed adherent cells from as early as three days after the culture and those cells gradually formed colonies. We could isolate these cells with very high efficiency. Moreover, we have also confirmed that these cells can be differentiated into osteogenic, adipogenic, and myogenic-lineage cells. We succeeded in generating iPS cells with these cells. Our method with these cells showed enhanced efficiency of gene transduction, compared to that with the human dermal fibroblast. We obtained reprogrammed colonies in seven days after 4 factor virus transduction without feeder cells. We identified our iPS cells 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 than90 iPS cell lines from PBMC of patients with diverse diseases and normal volunteers. Our studies showed new method to isolate stem cells from human peripheral blood and to generate iPS cells with high efficacy. This result suggests that our new method could be used for clinical application of iPS cells in the upcoming future.

F2120

THE IMPACT OF INFLAMMAGING, A KEY HALLMARK OF AGING, ON iPSC REPROGRAMMING.

Mahmoudi, Salah, Mancini, Elena and Brunet, Anne, Stanford University, Stanford, CA, U.S.

Aging is characterized by a general functional decline of the whole organism, and is associated with a marked increase in a number of diseases, including neurodegenerative and cardiovascular diseases, metabolic disorders, and cancer. A key aspect of aging, which is also shared across many age-related diseases, is a chronic inflammatory state. ‘Inflammaging’ refers to the chronic, low-grade, systemic inflammation that accompanies aging, and is characterized by changes in intercellular communication and increased circulatory levels of inflammatory cytokines. Reprogramming of adult somatic cells into embryonic-like induced pluripotent stem cells (iPSCs) holds great promise for the study, and potential treatment, of aging and age-related diseases. However, a better understanding of the impact of age on iPSC generation and quality is crucial to harness the full therapeutic potential of these cells. To date, little is known about how age-dependent changes in intercellular communication, which includes increased secretion of inflammatory cytokines, impact the quality and generation of iPSCs. Using a systematic high-throughput approach, we show that fibroblasts derived from chronologically old mice and cultured *in vitro*, still maintain inflammaging features associated with their *in vivo* milieu. The old cells secrete more inflammatory cytokines, and have distinct transcriptomic and metabolic profiles compared to their young counterparts, but that these inflammaging features are erased in the resulting iPSCs. However, we show that this age-dependent inflammatory state impact the transition of somatic cells into pluripotent stem cells, manifesting itself as increased variability in reprogramming efficiency as a function of age. Furthermore, we identify a number of naturally secreted cytokines that play a role in the reprogramming process, including IL6, IL1b and TNFa, and which may contribute to the observed variability in reprogramming with age. This study is the first to examine the contribution of inflammaging, which is a key hallmark of aging, to IPSC reprogramming, and could help us to identify novel approaches to improve iPSC generation and quality using cells from old individuals.

F2122

IN VITRO DIFFERENTIATION OF T CELLS FROM HUMAN ES CELL AND INDUCED PLURIPOTENT STEM CELL WITH FEEDER- AND FBS-FREE CULTURE CONDITION.

Yasui, Yutaka[1], Iriguchi, Shoichi[1], Nakauchi, Hiromitsu[2] and Kaneko, Shin[1], [1]Center for iPS cell Research and Application (CiRA), Kyoto University, Kyoto, Japan, Kyoto, Japan, [2]Institute of Medical Science, University of Tokyo, Tokyo, Japan

iPS cell technologies are progressing for clinical research and regenerative medicine. Mouse feeder cells, FBS, and other animal components should be eliminated from culture condition for safe and stable clinical cell processing. However, a lot of culture protocols to obtain terminally differentiated cells from iPS cells still contain animal serum and/or murine feeder cells and so on.

We have previously reported that human iPS cells derived from peripheral T cells (T-iPS cells) were efficiently differentiated into T-lineage cells without using mouse feeder cells and FBS. T-iPS cells had genetically rearranged T cell receptor (TCR) in their genome and differentiated more efficiently into T-lineage cells on OP9-DLL1 feeder cells than normal human iPS cells did. Differentiated T cells from T-iPS cells without feeder cells and FBS were almost identical to the T cells differentiated on OP9-DLL1 feeder cell. On the other hand, whether normal human iPS cells and/or ES cells could be differentiated into T cells without feeder cells and FBS have not been elucidated.

We used Fc flagged DLL4 protein coated plates instead of a murine stromal feeder cell line expressing Notch ligand; OP9/delta like 1, to induce T-lineage cells. Specifically, normal human iPS cells and ES cells were initially differentiated to hematopoietic cells via embryoid body under feeder cell- and serum-free conditions. Then, the resulting hematopoietic cells were transferred onto a Fc-DLL4 coated plate and further cultured with thymopoietic cytokines. Few weeks later, differentiating cells were confirmed to express both CD4 and CD8, and other T cell markers by flow-cytometry. Besides, the cells were confirmed to have gene expression profiles similar to T cells. Thus, we have confirmed the feeder- and FBS-free culture conditions were applicable for T cell processing from normal human iPS cells and ES cells.

F2124

DEVELOPMENT OF NOVEL SMALL MOLECULE AUTOPHAGY INDUCERS FOR TREATMENT OF ALS

Goyal, Piyush[1], Shah, Kevan[2], Mount, Elliot[2], Pleiss, Mike[2], Greenhouse, Robert[3], Javaherian, Ashkan[2] and Finkbeiner, Steve[2,4], [1]Gladstone, San Francisco, CA, U.S., [2]J. David Gladstone Institutes, San Francisco, CA, U.S., [3]Nanosyn Inc, Santa Clara, CA, U.S., [4]University of California San Francisco School of Medicine, San Francisco, CA, U.S.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that primarily affects motor neurons. It results in paralysis and loss of control of vital functions, such as speech, swallowing and breathing, leading to premature death. Life expectancy of ALS patients averages 2–5 years from diagnosis. Riluzole is the only FDA-approved drug to treat ALS, and it prolongs life only a few months. Mutations in a number of genes including SOD1, TDP43, and FUS can cause familial ALS. Furthermore, mislocalization and aggregation of wildtype TDP43 has been observed in motor neurons from nearly all sporadic ALS patients. It has been suggested that in ALS and other neurodegenerative diseases, misfolded proteins cause cellular dysfunction that leads to neurodegeneration. Autophagy is a cellular mechanism by which misfolded proteins, toxic protein aggregates, and injured organelles are directed to the lysosome to be degraded and recycled as nutrients to the cell. Therefore drugs that can induce autophagy in neurons could be beneficial for ALS and neurodegenerative diseases in general. We have invented a novel automated robotic microscopy and single-cell longitudinal imaging platform to investigate neurodegeneration in iPSC-derived motor neurons from ALS patients. We utilized our imaging system to create HTS-amenable patient iPSC-derived cellular models for ALS and test libraries of novel small molecules with therapeutic potential. We designed a series of novel small molecule autophagy inducers based on a previously discovered pharmacophore and carried out structure-activity relationship studies to identify lead compounds that can induce autophagy in neurons and rescue neurodegeneration in patient-derived cellular models of ALS. These molecules are blood brain penetrant and could be developed as therapeutics for ALS and other neurodegenerative diseases.

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F2126

ACCESSING POPULATION BIOLOGY THROUGH LARGE SCALE GENERATION OF HUMAN iPSCs FROM DISEASE COHORTS

Jones, Eugenia[1], Mack, Amanda[2], Novak, Thomas[1], Broeckel, Ulrich[3], McMahon, Chris[1] and Burke, Thomas J.[4], [1]Cellular Dynamics, Madison, WI, USA, [2]Cellular Dynamics International, Inc- a Fujifilm company, Madison, WI, USA, [3]Medical College of Wisconsin, Milwaukee, WI, USA, [4]Cellular Dynamics International, Madison, WI, USA

Induced pluripotent stem cell (iPSC) technology brings undisputed value to understanding disease pathology and therapeutic intervention/prevention. Large scale reprogramming efforts are building on this value by moving from individual examples of iPSC-based disease recapitulations to population-based investigations. Cellular Dynamics International (CDI) has pioneered these efforts by developing processes to generate iPSC lines from over 3200 donors from control and disease cohorts through the initiatives of the US-National Heart, Lung, Blood Institute (NHLBI) and The California Institute of Regenerative Medicine (CIRM). The industrialized processes developed at CDI are meeting the demands of these initiatives where, to date over 1150 samples have been reprogrammed collectively from both programs with the remaining ~2100 samples from CIRM on schedule to be reprogrammed before the end of the initiative (December 2016). The first 300 iPSC lines from the CIRM effort have been deposited into the Coriell Institute as of September 1, 2015 and the iPSCs from the NHLBI initiative will be deposited into WiCell Research Institute. Both repositories will provide iPSCs and links to varying degrees of relevant descriptive and clinical information. In addition to reprogramming and banking, CDI has also generated cardiomyocytes from over 230 of the NHLBI-sourced donors for use with in-vitro investigations. Ultimately, the lessons learned from these large scale reprogramming and differentiation efforts and the availability of the iPSC material will aid in future population-based reprogramming efforts, investigations of disease etiology, and potential therapies.

IPS CELLS: DIRECTED DIFFERENTIATION

F2128

INVESTIGATING THE INFLUENCE OF PARENTAL CELL TYPE ON DIFFERENTIATION POTENTIAL OF MOUSE AND HUMAN INDUCED PLURIPOTENT STEM CELLS TO RENAL PROGENITORS

Chow, Theresa[1], Whiteley, Jennifer[2], Monetti, Claudio[1], Nagy, Andras[1] and **Rogers, Ian**[1], [1]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, ON, Canada, [2]Mount Sinai Hospital, Toronto, ON, Canada

Currently, the only effective treatment for end-stage kidney disease is kidney transplantation. However, this curative treatment is not available for many patients due to the lack of genetically-matched donors. Cell therapies are promising treatment options but the challenges that impede the development of an effective kidney cell therapy include identification and production of patient-matched renal progenitors capable of integrating into and restoring function to damaged regions. To understand the influence of transcriptional and epigenetic memory on the differentiation potential of iPSC to renal progenitors, we sought to determine the differentiation propensity of proximal tubule (PT)-, blood- and fibroblast-iPSC and the functionality of renal progenitors produced from each iPSC type. *Mouse model*: iPSC from PT, CD3+ T cells and tail tip fibroblast were established from a novel doxycycline-inducible, secondary reprogramming system, OKMS-250 mouseline, developed by Dr. Andras Nagy. To direct the differentiation of these iPSC lines to renal progenitors, we developed a protocol to sequentially differentiation pluripotent stem cells T+ mesoderm, Pax2+ intermediate mesoderm and Six2+ renal progenitors at efficiencies of approximately 80%, 80% and 15%, respectively. We are now applying this differentiation protocol to the PT-, T cell-, and fibroblast-iPSC. *Human model*: We are establishing iPSC lines from urine, peripheral blood and skin fibroblast collected from the same healthy donor using the integration-free, episomal reprogramming system. Similar to the mouse model, we have developed a human protocol to direct the differentiation of pluripotent stem cells to T+ mesoderm, PAX2+ intermediate mesoderm and SIX2+ renal progenitors at efficiencies of approximately 90%, 90% and 30%, respectively. Once the urine-, blood-, and skin fibroblast-iPSC are established, we will apply this protocol to the different lines. Results from our study will 1) help us better understand the influence of transcriptional and epigenetic memory on iPSC differentiation to renal lineages, and 2) help identify the best cell source for generating patient-specific iPSC for kidney cell therapy.

F2130

DERIVATION OF NATURAL KILLER CELLS FROM HUMAN INDUCED PLURIPOTENT STEM CELLS FOR UNIVERSAL, OFF-THE-SHELF TUMOR IMMUNOTHERAPY

Bjordahl, Ryan[1], Clarke, Raedun Laurie[2], Groff, Brian[2], Rogers, Paul[2], Moreno, Stacey[2], Abujarour, Ramzey J.[2], Robinson, Megan[2], Kim, William[2], Huang, Xiaosong[2], Robbins, Dave[2], Rezner, Betsy[2], Abbot, Stewart[2], Shoemaker, Daniel[2] and Valamehr, Bahram[2], [1]Fate Therapeutics, San Diego, CA, U.S., [2]Fate Therapeutics, Inc., San Diego, CA, U.S.

In comparison to other lymphocytes, natural killer (NK) cells are unique in their capability to elicit tumoricidal responses without prior sensitization and have shown considerable promise as an adoptive cellular therapy. Unlike native T cells, in which tumoricidal activation is restricted by major histocompatibility complex (MHC)-mediated peptide presentation, NK cells directly recognize malignant cells through a combination of the presence of activating and absence of inhibitory receptor ligands, in an essentially MHC unrestricted manner. Clinical data from bone marrow transplant and allogeneic NK immunotherapy suggest that MHC mismatch is advantageous in driving anti-tumor effects of NK cells, promoting enhanced NK cell recognition of tumor cells. Were it not for the limited in vitro expansion of NK cells they could be considered an ideal candidate for a universal, "off-the-shelf" cellular immunotherapy product. The ability to generate NK cells from continuous human induced pluripotent stem cell (hiPSC) lines could overcome the limited expansion of traditional sources of NK cells. While their differentiation from hiPSC has been demonstrated, the scalability and robustness of this process has been limited by skewed development towards primitive hematopoiesis and the cumbersome use of embryoid bodies (EBs). Here we extend our reported ability to drive definitive hematopoiesis in hiPSC differentiation to demonstrate the robust and highly scalable generation of functionally mature NK cells without the need for EBs in both stroma-dependent and stroma-free culture. Our hiPSC-derived NK cells are phenotypically and functionally mature (including expression of CD16, KIR3DL1, NCRs and CD94), exhibit the canonical NK functions of tumor cytotoxicity and production of effector cytokines and are functional in vivo mediating both direct and ADCC-mediated killing of tumor targets. Furthermore, genetic modifications made at the clonal hiPSC-level are active in differentiated NK cells, including inducible suicide genes and constitutive expression of targeting modalities for enhance tumor recognition. In summary, this study demonstrates the application of a scalable, genetically tractable hiPSC differentiation platform for the development of "off-the-shelf" NK cell-based immunotherapeutics.

F2132

QUALITY CONTROL OF IPSC-DERIVED DOPAMINERGIC CELL GRAFTS FOR PARKINSON'S DISEASE PATIENTS

Doi, Daisuke, Kikuchi, Tetsuhiro, Morizane, Asuka and Takahashi, Jun, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

We are going to start a clinical trial in Japan, which is allo-transplantation of iPSC-derived dopaminergic progenitors (DAPs) to Parkinson's disease patients, mainly to check their safety. In the clinical trial, we plan to use the "Stock iPSCs" established in the cell processing center in CiRA (Center for iPS cell Research and Application), to differentiate them to DAPs as donor cells, and to transplant to the patients as allo-transplantation. "Stock iPSC" is established from peripheral blood of a HLA-homozygous healthy volunteer donor, which covers about 17% of Japanese population. The master cell bank and working cell bank derived from the stock iPSCs have been already made. The differentiation protocol of DAPs from iPSCs was already fixed, and we set the quality checkpoints of DAPs, first in iPSC state and second in the post-sorting, and third in the final product. In these checkpoints we set the benchmarks based by the results of non-clinical (rats and monkeys) study for the safety and the efficacy of DAPs. In this poster, I discuss about the quality control of iPSC-derived cell products.

F2134

GENERATION OF SPECIFIC NEURONAL SUBTYPES FROM HUMAN PLURIPOTENT STEM CELLS

Imaizumi, Kent[1], Sone, Takefumi[1], Akamatsu, Wado[1,2] and Okano, Hideyuki[1], [1]Department of Physiology, Keio University School of Medicine, Tokyo, Japan, [2]Center for Genomic and Regenerative Medicine, Juntendo University School of Medicine, Tokyo, Japan

Disease-specific human pluripotent stem cells (PSCs) have great potential to explore the mechanisms of neurological diseases and to perform drug discovery. However, it is currently difficult to model neurological diseases that affect various neuronal subtypes because not all subtypes can be induced from PSCs. To overcome this issue, we here established a culture system to induce any desired neuronal subtypes based on the identical protocol. We controlled the regional identity of PSC-derived neural progenitors along the anteroposterior (A-P) and dorsoventral (D-V) axes by combinatory treatment of various patterning factors. Using this system, we were able to induce specific neuronal subtypes, including cortical projection neurons, cortical interneurons, cerebellar Purkinje neurons, midbrain dopaminergic neurons, hindbrain sero-

tonergic neurons, spinal cord sensory interneurons, and spinal cord motor neurons. Furthermore, we reproduced subtype-specific phenotypes of amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD) by comparing various subtypes induced by this method. This culture system will facilitate highly precise neurological disease modeling using PSCs.

F2136

CREB3L1 IS NECESSARY FOR HUMAN BONE DEVELOPMENT AS EVIDENCED BY MUTATIONS THAT CAUSE OSTEOGENESIS IMPERFECTA

Keller, Rachel B, Tran, Thao T and Byers, MD, Peter H, University Of Washington, Seattle, WA, U.S.

Insufficiency or defectiveness of type I collagen is the cause of osteogenesis imperfecta (OI), an inherited disorder characterized by abnormal bone development with an increased lifetime risk of fractures. Exome sequencing of a family presenting with OI phenotypes ranging from mild with few fractures to prenatal lethality revealed a mutation in CREB3L1, encoding a basic leucine zipper (bZIP) transcription factor. CREB3L1 has been reported once before in association with OI but the disease mechanism is unclear. Collagen proteins are quite large and their transport from the endoplasmic reticulum (ER) to the Golgi for packaging and eventual secretion is achieved using specialized secretory machinery. A closely related protein, CREB3L2, has a role in regulating one such pathway in chondrocytes and is necessary for type II collagen secretion and proper cartilage formation. Impaired functionality of CREB3L1 due to mutation may perturb the same or a related pathway in osteoblasts and lead to reduced type I collagen in bone, explaining the OI. There is also evidence that CREB3L1 operates in osteoblast-specific physiological ER stress pathways that may contribute to the phenotype. Fibroblasts from the proband are available but CREB3L1 is not expressed in skin. CREB3L1-associated OI was modeled using induced pluripotent stem cell (iPSC)-derived osteoblasts from patient cells bearing the family deletion. This model will be used to look for evidence of the impaired secretion that is hypothesized and to elucidate the role of CREB3L1 in various pathways that operate during bone development. The model will serve as confirmation of CREB3L1 as an OI disease gene and potentially as a tool for design of new drugs or treatments for brittle bone disease.

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F2138

FUNCTIONAL IMPROVEMENT OF MESENCHYMAL STEM CELLS THROUGH DIRECT DIFFERENTIATION OF HUMAN iPSC WITH p38 INHIBITOR TREATMENT

Lee, Seung Bum, Shin, Hye-Yun, Han, Sung-Hoon, Kim, Min-Jung, Shim, Sehwan, Seo, Seong-Won, Seong, Ki Moon, Jang, Won-Suk, Lee, Sun-Joo, Jin, Young-Woo, Lee, Seung-Sook and Park, Sunhoo, Lab. of Radiation Exposure & Therapeutics, National Radiation Emergency Medical Center,Korea Institute of Radiological & Medical Science, Seoul, Korea

Human mesenchymal stem cells (hMSC) have proven therapeutic efficacy in treating various diseases and acute radiation syndrome (ARS), but clinical application with adult MSCs from the elderly and patients are limited by their poor self-renewal potential and functional competency. In this study, we generated the functionally improved MSC from human induced pluripotetn stem cells (hiPSC) by transient treatment of p38 inhibitor. Treatment with or without p38 inhibitior show fibroblast-like morphology and expression of hMSC surface antigen by FACS analysis, whereas transient treatment of p38 inhibitor during differentiation of iPSC derived-MSC (iMSC) displayed the superior colony forming unit (CFU) compared to non-treatment of p38 inhibitor, indicating that iMSC (p38 inhibitor +) retain higher self-renewal. Furthermore, iMSC generated by p38 inhibitor treatment showed the more efficient differentiation capacity into mesodermal lineage cell types (adipocytes, osteocytes and chondrocytes) than iMSC derived from non-treatment of p38 inhibitor, even than human umbilical cord blood-derived MSC. Therefore, our finding suggested that transient treatment of p38 inhibitor induce more functionally improved MSC derived from hiPSC.

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F2140

ENHANCED LYMPHOCYTE GENERATION FROM T-iPSC BY COORDINATED MANIPULATION OF WNT AND TGF-BETA SIGNALING

Patel, Dharmeshkumar[1], Webber, Beau R.[1], Dunmire, Samantha K[1], Stefanski, Heather[1], Osborn, Mark J.[1], Eide, Cindy[1], Tolar, Jakub[1,2] and Blazar, Bruce R.[1], [1]University of Minnesota, Minneapolis, MN, U.S., [2]Stem Cell Institute, University of Minnesota, Minneapolis, MN, U.S.

Derivation of T-lymphocytes from human pluripotent stem cells (PSC) is an inefficient process that is limited by the inherent bias of PSC toward primitive hematopoiesis in vitro. While others have demonstrated that inhibition of Activin/TGF-beta signaling or augmentation of Wnt signaling individually can enhance definitive hematopoietic development and subsequent T-lymphocyte specification, we hypothesized that concurrent manipulation of both pathways would have a synergistic effect on this process. We found that combinatorial modulation of Wnt and TGF-beta signaling during the mesoderm phase of development enhances lymphocyte generation from T-cell derived iPSC (in comparison to modulation of either pathway alone). Using both a Wnt signaling agonist (CHIR 99021) and a TGF beta signaling inhibitor (SB 431542) we observed a ~10 fold increase in $CD45^+$ (~97%) and $CD7^+/CD3^+$ (~43%) population, and a ~9 fold increase in $CD5^+/CD7^+$ (~27%) and $CD3^+/CD5^+$ (~16%) T lymphocyte progenitors at day 16 of differentiation compared to CHIR 99021 alone. We show that in vitro generated T-cells are functionally active and proliferate (proliferation index ~3 at day 10) upon stimulation with anti CD3/28 beads and IL-2. In addition, in vitro generated T-cells from T-iPSC have cytolytic activity as evidenced by the presence of cytolytic granules and the expression of Perforin (~32%), Granzyme A (~58%), Granzyme B (~18%) and CD56 (~36%). Our finding that combined modulation of Activin/TGF-beta and Wnt signaling enhances definitive hematopoiesis and subsequent specification to T-lymphocytes from iPSC should foster efforts toward investigating molecular mechanisms for human T-lymphocyte development, scalable production of blood cells in vitro, as well as the development of iPSC-derived T-cell based immunotherapies.

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F2142

DIFFERENTIATION OF HUMAN INDUCED PLURIPOTENT STEM CELLS TO MAMMARY EPITHELIAL-LIKE CELLS

Resch, Zachary, Gray, Catherine, Roy, Bhaskar, Stelzig, Kimberly, Nelson, Timothy J. and Wigle, Dennis, Mayo Clinic, Rochester, MN, U.S.

Methods describing the differentiation of induced pluripotent stem cells (iPSC) to various tissues found in the human breast have been limited to date. Mammary epithelial-like cells derived from iPSC (iPSC-MEC) could serve as a valuable resource in many research activities, in particular studies of tumor initiation and therapeutic response. We enlisted a novel iPSC-MEC differentiation methodology to differentiate human iPSCs from females aged 32 to 48 (n=7) with no known history of breast cancer. Cells were maintained in serum- and feeder-free conditions and maintained on Geltrex-coated plates throughout the protocol. iPSCs underwent ectodermal differentiation for 4 days prior to a 28 day, MEC-specific differentiation protocol, which uses a serum-free media base with the addition of a combination of growth factors and tissue extracts. Cells were assayed at weekly intervals during the differentiation for the expression of representative MEC-related markers (Cytokeratins 14, 18, 19 as well as vimentin, MUC1, EpCam, Int6, CD10) by flow cytometry and immunohistochemistry, and compared to commercially available primary human MEC lines. Additionally, at the completion of differentiation, iPSC-MECs were assayed for their ability to form mammospheres. iPSC-MEC were found to express higher levels of luminal MEC-specific markers (Cytokeratin 18 and 19, MUC1) when compared to the primary human MEC which express medial MEC specific markers (Cytokeratin 14, Int6, and vimentin). Differentiated cells also formed mammospheres when cultured in ultra-low cell binding plates. These initial studies suggest we have developed a novel method of generating MEC-like cells from iPSCs using a two-step, monolayer process under serum-free conditions. In the future, we aim to further examine our ability to manipulate the differentiation of iPSCs to luminal or medial iPSC-MEC fates. We will continue screening the Biotrust collection of over 250 patient-derived iPSC lines for individuals based on their genetic background that may be susceptible to breast cancer, and look for factors that induce tumorigenesis and/or chemoprevention agents that may prevent induced or spontaneous oncogenesis.

F2144

EARLY DEVELOPMENTAL PERTURBATIONS IN A HUMAN STEM CELL MODEL OF MODY5/ HNF1B PANCREATIC HYPOPLASIA

Teo, Adrian Kee Keong[1], Lau, Hwee Hui[2], Valdez, Ivan[3], Dirice, Ercument[3], Tjora, Erling[4], Raeder, Helge[4] and Kulkarni, Rohit[3], [1]Institute of Molecular and Cell Biology, Singapore, Singapore, [2]Institute of Molecular and Cell Biology (IMCB), A*STAR, Singapore, Singapore, [3]Joslin Diabetes Center, Boston, MA, U.S., [4]Haukeland University Hospital, Bergen, Norway

Patients with a HNF1B$^{S148L/+}$ mutation (MODY5) typically exhibit pancreatic hypoplasia. However, the molecular mechanisms are unknown due to the inaccessibility of patient material and because mouse models do not fully recapitulate MODY5. Here we differentiated MODY5-human induced pluripotent stem cells (hiPSCs) into pancreatic progenitors and show that the HNF1B$^{S148L/+}$ mutation causes a compensatory increase in several pancreatic transcription factors, and surprisingly, a decrease in *PAX6* pancreatic gene expression. The lack of suppression of PDX1, PTF1A, GATA4 and GATA6 indicates that MODY5-mediated pancreatic hypoplasia is mechanistically-independent. Overexpression studies demonstrate that a compensatory increase in PDX1 gene expression is due to mutant HNF1B$^{S148L/+}$ but not wild-type HNF1B or HNF1A. Further, HNF1B does not appear to directly regulate PAX6 gene expression necessary for glucose tolerance. Our results demonstrate compensatory mechanisms in the pancreatic transcription factor network due to mutant HNF1B$^{S148L/+}$ protein. Thus, patients typically develop MODY5 but not neonatal diabetes despite exhibiting pancreatic hypoplasia.

F2146

LONG-TERM CULTURE OF ALVEOLAR EPITHELIAL TYPE 2 CELLS DERIVED FROM HUMA INDUCED PLURIPOTENT STEM CELLS

Yamamoto, Yuki, Gotoh, Shimpei, Korogi, Yohei, Konishi, Satoshi, Nagasaki, Tadao, Matsumoto, Hisako, Muro, Shigeo, Hirai, Toyohiro, Ito, Isao and Mishima, Michiaki, Kyoto University, Kyoto, Japan

Alveolar epithelial type 2 (AT2) cells produce pulmonary surfactant and maintain lung homeostasis by self-renewal and differentiating into alveolar type 1 (AT1) cells as tissue stem cells. Their dysfunction is associated with various life-threatening lung diseases, such as COPD, pulmonary fibrosis and lung cancer. The use of human AT2 cells for elucidating their pathogenesis has been quite restricted by limited availability of primary cells and difficulty in expansion. Although human induced pluripotent stem cell (hiPSC) technologies have been expected to overcome the limitation, efficient induction of AT2 cells from hiPSCs and their expansion have been difficult. In this study, ventral anterior foregut endoderm cells (VAFECs) were induced from hiPSCs stepwisely in 2D culture. Following treatment with distal lung specification medium, CPM$^+$NKX2.1$^+$ distal lung progenitor cells were isolated and co-cultured with fibroblasts in 3D Matrigel. After two weeks of 3D co-culture, SFTPC$^+$ hiAT2 cells were induced with an efficiency of 50% in spheroid structures. Immunocytochemisty revealed that other AT2 cell markers such as SFTPA, SFTPB, SFTPD, and DCLAMP were co-stained with SFTPC$^+$ cells. Lamellar bodies, which are the specific organelle of AT2 cells, were identified in spheroids and isolated hiAT2 cells by transmission electron microscopy. By microarray analysis, gene expression pattern of isolated hiAT2 cells was compatible with that of AT2 cells. Next, passaging of isolated hiAT2 cells was repeated in the 3D co-culture system. hiAT2 cells were expandable with keeping their gene expression pattern for at least ten weeks. In conclusion, we established the method for efficient induction of hiAT2 cells as well as their long-term expansion of hiAT2 cells. These methods might break through the limitation of the research for human AT2 cells enabling disease modeling, toxicologic studies, and regenerative medicine.

IPS CELLS: EPIGENETICS

F2150

UNDERSTANDING THE ROLE OF X-CHROMOSOME ACTIVATION PROCESS IN HUMAN PLURIPOTNET STEM CELLS

Ghazvini, Mehrnaz, Erasmus Medical center, ROTTERDAM, Netherlands

Understanding the role of X-linked activators of X-chromosome inactivation process in human Pluripotent Stem Cells

Mehrnaz Ghazvini, Tracy Li & Joost Gribnau

Developmental Biology department, Erasmus Medical Center, Rotterdam, The Netherlands

X-chromosome inactivation (XCI) is an epigenetic process utilized by eutherian female cells to compensate for potential dosage differences of X chromosomal genes between female XX and male XY cells (Lyon, 1961). XCI regulation has been extensively studied during mouse development and in female mouse embryonic stem cells (ESCs). These cells, derived from the inner cell mass (ICM) of the blastocyst, contain two active X chromosomes (Xa) and undergo XCI upon in vitro differentiation. The non-coding Xist RNA is crucial for XCI and becomes up-regulated upon differentiation of mouse ESCs. Xist coats the future Xi attracting chromatin remodeling enzymes which infer the transcriptional shut-down of the Xi (Barakat and Gribnau, 2012). Initiation of XCI is directed by X-linked

activators and autosomaly encoded inhibitors of the XCI process. Several components of this complex regulatory network directing XCI are conserved between mouse and human, but many questions regarding their role in human XCI remain unanswered. Here, we have studied the role of factors, including RNF12, REX1 and other putative regulators, in human XCI, using human pluripotent stem cells (PSCs) as a model system applying the CRISPER/Cas9 technology to perform loss of function studies.

F2152

EPIGENETIC VARIATIONS AT IMPRINTING CONTROL REGIONS ARE DEPENDENT ON CLONE AND GENE LOCI BUT NOT ON PLURIPOTENT STATES

Yagi, Masaki, Kyoto University Center for iPS cell research and application, Kyoto, Japan

Induced pluripotent stem cells (iPSCs) can be generated by enforced expression of transcription factors such as Oct3/4, Sox2, Klf4 and c-Myc in somatic cells. Dynamic epigenetic alterations occur during the reprogramming process. In order to achieve medical applications using iPSCs, it is important to understand epigenetic variations of iPSCs, which potentially affect characteristics of iPSCs. Here, we examined DNA methylation status at H19 differentially methylated region (DMR) and Peg3 DMR in mouse pluripotent stem cells. We have established ESCs and iPSCs derived from F1 embryos between 129/Sv and MSM/Ms genetic background to discriminate parental alleles by SNPs. We found that Igf2 is transcribed from both alleles in some ESC and iPSC clones, which is accompanied by the altered DNA methylation at H19 DMR. However, loss of imprinting at H19 DMR was not correlated with the expression level of Nanog. In contrast to H19 DMR, DNA methylation status at Peg3DMR was maintained in most ESCs and iPSCs. Furthermore, we found that the altered imprint status is inherited into differentiated cells. In this presentation, we will discuss the role of altered imprinting regulation on the quality of iPSCs based on our recent results in comprehensive imprinting analyses of F1 PSCs.

CHROMATIN IN STEM CELLS

F2154

EPIGENETIC PROFILES SIGNIFY CELL FATE PLASTICITY IN UNIPOTENT MAMMALIAN SPERMATOGONIAL STEM AND PROGENITOR CELLS

Liu, Ying, Weill Cornell Medical College, New York, NY, U.S.

Mammalian spermatogonial stem and progenitor cells (SSCs) are precursors of all subsequent germ cells in the adult male gonad. During in vitro expansion, these unipotent stem cells can spontaneously convert to multipotent adult spermatogonial-derived stem cells (MASCs) without ectopic expression of any transcription factors. The underlying mechanisms of this spontaneous conversion process are still poorly understood. Here, we report an integrative analysis of both the transcriptomes and histone modification-defined epigenomes of mouse SSCs and MASCs. We found in SSCs that many genes essential to maintenance and differentiation of embryonic stem cells (ESCs) are enriched with both histone H3 lysine 4 and lysine 27 trimethylation modifications (K4me3+K27me3) at promoter regions. Quantitative epigenomic analysis suggested that these ‘bivalent’ modifications are maintained at most somatic gene promoters after conversion, except for changes at a few signature genes for ESCs and germ cells. Thus, MASCs are bestowed with ESC-like promoter chromatin states. This phenomenon distinguishes MASC derivation from induced pluripotent stem (iPS) cell reprogramming, which involves global epigenetic changes and reconstitution of promoter bivalency. At enhancer regions, the core pluripotency circuitry was activated partially in SSCs and completely in MASCs, concomitant with global erasure of germ cell-specific enhancer activity and initiation of an embryonic-like program. Furthermore, long-term in vitro cultured SSCs maintained epigenomic characteristics reflective of germ cells in vivo. Our observations suggest that unipotent SSCs encode their innate developmental flexibility by means of the epigenome and that both the conversion of promoter chromatin states and the activation of cell type-specific enhancers are prominent features of SSC reprogramming.

F2156

DELETION OF TET1 OR TET2 IN MESC RESULTS IN ABERRANT H3K27ME3 DEPOSITION

Reimer, Michael Henry[1,2], Blinka, Steven Mark[2], Pulakanti, Kirthi[1] and Rao, Sridhar[1,2], [1]BloodCenter of Wisconsin, Milwaukee, WI, U.S., [2]Medical College of Wisconsin, Milwaukee, WI, U.S.

Ten-eleven translocation (TET) proteins play a central role in the DNA demethylation pathway in a variety of cell types. TET proteins are able to catalyze the modification of methylated cytosines leading to their removal from the genome. In mouse embryonic stem cells (mESC), Tet1 and Tet2 proteins are highly expressed and found at enhancers as well as at active and bivalent gene promoters. Bivalent promoters are marked by two opposing epigenetic marks; activating H3K4me3 and repressive H3K27me3. These genes are repressed in mESCs but are considered “poised” or able to be transcribed quickly following introduction of some differentiation cue. At bivalent promoters, TET proteins are often found with polycomb repressive complex 2 (PRC2) which is responsible for the deposition of the repressive epigenetic mark; H3K27me3.

An interaction between one member of the TET family, Tet1, with PRC2 was detected exclusively in mESCs and not in other cell types tested. Interestingly, deletion of TET proteins does not affect mESC maintenance but rather impairs differentiation. In order to study TET protein's role in differentiation, we generated Tet1 and Tet2 knockout mESC lines using CRISPR technology. In agreement with the published literature, these cell lines display a significant delay in differentiation. RT-qPCR and RNA-seq analysis showed no significant differences in gene expression in either cell line. ChIP-seq of the PRC2 mark H3K27me3, revealed higher levels of this repressive mark at bivalent promoters in the Tet1 and Tet2 knockout lines as compared to controls. This variability was enriched at promoters (76%) when examined on a genome-wide scale. Importantly, H3K27me3 variability was not found at genes important for stem cell maintenance (Oct4, Nanog, Sox2 etc.) and overall levels of PRC2 components (Suz12, EZH2) remain unchanged. This preliminary data suggests that TETs may function in regulating the amount of H3K27me3 at bivalent promoters and subsequent activation of these genes during differentiation. Further investigation into the relationship between PRC2 and TET proteins will help elucidate TET's role in differentiation.

GERMLINE CELLS

F2160

SDF1 AND CXCR4 REGULATED MIGRATION AND IN-VITRO ACTION OF PORCINE SPERMATOGONIAL STEM CELLS (PSSC)

Lee, Ran[1], Lee, Won Young[2] and Song, Hyuk[1], [1]Konkuk University, Seoul, Korea, [2]Konkuk University, Chung-ju, Korea

The spermatogonial stem cells (SSCs) are responsible for the maintenance of spermatogenesis. Although the some of markers of SSCs were investigated in porcine including protein gene product 9.5 (PGP9.5), Nanog, glial cell line-derived neurotrophic factor receptor alpha 1 (GFR-α1), lectin Dolichos biflorus agglutinin, CD14, CD209 and undifferentiated embryonic cell transcription factor 1 (UTF1), these markers are insufficient to define characteristics of porcine SSC (pSSC). Stromal cell derived factor 1 (SDF-1) and chemokine (C-X-C) receptor type 4 (CXCR4) play an essential role for migration and colonization of mice SSCs. The objective of this study is to identify the expression of SDF1 and CXCR4 in porcine testis and to characterize the SDF1 and CXCR4 expression in cultured pSSCs. SDF1 was expressed in PGP9.5 positive spermatogonial stem cells, and CXCR4 was expressed in GATA4 positive early type of sertoli cells in porcine testis tissues. In vitro culture of pSSCs from 5 day old testis revealed that expression of PGP9.5 and SDF1 was identically determined in the pSSC colonies, and CXCR4 was identified in the feeder cells. The migration of CXCR4 positive feeder cells was significantly increased by exogenous SDF1, and was significantly decreased by AMD3100 treatment. These data suggest that interaction between SDF1 and CXCR4 may regulate the migration and colony formation of in-vitro cultured pSSCs, and in-vivo testis development.

TOTIPOTENT/EARLY EMBRYO CELLS

F2164

DISTINCT FEATURES IN ESTABLISHING H3K4me3 AND H3K27me3 IN PRE-IMPLANTATION EMBRYOS

Liu, Xiaoyu[1,2], Wang, Chenfei[3], Gao, Shaorong[3] and **Gao, Yawei**[3], [1]National Institute of Biological Sciences, Beijing, Beijing, China, [2]Graduate School of Peking Union Medical College, Beijing, China, [3]Tongji University, Shanghai, China

Histone modifications play critical roles in regulating the expression of developmental genes during embryo development in mammals. However, genome-wide analyses of histone modifications in pre-implantation embryos have been impeded by technical difficulties and scarcity of the required materials. Here, by using a small-scale chromatin immunoprecipitation sequencing (ChIP-seq) method, for the first time, we mapped the genome-wide profile of histone H3 lysine 4 trimethylation (H3K4me3) and histone H3 lysine 27 trimethylation (H3K27me3), which are associated with gene activation and repression, respectively, in mouse pre-implantation embryos. We found that the re-establishment of H3K4me3 occurs much more rapidly than that of H3K27me3 following fertilization, which is consistent with the major wave of zygotic genome activation (ZGA) at the 2-cell stage. Furthermore, H3K4me3 and H3K27me3 possess distinct features of sequence preference and dynamics in pre-implantation embryos. Although H3K4me3 modifications exist constantly on transcription start site (TSS) regions, the breadth of the H3K4me3 domain is a highly dynamic feature. Interestingly, the broad H3K4me3 (wider than 5 kb) is associated with higher transcription activity and cell identity not only in pre-implantation embryos but also in the process of deriving embryonic stem cells (ESCs) from the inner cell mass (ICM) and trophoblast stem cells (TSCs) from the trophectoderm (TE). Unlike in ESCs, we found that the bivalency (co-occurrence of H3K4me3 and H3K27me3) is unstable and infrequent in early cleavage-stage embryos until the morula-to-blastocyst transition. Taken together, our results provide a genome-wide map of H3K4me3 and H3K27me3 modifications in pre-implantation embryos, facilitating further exploration of the epigenetic regulation mechanism in early embryo development.

F2166

OXIDATIVE STRESS OF FBS (FETAL BOVINE SERUM) IN EARLY DEVELOPMENT AND EARLY EMBRYOGENESIS IN PIG

Sim, Bo-Woong[1], Choi, Seon-A[1], Yoon, Seung-Bin[1,2], Jeong, Pil-Soo[1], Yang, Hae-Jun[1], Kim, Joo-Young[1], Park, Young-Ho[1], Song, Bong-Seok[1], Kim, Ji-Su[1,2] and Kim, Sun-Uk[1,2], [1]Korea Research Institute of Bioscience and Biotechnology (KRIBB), Cheongju-si, Korea, [2]University of Science and Technology, Daejeon, Korea

Despite the application of numerous supplements to improve in vitro culture (IVC) conditions of mammalian cells, relatively a few or no have been studied concerning the effect of fetal bovine serum (FBS) on early development of mammalian embryos. Thus, the current study was conducted to investigate the effect of FBS on the developmental competence of porcine embryos and to establish the optimal treatment conditions using parthenogenetic activation (PA), in vitro fertilization (IVF), somatic cell nuclear transfer (SCNT) embryos. The rates of blastocyst formation and hatching were significantly increased by addition of FBS during the late-phase of IVC compared to control, which was further evidenced by the improvements in cellular survival, total cell number and tropectoderm (TE) proportion. Moreover, reactive oxygen species (ROS) levels were markedly reduced in FBS treatment group compared to control, which appeared to be closely associated with increase of TE cells in blastocysts. Indeed, addition of hydrogen peroxide greatly reduced the developmental competence parameters including TE cell proportion, whereas the defects were significantly restored by supplementation of FBS. More interestingly, the beneficial effects of FBS in late-phase of IVC were consistently found in the embryos produced by IVF or SCNT embryos. Taken together, these results suggest that FBS can be efficiently used as a useful IVC supplement for massive production of porcine embryos with high developmental competence.

EMBRYONIC STEM CELL DIFFERENTIATION

F2170

DEFINED CULTURE CONDITIONS FOR EFFICIENT DERIVATION, EXPANSION AND CRYOPRESERVATION OF MESENCHYMAL PROGENITOR CELLS FROM HUMAN PLURIPOTENT STEM CELLS

Wagey, Ravenska[1], Elliott, Melissa[1], **Sampaio, Arthur V**[1], Szilvassy, Stephen J[1], Kardel, Melanie Dawn[1], Hadley, Erik[1], Thomas, Terry E.[1] and Eaves, Allen C.[1,2], [1]STEMCELL Technologies Inc., Vancouver, BC, Canada, [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

The invasive nature of the harvesting procedure and heterogeneity of mesenchymal progenitor cells (MPCs) isolated from adult tissues have prompted researchers to use human pluripotent stem cells (hPSCs) as an alternative source. Current protocols for deriving MPCs from hPSCs involve culturing cells in serum-containing medium on animal feeders and require prolonged culture time. We have developed a highly efficient method of inducing differentiation of hPSCs into cells with MPC-like properties under completely defined culture conditions. Briefly, hPSCs were seeded as single cells in mTeSR™ or TeSR™ E8™-medium with Y-27632 for 2 days. Differentiation was then initiated by replacing the medium with MesenCult™-ACF Induction Medium (ACF-IM). The cells reached confluency after 4 days in ACF-IM and the medium was then replaced with MesenCult™-ACF (MACF) medium. The cells in MACF medium reached 80% confluency after 2-3 days and were dissociated using animal component-free (ACF) dissociation reagents. These cells were seeded at 2.5 – 7.5 x10^4 cells/cm^2 in MACF medium on ACF substrate. The proliferative potential of hPSCs-derived MPCs was measured and phenotype was analyzed by flow cytometry. After 1 week in MACF medium, hPSCs adopted MPC-like morphology and could be expanded for up to 20 passages. Data from two experiments using one induced hPSC line and one Embryonic Stem Cell (ESC) line (H9) indicated the average fold expansion at each subculture (P1-P20) was ~11- fold and ~12-fold, respectively. These cells were also cryopreserved at different stages of differentiation using MesenCult™-ACF cryopreservation medium with high viability (>90%) upon thawing. More than 90% of MPC-like cells expressed CD73, CD105, CD90 and CD146, and lacked expression of hematopoietic (CD34, CD45) and endothelial (CD144) cell markers at 21 days. The hPSCs-derived MPCs were able to differentiate into adipocytes, osteogenic cells and chondrocytes in vitro. These results indicate that MPC-like cells can be derived efficiently from hPSCs using completely defined culture

conditions. Current studies are evaluating additional hPSC lines using this protocol.

F2172

CONTROLLING CARDIOVASCULAR PROGENITOR CELLS CULTURE FROM HUMAN PLURIPOTENT STEM CELLS BY CHEMICAL COMPOUNDS

Bolesani, Emiliano, Kempf, Henning and Zweigerdt, Robert, Hannover Medical School, Hannover, Germany

Cardiovascular progenitor cells (CPCs) may represent a favourable cell type for cell-based heart repair and a valuable model to investigate heart development. Human pluripotent stem cell (hPSC)-derived CPCs might be expandable whilst maintaining their multi-lineage differentiation potential, comprising cardiomyocytes, endothelial cells and smooth muscle cells. In this study we aim at characterizing markers and pathways of hPSCs' cardiovascular differentiation to ultimately establish maintained CPCs propagation in vitro. Directed cardiac differentiation of hPSCs was achieved by means of chemical Wnt pathway modulators CHIR99021 and IWP1. Mesendodermal induction was monitored using a MIXL1-eGFP reporter line and expression of endogenous MESP1, marking formation of cardiac mesoderm. In our differentiation protocol the second heart field multipotent progenitors' marker ISL1 is upregulated shortly before NKX2.5, which marks the appearance of functional cardiomyocytes. A close interplay between ISL1 expression and Wnt pathway activity was reported in the mouse embryo, in which ISL1 expressing cardiac progenitor cells depend on Wnt signalling for proliferation and survival. We therefore hypothesize that Wnt pathway control could allow propagation and expansion of hPSC-derived CPCs. Monitoring endogenous ISL1 and transgenic NKX2.5-eGFP expression we have screened for small molecules supporting maintained ISL1 expression, thereby avoiding progression of differentiation into NKX2.5-positive cardiomyocytes. Presented results provide a better characterization of in vitro-derived human CPCs, support the understanding of pathways involved in their commitment and maintenance, and might enable the long term in vitro expansion of CPCs in larger scale.

F2174

REGULATING THE CELL FATE SPECIFICATION OF HUMAN PLURIPOTENT STEM CELLS

Chetty, Sundari, Stanford University, Stanford, CA, U.S., Ziller, Michael, Max-Planck Institute of Psychiatry, Munich, Germany, Gifford, Casey, Gladstone Institute of Cardiovascular Disease, San Francisco, CA, U.S., Melton, Douglas A., Harvard University, Cambridge, MA, U.S. and Meissner, Alexander, Harvard University/ Broad Institute, Cambridge, MA, U.S.

Despite recent advances in generating specialized cell types from human pluripotent stem cells (hPSCs), many studies have noted that pluripotent stem cell lines often have an inherent inability to differentiate even when stimulated with a proper set of signals. Significant variations in differentiation propensity have even been noted within a cell line due to differences in culture conditions. Furthermore, generating terminally differentiated cells with functional capacity in a robust and efficient manner has proven challenging. Understanding the mechanisms that regulate the differentiation of hPSCs is therefore critical to effectively drive their fates into desired cell types. Here, we show that the effectiveness of differentiated cells can be greatly improved by simply altering the "starting state" of hPSCs. Specifically, we demonstrate that changes to the cell cycle and epigenetic dynamics of hPSCs have significant long term impacts on enhancing the functionality of differentiated progeny following transplantation *in vivo*. Using transcriptional and epigenetic profiling at the population and single cell level, we show that changes to the cell cycle by activating checkpoint controls promotes an opening up of the chromatin state at early developmental genes. These chromatin changes occur prior to gene activation, epigenetically priming hPSCs for differentiation. Using ChIP-seq, we show that histone modifications (e.g. gain of H3K27ac and H3K4me3, and loss of H3K27me3) particularly target early developmental genes involved in orchestrating the initial steps of embryogenesis, such as gastrulation, primitive streak formation, patterning, neural tube development, and cell fate specification. Further investigation of chromatin accessibility shows unique events associated with the "primed" pluripotent state and that of the cell cycle, particularly in the G1 phase. Together, these results show that important changes to the cell cycle and epigenetic state occur prior to differentiation, paralleling those that occur in normal embryonic development. Using these mechanistic insights, we develop tools to prime hPSCs into a state competent for differentiation and subsequently enhance the generation of differentiated cell types for therapeutic applications.

F2178

INDUCIBLE GENE EXPRESSION IN T LINEAGE CELLS DERIVED IN VIVO FROM HUMAN EMBRYONIC STEM CELLS

Scripture-Adams, Deirdre[1], Zhong, Nianxin[1], Chen, Hongying[1], Zack, Jerome A.[1,2] and **Galic, Zoran**[1,2], [1]David Geffen School of Medicine at UCLA, Los Angeles, CA, U.S., [2]UCLA Broad Stem Cell Research Center, Los Angeles, CA, U.S.

Stem cell therapy holds promise for the treatment of a broad array of conditions, including cancer, HIV infection, and immune insufficiency disorders. For some of these diseases, therapeutic transfer of cells expressing specialized receptors or carrying corrective genes designed to combat particular disorders has been proposed. These gene therapy strategies may someday be improved through the use of human embryonic stem cells (hESC), and induced pluripotent stem cells (iPSC), both of which can in theory expand indefinitely without losing pluripotenitality, and which can be protected against malignant transformation via targeted selection of a single, known integration site wherein the genes of interest are introduced. Some genes with potential therapeutic benefit in mature T cells, such as a specialized T cell receptors targeting specific antigens, or alternatively transcription factors which drive particular T effector phenotypes, may not be either safe or practical to express constitutively from the hESC stage, and may therefore require a more directly regulated expression system to maintain safety and efficacy. To address these concerns and to generate a model system in which genes of interest can be turned on and off at will, we have modified the H1 hESC line such that genes of interest can be regulated by administration of doxycycline. Using these modified hESC lines we generated thymocytes in vivo in humanized mice, and subsequently used doxycycline to induce expression of both a membrane bound reporter (NGFR) and a nuclear transcription factor (FoxP3) from these cells in vivo and ex vivo. This work constitutes the first demonstration of a working genetic regulation scheme for T lineage cells derived in vivo from hESC

F2180

PRIMARY CILIUM-AUTOPHAGY-Nrf2 (PAN) AXIS ACTIVATION COMMITS HUMAN EMBRYONIC STEM CELLS TO A NEUROECTODERM FATE

Jang, Jiwon, Wang, Yidi, Lalli, Matthew A, Guzman, Elmer, Godshalk, Sirie, Zhou, Hongjun and Kosik, Kenneth S., University of California Santa Barbara, Santa Barbara, CA, U.S.

Resolving epigenetic loci and histone modification has been extensively studied to understand stem cell differentiation. However, the earliest mechanisms preceding epigenetic changes remain elusive. G1 elongation in the cell cycle occurs during stem cell differentiation and it was proposed that G1 elongation initiate differentiation. However, whether this event occurs during general stem cell differentiation or happens in a lineage-specific manner as a cell fate determinant is unknown. Thus a clear role for this well-studied phase of the cell cycle has not been fully delineated in stem cell biology. Here, we show G1 elongation is a lineage specific event that drives the NE fate. Furthermore, the intertwined relationship between G1 and the elaboration of primary cilia serves as an elegant means of directing NE differentiation translating cell cycle patterns into cellular signaling for lineage specification. Coupled with lineage-specific G1 lengthening a divergent ciliation pattern emerged within the first 24 hours of induced lineage specification and these changes heralded a neuroectoderm decision before any neural precursor markers were expressed. By day 2, increased ciliation in NE precursors induced autophagy that resulted in the inactivation of Nrf2 and thereby relieved transcriptional activation of OCT4 and NANOG. Nrf2 binds directly to upstream regions of these pluripotency genes to promote their expression and repress NE derivation. Nrf2 suppression was sufficient to rescue poorly neurogenic iPSC lines. Only after these events had been initiated do neural precursor markers get expressed at day 4. Thus we have identified a primary cilium-autophagy-Nrf2 (PAN) control axis coupled to cell cycle progression that directs hESCs toward NE.

F2182

REGENERATION OF FUNCTIONAL ORGAN BY USE OF AVIAN STEM CELLS

Kagami, Hiroshi, Shinshu University, Kamiina, Japan

Pluripotent avian stem cells were isolated from early embryos; stage X blastoderm. The stem cell clusters were dissociated into single cell. The cells were used for production of somatic and germline chimeras. The donor stem cells could be regenerated into functional legs. The cells were also differentiated into skin, muscle and feather and fertile gametes. As above, frontier strategies have been established for developmental regulation of the pluripotent stem cells. The established strategies could be contributed for developmental biology and regenerative medicine.

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F2184

THE ROLE OF GATA FACTORS IN HUMAN PANCREATIC DEVELOPMENT

Lee, Kihyun, MSKCC, New York, NY, U.S.

GATA factor haploinsufficiency has been associated with a wide-range of clinical phenotypes from normal pancreatic development and function to pancreatic agenesis or adult-onset diabetes. Unfortunately, haploinsufficiency of the GATA factors is not well recapitulated in the mouse system. To investigate the exact role of GATA factors in human pancreatic development we combined a robust genome editing technology, clustered regularly interspaced short palindromic repeat (CRISPR), with human pluripotent stem cell (hPSC) directed pancreatic differentiation. GATA6 or GATA4 deficient hPSC showed compromised pancreatic specification, which was not previously seen in mice. GATA6 heterozygous hPSCs also showed dysregulation of genes essential for endoderm and pancreatic development, suggesting that patients with GATA6 heterozygous mutations have impaired or defective pancreatic development. However, the severity of differentiation phenotypes varied among hPSC lines, similar to the wide-range of pancreatic phenotypes identified in GATA6 deficient patients. Our data suggests that GATA6 has a critical role in human endoderm and pancreatic development, while genetic background influences the disease severity. Our finding places emphasis on the broader use of hPSCs and CRISPR/Cas9 technology to better understand complex disease etiology.

F2186

DIFFERENTIATION CAPACITY OF hESC LINES CARRYING A 20q11.21 AMPLIFICATION

Markouli, Christina[1], Geens, Mieke[2], Dziedzicka, Dominika[2], De Kock, Joery[2], Spits, Claudia[2] and Sermon, Karen[2], [1]Vrije Universiteit Brussel (VUB), Brussels, Belgium, [2]Vrije Universiteit Brussel, Brussels, Belgium

Being present in over 20% of human embryonic stem cell (hESC) lines worldwide, a gain of 20q11.21 is one of the most recurrent chromosomal abnormalities in hESC. The smallest region of amplification comprises 3 genes: BCL2L1, ID1 and HM13. Bcl-xL, the predominantly expressed isoform of BCL2L1, has an anti-apoptotic function and is responsible for the selective advantage during culture of hESC carrying the 20q11.21 amplification. ID1 plays an important role in maintaining pluripotency while HM13 has no known implication in this phenomenon. Two independent studies have thoroughly described the mechanism behind the selective advantage of the mutation, but important information on whether it influences differentiation is nevertheless still lacking. We aimed to compare the behavior of genetically normal hESC lines to that of lines carrying a gain of 20q11.21, after spontaneous and lineage directed differentiation. We studied 3 pairs of in-house derived hESC lines for their differentiation capacity towards the mesodermal lineage (osteo-progenitor-like cells) and endodermal lineage (definitive endoderm), as well as after embryoid body formation. We used gene expression data (mRNA and protein levels) to assess the overall onset of differentiation and the probability of the mutant lines retaining higher numbers of undifferentiated cells after differentiation. We observed comparable differentiation efficiency among all pairs of lines with small inter-line variability, which is a frequently observed phenomenon. Moreover, the mutant lines consistently expressed low levels of pluripotency markers (POU5F1 and NANOG) after differentiation excluding an influence of the extra copy(ies) of ID1. However, immunohistochemistry suggested an improved cell survival of the mutant cells during differentiation. Moreover, mutant cells appear to survive in the differentiation media better than their normal counterparts, regardless of whether the cells differentiated to the intended cell type or not. Further quantification of apoptosis to confirm this hypothesis is ongoing. Overall our study suggests that aberrant cells display similar differentiation ability (whether in a spontaneous or lineage-specific way), but maintain a character that is less prone to apoptosis throughout the process of differentiation.

F2188

ENFORCED EXPRESSION OF PAX5 DURING HEMATOPOIETIC DIFFERENTIATION FROM HUMAN PLURIPOTENT STEM CELLS INDUCES EXPRESSION OF CD19 BUT NOT CD10

Motazedian, Ali[1,2], Labonne, Tanya[3], Ng, Elizabeth S[3], Elefanty, Andrew George[1,3] and Stanley, Ed[1,3], [1]University of Melbourne, Melbourne, Australia, [2]University of Melbourne, Melbourne, Victoria, Australia, [3]Murdoch Children's Research Institute, Melbourne, Australia

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) represent a tractable system for medical research and have sparked hope for the rapid development of personalised treatments and regenerative medicine. Pluripotent stem cells (PSCs), including ESCs and iPSCs, can undergo unlimited self-renewal and, in theory, have the ability to give rise to all of the different cell types in body. The properties of PSCs make them an attractive platform for generating a variety of normal and mutated human cell types for medical related applications. For example, hematopoietic cells generated from the in vitro differentiation of PSCs could be used for drug screening and potentially for cell replacement therapies. In this study, we have investigated a method for generating B-lymphocytes from PSCs. B-cells are of particular interest as their derivation would be indicative of prog-

ress towards the development of conditions that support HSC formation, the cell type which is likely to have the most immediate clinical applications. We and others have developed culture conditions suitable for primitive and definitive hematopoietic differentiation of PSCs. We have used these systems to examine B-lymphocyte generation in the context of over-expressing B-lineage specific transcription factors in a controllable and inducible manner. Our data suggest that, during the induction of primitive blood cells, forced expression of PAX5 alone in CD34+ hematopoietic progenitors can up-regulate the B-cell marker CD19 and promote the appearance of Glycophorin A expressing erythroid lineage cells. During definitive blood induction, PAX5 induction in CD34+ hematopoietic progenitors co-cultured on OP9-DLL4 stromal cells, blocks the formation of T-cell progenitors. Furthermore, although premature forced expression of PAX5 blocks the formation of blood cells, delayed PAX5 induction, results in significant up-regulation of CD19 expression specifically in CD45+ & CD14- population. However, these CD19+ cells fail to persist in our culture conditions and do not move on to express CD10, another B-cell marker which is co-expressed with CD19 at most stages of B-cell development. These results suggest that PAX5 induction alone may not be sufficient for complete B-cell induction of PSC derived hematopoietic progenitors.

F2190

HIGH THROUGHPUT SINGLE CELL GENE EXPRESSION PROFILING BY MULTIPLEX qPCR

Okino, Steven[1], Kong, Michelle[1], Ma, Jason[1,2], Fenrich, Joshua[1], Boveri, Luca[1], Jouvenot, Yann[1] and Wang, Yan[1], [1]Bio-Rad Laboratories, Hercules, CA, U.S., [2]Biorad Laboratories, Hercules, CA, U.S.

Single cell gene expression analysis is a powerful technique that provides a unique and insightful perspective on biological pathways and processes. Here we present a robust workflow that enables fast and accurate analysis of up to 100 genes in isolated single cells. Our workflow is highly sensitive, by assessing RNA reference standards we find that a single RNA transcript is detected with about 80% efficiency. We used this workflow to study differentiation in cultured NTera2 cells (NT2), a human embryonic stem cell model system. We analyzed untreated NT2 cells, and NT2 cells treated with low and high doses of retinoic acid (RA) for 10 days to initiate differentiation to a neuronal lineage. The expression levels of 16 genes were quantified in 164 single cells by multiplex real-time qPCR with two technical replicates. The entire experiment, from cultured cells to results, can be completed in 2-3 days and requires four 384-well qPCR plates for gene expression quantification. We find that control cells and cells treated with a high dose of RA (10 uM) are relatively homogeneous in the expression levels of the targeted genes. However cells treated with a low dose of RA (0.25 uM) exhibit significant heterogeneity with respect to gene expression; about half of the cells are similar to the high-dose RA cells, the other cells exhibit a wide range of partial differentiation. Interestingly, we find that LEFTY2 expression is almost exclusive to the low dose RA cells and strongly correlates with partial differentiation. A time-course study analyzing cell populations reveals that LEFTY2 is only transiently expressed in differentiating NT2 cells with peak expression at 3 days of high dose RA treatment. These findings imply that, in NT2 cells, LEFTY2 is a potential biomarker of early differentiation. In summary, we present an accurate, sensitive and robust single cell analysis procedure that uses standard reagents and platforms. We envision that this workflow will enable researchers to investigate cell heterogeneity in biological pathways in a cost-effective way.

Funding Source: None

F2192

A DEFINED, 3D BIOMATERIAL SYSTEM FOR DIRECTED DIFFERENTIATION OF OLIGODENDOCYTE PRECUSSOR CELLS FROM HPSCS

Rodrigues, Goncalo[1,2], Gaj, Thomas[1], Adil, Maroof[3], Diogo, Margarida[2], Sampaio Cabral, Joaquim[2] and V. Schaffer, David[1], [1]University of California Berkeley, Berkeley, CA, U.S., [2]SCBL-RM, Lisbon, Portugal, [3]University of California, Berkeley, Berkeley, CA, U.S.

Oligodendrocyte precursors cells (OPCs) initially migrate along the developing neural tube to populate the central nervous system, then differentiate into mature, non-dividing myelinating oligodendrocytes. Due to their ability to migrate, engraft, and differentiate after transplantation, cell replacement therapies based on OPCs have the potential to treat numerous demyelinating diseases, including multiple sclerosis and hypomyelinating leukodystrophies. However, it is challenging to generate reliable and sustainable source of clinically compliant OPCs. While pluripotent stem cells represent an alternative source for OPC generation, inefficient differentiation protocols that exceed 100 days can hamper their large-scale production and application. Using a synthetic thermoresponsive, biomaterial system, we adapted a neural differentiation protocol based on dual-SMAD inhibition to generate pre-OPCs in a 3D system that enables large-scale production of cells. By varying the concentrations and time-points for addition of SHH, CHIR, SAG and retinoic acid (RA), high levels of pre-OPC differentiation were achieved. We also used CRISPR/Cas9-mediated genome engineering to generate a NKX2.2-EGFP hESC reporter line. Because we could faithfully monitor NKX2.2 expression in a simple manner, this reporter line enabled precise tuning of OPC specification using a more comprehensive set of differentiation conditions. After 18 days of differentiation, ~70% OLIG2-positive and ~50% NKX2.2-positive pre-OPCs were generated, in an optimized condition, where only RA and

SAG were used together. At later stages of the differentiation, cells started to express OPC markers, such as O4, on day 50, and oligodendrocyte markers, such as MBP, on day 70. Importantly, this strategy enabled the rapid generation of Olig2- and NKX2.2-positive cells that can be further differentiated into OPCs suitable for transplantation. Moreover, *in vitro*, these OPCs matured quickly into MBP-positive oligodendrocytes. A chemically defined, 3D biomaterial system for directed OPC differentiation, enabled by reporter cell lines, can expedite the generation of OPCs for stem cell-based therapies.

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F2194

THE ROLE OF THE SIGNALING FACTORS ON DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELL-DERIVED NEURAL CREST STEM CELLS

Suga, Mika[1], Mimura, Sumiyo[1,2], Okada, Kaori[1], Kinehara, Masaki[1,2], Nikawa, Hiroki[2] and Furue, Miho Kusuda[1], [1]National Institutes of Biomedical Innovation, Health and Nutrition, Osaka, Japan, [2]Hiroshima University, Hiroshima, Japan

Neural crest (NC) cells are a group of cells located in the neural folds at the boundary between the neural and epidermal ectoderm. Cranial NC cells migrate to the branchial arches and give rise to majority of the craniofacial region, whereas trunk and tail NC cells contribute to the heart, enteric ganglia of the gut, melanocytes, sympathetic ganglia, and adrenal chromaffin cells. Positional information is indispensable for the regulation of cranial or trunk and tail NC cells. However, the mechanisms underlying the regulation of positional information during human NC induction have yet to be fully elucidated. In the present study, it was tested whether signaling factors induce the expression of HOX genes during NC cell differentiation from human embryonic stem cells (hESCs) under defined serum-free culture condition. We found that bone morphogenetic protein 4 promotes craniofacial mesenchyme induction from hESC-derived NC stem cells. We will discuss about the role of the signaling factors on positional information control in the hESC-derived NC stem cells under the chemically defined culture condition.

F2196

PRIMORDIAL FOLLICLE OOCYTES DERIVED FROM HUMAN PLURIPOTENT STEM CELLS BY TWO-STEP INDUCTION PROCEDURE

Wang, Ning[1,2] and **Wang, Huayan Hugh**[2], [1]Northwest A&F University, Yangling, China, [2]College of Vet Medicine, Northwest A&F Univ, Yangling, Xi'an, China

The previous studies have demonstrated that human ES and iPS cells can convert to human primordial germ cell-like cells (hPGCLCs) in vitro, which show the transcriptome profile and epigenetic status similar to their counterparts in vivo. However, this conversion was time consuming and inefficiency. In this study, we optimize the two-step induction procedure reported previously to convert ES and iPS cells to oocyte-like cells (OLCs). First, to generate hPGCLCs, the hESCs and iPSCs were cultured in a germ-cell-medium (alpha-MEM supplemented with bovine follicle fluid (bFF) and cytokines LIF, BMP4, SCF, EGF) for 10 days. At this stage, the differentiated cells show the morphology similar to hPGCLCs, and expressed germ cell markers DAZL, SOX17 and BLIMP1. To generate OLCs, the hPGCLCs were then carefully collected and re-plated in a oocyte-medium (M199 supplemented with PMSG, FSH, EGF and ITS) for additional 7 to 10 days. The cumulus-oocyte-complexes (COCs) structures were formed and the OLCs in different sizes (range from 50-120 μm) with the thin and fragile zona pellucida were observed, some OLCs attached to the culture plate and some floated in the medium. At this stage, some OLCs could develop spontaneously into multi-cell structures similar to preimplantation embryos, presenting high cytoplasmic-to-nuclear ratio and indicating that OLCs could be parthenogenetically activated at current culture condition. In conclusion, our data demonstrated that hESCs and iPSCs possess the differentiation potential into germ cells. Using the two-step procedure, ES cells can be induced into OLCs in a short term (<20 days) and high efficiency (>2%) in vitro. This study may provide a new desirable model for study human germ cell formation and clinical application.

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F2198

EXTENDED IN VITRO DEVELOPMENTAL POTENTIAL OF EARLY HUMAN HEMATOPOIETIC CELLS

You, Hao, Wang, Cuihua, Filonenko, Elena and Samokhvalov, Igor, Guangzhou Institutes of Biomedicine and Health, CAS, Guangzhou, China

In vitro differentiation of human pluripotent stem cells (hPSCs) represents an exclusive research model for studying

cellular and molecular mechanisms of human hematopoietic development. Using recapitulative approach for modeling human mesodermal ontogeny we have developed an efficient protocol for hematopoietic differentiation of hPSCs in well-defined culture conditions and in absence of exogenous hematopoietic cytokines. The earliest hematopoietic cells begin to express CD43 (leukosialin) at Day 5 of differentiation, and around Day 10 CD43$^+$ cells start to upregulate a late hematopoietic marker CD45. The CD43$^+$ hematopoietic cells co-express low levels of VE-cadherin, whereas the endothelial cell lineage, which segregates at the onset of hematopoietic development, is characterized by high levels of VE-cadherin and CD31 expression. The CD43$^+$VE-cadherin^{low} cell population contains a full complement of erythro-myeloid hematopoietic progenitors generated by differentiating hPSCs. Upon bi-allelic inactivation of *RUNX1* by homologous TALEN-supported recombination, hPSC severely reduce their capacity to generate CD43$^+$ and CD43$^+$CD45$^+$ cells, which suggests that these cells belong to the definitive wave of hematopoiesis. Surprisingly, Day 6–20 CD43$^+$ cells, when replated in the same culture conditions, undergo spontaneous concomitant differentiation into three mesodermal lineages: CD146$^+$CD31$^-$ mesenchymal progenitors, CD146$^+$CD31$^+$ endothelial cells, and CD43$^+$/CD45$^+$ blood cells. On contrary, sorted CD43$^-$CD146$^+$CD31$^-$ mesenchymal precursors or CD43$^-$CD146$^+$CD31$^+$ endothelial cells demonstrate only unilineage developmental potential upon replating. The three-lineage potential of the early hematopoietic progenitors disappears in the presence of a cocktail of hematopoietic cytokines. Preliminary data suggest that the triple developmental potential is clonal and dependent on autocrine/paracrine signaling within CD43$^+$ population. Thus, early human blood cells may function as multilineage precursors – hemomesangioblasts – capable to facilitate the development of key mesodermal tissues.

EMBRYONIC STEM CELL PLURIPOTENCY

F2202

THE GENETIC STABILITY OF HUMAN PLURIPOTENT STEM CELLS DERIVED FROM THE SAME SOMATIC CELLS BY SOMATIC CELL NUCLEAR TRANSFER AND INDUCED REPROGRAMMING

Lee, Jeoung Eun[1], Shim, Sung Han[1], Park, Ji Eun[1], Park, Sang Hee[1], Lee, Jin Il[1], Lee, Sung-Geum[1], Jun, Sung-Min[1], Jung, Sookyung[1], Go, Eun Sol[1], Chung, Young Gie[2] and Lee, Dong Ryul[1], [1]CHA University, Gyeonggi-do, Korea, [2]CHA Health Systems, Los Angeles, CA, U.S.

Since the first report of human embryonic stem cell (hESC) establishment, hESC have been considered as the best cell sources for the clinical application in spite of allogenicity issues. Patient specific PSCs such as induced pluripotent stem cells (iPSC) and somatic cell nuclear transfer ES cells (SCNT-ESC) give a solution for the immune problems as autologous cell replacement therapy. Before the clinical application, confirmation of genetic stability of hPSC and their derivatives should be proven. There are some reports that a high frequency of genetic and epigenetic abnormalities has been observed in induced reprogramming procedure, and it is not understood whether genomic changes and/or epigenetic aberrance during reprogramming are normal variation or critical errors to affect cell genomic integrity and characters. In this study, we examined the genomic integrity of SCNT-ESC and iPSC derived from the same somatic cells in the process of reprogramming and extended long-term in vitro culture. Three SCNT-hESC lines (CHA-hES NT2, NT4 and NT5), 6 iPSC lines (iPSC-NT2-S1 and S2, iPSC-NT4-S1 and L1, iPS-NT5-S1 and S9), and 3 human dermal fibroblasts (hDF-NT2, hDF-NT4 and hDF-NT5) were compared the genetic stability using SNP-array, CNV, STR analysis and G-banding karyotype. Based on the SNP analysis, matching percentages to somatic cells were 99.983%, 99.659% and 99.957% for set 1 (hDF-NT2), 99.995%, 99.978% and 99.714% for set 2 (hDF-NT4), and 99.964%, 99.954% and 99.968% for set 3 (hDF-NT5) at early passage, respectively. CNV analysis showed that iPS-NT4-L1 has LOH (loss of heterozygosity) on 16q. This LOH caused relatively high mismatching % in iPS-NT4-L1 than in CHA-hES NT4 and iPS-NT4-S1, and change the STR marker expression. From the results, there are no significant differences between SCNT-ES and iPSC in CNV and SNP analysis, but SCNT-ES have lower difference of CNV and SNP than iPSC.

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F2204

AN ERNA-PRODUCING SUPER-ENHANCER AT THE EXTENDED NANOG LOCUS DIFFERENTIALLY REGULATES NEIGHBORING PLURIPOTENCY-ASSOCIATED GENES

Blinka, Steven Mark[1], Reimer, Michael Henry[1,2] and Rao, Sridhar[1,2], [1]Medical College of Wisconsin, Milwaukee, WI, U.S., [2]BloodCenter of Wisconsin, Milwaukee, WI, U.S.

Super-enhancers are tissue specific *cis*-regulatory elements (CREs) that drive expression of genes associated with cell identity and malignancy. A cardinal feature of super-enhancers is that they are bidirectionally transcribed to produce long non-coding RNAs, termed eRNAs. It remains unclear which enhancer functions are attributable to eRNAs or the CRE. CRISPR-mediated deletion of a super-enhancer 45 kb upstream of Nanog (-45 enhancer)

in embryonic stem cells (ESCs) results in decreased expression of both nearest neighbor pluripotency-associated genes (Nanog, Dppa3); however, the -45 enhancer is required for robust expression of only Dppa3. Nanog is unique from other ESC master transcription factors as it lies between two distal super-enhancers. By chromosome conformation capture (3C) we show a novel interaction between Nanog and a second super-enhancer 60 kb downstream (+60 enhancer), suggesting that the +60 enhancer is sufficient to maintain pluripotency in -45 enhancer deleted ESCs. To compare function of the -45 enhancer with eRNAs produced at this CRE, we depleted eRNAs using antisense oligonucleotides. In contrast to -45 enhancer deletion, eRNA depletion results only in decreased Dppa3 expression. To understand the mechanism by which eRNAs specifically regulate Dppa3, we demonstrate (by 3C) that eRNAs stabilize looping between the -45 enhancer and *Dppa3*. eRNA depletion does not result in alteration of active epigenetic marks at the -45 enhancer. These results demonstrate that two distal super-enhancers regulate the extended Nanog locus and a single super-enhancer (-45 enhancer) can regulate expression of multiple genes. Furthermore, we show that only a subset of genes regulated by the -45 enhancer is dependent upon cis-acting eRNAs to facilitate chromatin looping. Our work points to a method to selectively target a subset of loci regulated by super-enhancers in normal and pathologic states.

F2206

REGULATION OF PROMOTER AND ENHANCER HISTONE LANDSCAPE BY DNA METHYLATION IN MOUSE EMBRYONIC STEM CELLS

Fan, Guoping, UNIVERSITY OF CALIFORNIA, LOS ANGELES, Los Angeles, CA, U.S.

DNA methylation is one of multiple modes of epigenetic gene regulation and is found to be highly regulated during early development and stem cell differentiation. A major question is how changes in the DNA methylome contributes to transcriptional programs associated with cell fate commitment and differentiation. In this study, we profiled the DNA methylome, mRNA transcriptome, and global occupancy of histone modifications (H3K4me1, H3K4me3, H3K27me3, and H3K27ac) in a series of mouse embryonic stem cells (mESCs) varying in DNA methylation levels based on knock-out and reconstitution of DNA methyltransferases (DNMTs) to understand the interrelationship between DNA methylation and histone modifications in gene regulation. Our results reveal complex cross-talk between DNA methylation and various histone modifications at promoters and enhancers, which collectively exerts influence on gene expression. We find different isoforms of DNMT family members contribute to different levels of DNA methylation and histone modifications. Our data shows that DNA methylation is essential and instructive for the maintenance and establishment of H3K27me3 at gene promoters. DNA methylation acts directly to oppose H3K27me3 deposition at a subset of silent promoters, while acting indirectly at bivalent promoters, indicating that cross-talk at promoters is sensitive to chromatin context. Furthermore, global demethylation causes both H3K27me3 and H3K27ac changes at tissue-specific enhancers. The regulation of histone modifications at promoters and tissue-specific enhancers indicates that DNA methylation can impact gene expression patterns through multiple genomic elements and modalities.

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F2208

A FLUORESCENT FUSION-PROTEIN LIBRARY IN MOUSE ES CELLS IDENTIFIES NOVEL REGULATORS OF PLURIPOTENCY

Harikumar, Arigela, Hebrew University, Jerusalem, Israel and Meshorer, Eran, The Hebrew University of Jerusalem, Jerusalem, Israel

Embryonic stem cells (ESCs) have the dual capacity to differentiate into any cell type in the adult body, and to self-renew indefinitely. During differentiation, ESCs undergo dramatic changes both functionally and morphologically, accompanied by restriction of chromatin protein dynamics. In order to better understand the mechanism behind the differentiation process, it is important to study protein dynamics at the single cell level. For this purpose, we used a retroviral based CD-tagging approach to label genes in a non-directed manner, by integrating a DNA sequence coding for a fluorescent tag flanked by strong splicing signals, into genomic loci. When the tag is integrated inside an expressing gene, it is recognized as a novel exon by the splicing machinery, producing a fluorescently tagged fusion protein. Using this method, we have generated a small library of 200 YFP/mCherry tagged unique clones. Library statistics show that the virus has a preferential integration towards the first exon or first intron, making most of the proteins N-terminally tagged. This library enabled us to screen for proteins that are expressed heterogeneously and to compare the dynamics of the endogenously labeled proteins during differentiation using photobleaching methods in single living cells; to screen for proteins that are down-regulated during differentiation. Using later approach, We identified NASP, which displayed rapid and significant down regulation during early differentiation. NASP (Nuclear autoantigenic sperm protein-histone binding) function as Histone(H1) chaperone and also involved in histone storage and transport especially H3/H4. It has important roles in DNA replication, cell proliferation, cell cycle progression, cellular growth, blastocyst development, DNA repair, cancer. Our Preliminary results suggest that loss of NASP does not have any effect on ESC proliferation, however

result in aberrant EB formation as well as neuronal differentiation. Taken together we identified a novel chromatin factor that regulates differentiation.

F2210

CELL SURFACE-EXPRESSED ADENOVIRUS EARLY REGION 1B-ASSOCIATED PROTEIN 5 REGULATES PROLIFERATION OF HUMAN PLURIPOTENT STEM CELLS AND CANCER CELLS VIA WNT/GSK3 β/β-CATENIN SIGNALING

Kim, Won-tae[1], Choi, Hong Seo[2], Lee, Hyun Min[2], Jang, Young-Joo[3] and Ryu, Chun Jeih[2], [1]Sejong Univ, Seoul, Korea, South, [2]Sejong University, Seoul, Korea, South, [3]Dankook University, Cheonan, Korea, South

Adenovirus early region 1B-associated protein 5 (E1B-AP5) is known as a heterogeneous nuclear ribonucleoprotein and its role is involved in multifunctional RNA processing. The role of E1B-AP5 in human pluripotent stem cells (hPSCs) is still unclear. Previously, we generated murine monoclonal antibodies (MAbs) specific to surface antigens of undifferentiated human pluripotent stem cells (hPSCs) using a modified decoy immunization strategy. Furthermore, we showed that 57-C11, one such MAb, recognizes phosphorylated form of E1B-AP5 on the surface of hPSCs. Previous studies have shown that E1B-AP5 regulates cell cycle distribution through inhibition of p53 in cancer cells. However, the role of E1B-AP5 is still unclear on the surface and nucleus in hPSCs. In this study, we investigated on the functional roles of E1B-AP5 on the surface of hPSCs. To study on the role of E1B-AP5, we deprived expression level of E1B-AP5 using small interfering RNA technology. Knockdown of E1B-AP5 decreased cell proliferation, stem cell markers, and differentiation via downregulation of PI3K/AKT, MEK/ERK, and Wnt/GSK3β/β-catenin signaling in hPSCs. Knockdown of E1B-AP5 induces arrest cell cycle at G0/G1 phase through control of cyclin E and CDC25A. Similar effects were observed using shRNA in hPSCs. However, cancer cells (HeLa, A375, and U2OS) were not affected cell cycle regulation by E1B-AP5 knockdown. Interestingly, cell surface expression of E1B-AP5 inhibits apoptosis by siRNA except for HeLa cells. To further investigate the role of E1B-AP5 on the surface, we treated anti-E1B-AP5 antibodies. Treatment of anti-E1B-AP5 antibodies suppressed cell proliferation through Wnt/GSK3β/β-catenin signaling. Treatment of LiCl also induces expression level of E1B-AP5 on the surface of hPSCs. Simialr effects were observed in A375 cells. However, we could not observe both cell-cycle arrest and apoptosis. These data suggest that surface-E1B-AP5 is closely related to proliferation and Wnt/GSK3β/β-catenin signaling. These finding provide for the first time mechanistic insight into how E1B-AP5 regulates hPSCs proliferation on the surface.

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F2212

POTASSIUM: A FIFTH “ELEMENT” FOR THE REGULATION OF PLURIPOTENCY IN HUMAN PLURIPOTENT STEM CELLS

Lin, Victor[1], Koneru, Bhuvaneswari[1], Shi, Yi[1], Zolekar, Ashwini[1], Dimitrijevich, Dan[2,3], Garcia, Manuel[3], Kumar, Nalin[3], Di Pasqua, Anthony[1] and Wang, Yu-Chieh[1], [1]Pharmaceutical Sciences, University of North Texas Health Science Center, Ft Worth, TX, U.S., [2]Cardiovascular Research Institute, University of North Texas Health Science Center, Ft Worth, TX, U.S., [3]UHV Technologies Inc, Ft Worth, TX, U.S.

Many inorganic elements are critically involved in the modulation of biochemical reactions and cell signaling pathways, suggesting that cells in unique states may display distinct elemental profiles and have specific requirements for different elements. Using X-ray fluorescence (XRF) spectrometry and inductively coupled plasma mass spectrometry (ICPMS) techniques, we measured the amounts of 56 major and trace inorganic elements in undifferentiated human pluripotent stem cells (hPSCs), their isogenic differentiated derivatives, and somatic cells used for cell reprogramming. While the amounts of most elements that we analyzed did not appear correlated with the pluripotent state of cells, the amount of potassium cation in undifferentiated hPSCs was significantly lower than that in multiple types of non-pluripotent cells. This phenomenon was reproducibly and consistently shown by both XRF spectrometry and ICPMS analyses in multiple hPSC lines and differentiated cells. Flow cytometry analysis using a cell-permeable fluorescence indicator for potassium, APG2-AM, also suggested that higher percentages of cells in pluripotent populations have a low level of intracellular potassium than those in non-pluripotent populations. To test whether the cellular pluripotency could be influenced by the manipulation of intracellular potassium, we used pharmacological tools to alter the permeability and intracellular concentration of potassium in hPSCs. The treatment with two potassium channel blockers, tetraethylammonium and 4-aminopyridine, increased intracellular potassium in human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs), accompanied by the dose- and time-dependent downregulation of pluripotency markers POU5F1 and NANOG. In contrast, treatment with two types of potassium channel activators in these cells led to a decrease in intracellular potassium and the upregulation of POU5F1 and NANOG. Collectively, our data indicate that the amount of intracellular potassium is associated with the cellular states of hPSCs, and

that the manipulation of intracellular potassium with pharmacological tools has functional impact on the regulation of pluripotency signaling in hPSCs.

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F2214

QUALIFICATION AND STANDARDISATION OF A STREAMLINED EMBRYOID BODY-BASED ASSAY FOR SCREENING POTENTIAL PLURIPOTENCY POTENTIAL IN INDUCED PLURIPOTENT STEM CELL LINES IN THE EBISC IPSC BANK

O'Shea, Orla, Chapman, Charlotte and Stacey, Glyn, UK Stem Cell Bank, Potters Bar, U.K.

European Bank for induced pluripotent Stem Cells (EBiSC) is a large European public-private partnership project. The goal of EBiSC is to supply the scientific community with quality-controlled, disease-relevant research-grade iPSC lines, and robust quality control is a crucial element of the project. Here we describe the development of a routine assay for screening pluripotency potential in over 60 EBiSC cell lines, based on the generation of Embryoid bodies (EBs) as a paradigm for cell differentiation during early embryogenesis. The assay was measured at both day 7 and day 14, using a panel of germline specific markers with 7 being used for each germlayer. It was found that the assay could effectively show up regulation of markers for each germ layer, and show down regulation of stem cell markers at both day 7 and 14. It was found that the cells can be grouped by various parameters via PCA which appears to discriminate groups of iPSC lines based on their differential gene expression and potential to differentiate. It has proven possible streamline the assay; reducing the differentiation time to seven days, and the number of markers used for each germlayer to 3 or 4. As a result, this saves both time and cost whilst still providing robust data to confirm potential for generation of each germ layer. Based on the data collected, it was possible to generate a control panel of Ct values that can be used as a benchmark to compare all undifferentiated hESC and iPSC lines as a quality control.

Funding Source: Work funded by the EBiSC project

F2216

CELL-CELL CONTACT SUPER SENSITIVE CONNEXIN FOUND IN MOUSE EMBRYONIC STEM CELLS

Saito, Mikako, Asai, Yuma, Imai, Keiichi, Hiratoko, Shoya and Ogawa, Yoshihide, Tokyo University of Agriculture & Technology, Tokyo, Japan

Based on the dynamic expression patterns, 19 connexin (Cx) isoforms in mouse embryonic stem cells could be classified into pluripotent state specific, differentiating stage specific, and non-specific connexins. Cx30.3 was focused as a typical one of the first category. The expression of Cx30.3 was regulated by leukemia inhibitory factor, a specific agent for maintaining the pluripotent state. A much more striking finding was that Cx30.3 was regulated sensitively by the cell-cell contact. The expression level of Cx30.3 in ES cell colonies dramatically decreased immediately after dissociation of the colonies into single-cells. FACS analysis revealed that this decrease of the Cx30.3 expression level was not due to the unintended change of cell state into differentiating state. The Cx30.3 protein labeled with EGFP expressed by an overexpression vector located predominantly at the cell-cell contact region. Therefore the generation of Cx30.3 protein was thought to be a speedy response to the increase of the cell-cell contact region. Such a dynamic expression manner contrasted that of Cx43 that expressed ubiquitously. The speedy response of Cx30.3 was also supported by the higher rate of mRNA decay than that of *Cx43*. In conclusion Cx30.3 was a promising molecule for the study of signaling from the cell-cell contact to the intercellular molecule movement that should be of biologically substantial significance.

F2218

REGULATION OF PLURIPOTENCY BY NAT1 IN MOUSE EMBRYONIC STEM CELLS

Sugiyama, Hayami[1], Narita, Megumi[2], Iwasaki, Mio[2], Takahashi, Kazutoshi[2,3], Rand, Tim A.[3], Nakagawa, Masato[2] and Yamanaka, Shinya[2,4], [1]The Center for iPS Cell Research and Application, Kyoto, Japan, [2]Center for IPS Cell Research and Application, Kyoto University, Kyoto, Japan, [3]Gladstone Institute of Cardiovascular Disease, San Francisco, CA, U.S., [4]Gladstone Institutes, San Francisco, CA, U.S.

Novel APOBEC-1 target 1 (Nat1) is a known translation initiation factor that has high homology with the C-terminal of Eif4g1, a core translation initiation factor of cap-dependent translation. Nat1 is essential for the differentiation of mouse embryonic stem cells (mES cells). In previous studies we have shown that $Nat1^{+/-}$ mice have no detectable phenotypic changes from wild type (WT), but that $Nat1^{-/-}$ mice have embryonic lethality at the gastrulation

stage. In addition, Nat1-/- mES cells exhibit an impaired ability to differentiate into cells of all three germ layers. Furthermore, in cell culture with only LIF, Nat1-/- mES cells are undifferentiated and have a rounded, dome-like morphology that is similar to the ground state of WT mES cells cultured with 2i + LIF. However, there is little understanding of the relationship between the Nat1 knockout effect and ground state of mES cells. In the present work, we examined how Nat1 regulates pluripotency in mES cells. We revealed that Nat1-/- mES cells cultured with LIF expressed pluripotency-associated genes similarly with WT mES cells cultured with 2i + LIF, but enhanced the expressions compared with WT mES cells cultured with LIF. Re-introduction of Nat1 suppressed the enhanced expressions, and these mES cells showed morphology similar to WT mES cells. To demonstrate whether Nat1 regulates transcription directly or indirectly, we performed immunostaining of Nat1, finding that Nat1 was localized in the cytoplasm but not in the nucleus of mES cells. Lastly, to clarify Nat1 function in the cytoplasm, we performed immunoprecipitation and MS analysis, which allowed us to identify Nat1-binding proteins that reportedly regulate translation. These results indicate that Nat1 influences pluripotency through cooperation with other proteins in the cytoplasm and it indirectly regulates the transcription of pluripotency-associated genes.

F2220

MOUSE EMBRYONIC STEM CELL PENETRATION ON POLY(LACTIC-CO-GLYCOLIC ACID) SCAFFOLDS IN A STATIC CULTURE SYSTEM

Sperling, Laura Elena[1], Galuppo, Andrea G.[2], Chagastelles, Pedro[2], Gamba, Douglas[3], Braghirolli, Daikelly[2], Wendorff, Joachim[4] and Pranke, Patricia[5], [1]UFRGS, Porto Alegre, Brazil, [2]Hematology and Stem Cell Laboratory, Faculty of Pharmacy; Stem Cell Laboratory, Fundamental Health Science Institute, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, [3]Laboratory of Organic Synthesis and Polymers, Institute of Chemistry, Federal University of Rio Grande do Sul, Porto Alegre, Brazil, [4]Department of Chemistry Philipps University Marburg, Marburg, Germany, [5]Institute for Research with stem cells, Porto Alegre, Brazil

It has been proved that 3 dimensional (3D) cultivation provides an environment more similar to *in vivo* for ESCs. 3D structures can be obtained through the use of scaffolds produced by the electrospinning technique. The aim of this study has been to evaluate mouse embryonic stem cell (mESC) penetration on poly(lactic-co-glycolic acid) (PLGA) electrospun scaffolds in a static culture system. The scaffolds were produced using PLGA dissolved in dichloromethane:ethanol. For the electrospinning a high voltage of 14 kV, a collector to needle distance of 20 cm and a flow rate of 3.0 mL/h were used. The average diameter of the scaffold fibers was calculated by scanning electron microscopy. Half the scaffolds were hydrolyzed with NaOH (treated) and the other half were non-hydrolyzed (untreated). A total of 1.5×10^4 cells/cm^2 was seeded in the scaffolds and analyzed after 2, 7 and 14 days. For penetration evaluation, the cells were stained with DAPI/Phalloidin and analyzed by confocal microscopy. Cell viability was assessed by MTT test and the OCT-4 and Sox-2 were analyzed by immune staining. Statistical analyses were performed by the two-way ANOVA test. The average diameter of the fibers of the treated and untreated scaffolds was 3.6μm and 3.7μm, respectively. The penetration of the mESCs on the scaffolds presented no statistical differences in relation to the scaffold groups and cultivation times. It was expected that the cells would penetrate deeper in the treated scaffolds because the aim of the treatment was to produce a more suitable environment for the mESC cultivation. However, the data showed that the cells were unable to colonize the total area of both scaffolds. The MTT test showed that the mESCs were viable when cultivated in the scaffolds for 14 days. Although PLGA scaffolds can be used for mESC cultivation, the best viability results were obtained for the control from days 7 and 14 (cells seeded on the culture plates) ($P<0.05$). The pluripotency markers were positive for both groups. It could be concluded that both scaffolds are suitable for the maintenance of the mESCs. However, the 3D system for mESC cultivation can be considered an important tool for cell therapy and can contribute significantly to the studies of mESC pluripotency maintenance and differentiation.

Funding Source: CAPES, CNPq, FAPERGS and IPCT

EMBRYONIC STEM CELL CLINICAL APPLICATION

F2224

APPLICATION OF ALVEOLAR EPITHELIAL PROGENITOR CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS FOR EVALUATING CYTOTOXICITY OF CADMIUM

Heo, Hye-Ryeon[1], Kim, Jeeyoung[1], Kim, Eunbi[1], An, Borim[1], Kim, Woo Jin[1], Yang, Se-Ran[2] and Hong, Seok-Ho[1], [1]Kangwon National University, Chuncheon, Korea, South, [2]Kangwon National University, Chuncheon, Korea

Pluripotent stem cells (PSCs) have been considered as a good source for evaluating toxicities of harmful chemicals such as heavy metals and particulate matter. A variety of studies have been undertaken to clarify effects of cadmium (Cd) as a component of cigarette and microdust on

pathological mechanisms and preventions of various pulmonary diseases. We previously found that Cd induces inflammatory response, ER stress, and apoptosis in human bronchial epithelial cell line by modulating a set of genes. Thus, in this study, we asked if Cd induces similar set of genes and inflammatory responses in alveolar epithelial progenitor type II (AETII) cells derived from human PSCs using optimized stepwise protocols. AETII cells were treated with varying concentrations of Cd (1, 5, and 10 μM) for 24hrs and 48hrs and examined for phenotypic and genetic alterations using flow cytometry and real-time PCR. Frequencies of AETII cell specific markers (carboxypeptidase M, NKX2.1, and EPCAM) were significantly decreased after Cd treatment. In addition, Cd induced activation of inflammation- ER stress- and apoptosis-related genes. These results indicate that PSC-derived AETII cells could be useful materials for evaluating cytotoxicity of harmful materials and understanding pathological mechanisms of various pulmonary diseases as well as personalized regenerative medicine.

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F2226

IDENTIFYING PREDICTIVE MARKERS OF A SUCCESSFUL STEM CELL TRANSPLANT FOR PARKINSON'S DISEASE

Nolbrant, Sara[1], Kirkeby, Agnete[1], Tiklova, Katarina[2], Grealish, Shane[1], Heuer, Andreas[1], Cardoso, Tiago Bento[1], Kee, Nigel[2], Lelos, Mariah[3], Dunnett, Stephen[3], Perlmann, Thomas[2] and Parmar, Malin[1], [1]Lund University, Lund, Sweden, [2]Karolinska Institute, Stockholm, Sweden, [3]Cardiff University, Cardiff, U.K.

Stem cell based treatments for a number of neurodegenerative diseases are being developed and expected to reach clinical trials within a few years. A major challenge for cell production and clinical development is that transplantation is performed with immature progenitors which undergo terminal differentiation and functional maturation after transplantation *in vivo,* and no markers predicting functional efficacy of the cells after transplantation exist today. To address this issue we have performed a comprehensive retrospective analysis of grafting experiments performed using our cells at three different centers during the past five years, in order to directly compare the *in vivo* outcomes in terms of graft volumes, total tyrosine hydroxylase (TH)+ cell numbers, TH+ cell densities and functional characteristics. We have then taken an unbiased approach to identify early markers that predict successful graft outcome of transplanted DA neuron progenitors in an animal model of Parkinson's disease. Through RNAseq analysis of the >30 different batches of hESC-derived progenitors grafted, we identified a novel set of markers restricted to the caudal part of the VM that correlates with a successful *in vivo* outcome. The identification of novel markers that can better predict a successful grafting outcome will be a valuable addition to the pre-clinical work of securing the safety and efficacy of the transplantable cell product.

CANCER CELLS

F2228

INTERACTION OF HIV-1 TAT AND TIP30 PROMOTES CANCER STEM CELL PROPERTIES AND METASTASIS OF NON-SMALL CELL LUNG CANCER

Chen, Chao-Hsiung, Graduate Institute of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan and Liu, Yu-Peng, Graduate Institute of Clinical Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan Center for Infectious Disease and Cancer Research, Kaohsiung Medical University, Kaohsiung, Taiwan

In the post-combination antiretroviral therapy era, the human immunodeficiency virus (HIV)-infected patients are still suffering many other diseases, such as cancers. The incidence of HIV-infected lung cancer is increased and becomes one of the most common non-AIDS-defining cancers. Clinical evidences indicated that the non-small cell lung cancer (NSCLC) patients with HIV infection have worse prognosis compared with the NSCLC patients without HIV infection, although the pathological mechanism is largely unknown. Tat protein, which is encoded by the tat gene in HIV-1, contributed to the migration of lymphoma cells. Cancer stem cells (CSC) are responsible for tumor initiation, metastasis, and tumor recurrence. The high incidence and poor prognosis may be explain by the CSC hypothesis. In our study, we found that the ectopic expression of Tat-interacting protein 30kD, TIP30, inhibited transforming growth factor beta (TGF-b)-induced epithelial-to-mesenchymal (EMT), which was demonstrated by the morphological change and the expression levels of E-cadherin, N-cadherin, and vimentin in the NSCLC cell lines, H358 and PC13. In addition, overexpression of TIP30 also inhibited TGF-b–induced invasion of H358 and PC13 cells. In parallel, knockdown of TIP30 by shRNAs induced EMT of A549 and PC9, another NSCLC cell lines, and promoted the invasion of these cells. Besides, knockdown of TIP30 increased the number of tumor spheres and the expression of the stem-cell markers. On the other hand, treatment of recombinant HIV-1 Tat protein enhanced TGF-b-induced EMT and invasion of A549 in a dose- and time-dependent manner. Mechanistic investigations showed the direct binding of TIP30 with HIV-1 Tat, which promoted the nuclear translocation of Snail and the mRNA expression of the EMT-associated downstream genes. Together, our results provided

the molecular mechanism to explain the poor prognosis of NSCLC patients with HIV infection. Targeting of TIP30 may be a potential strategy to prevent the metastasis of HIV-infected NSCLC patients.

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F2230

ZEB2/CHROMATIN MODULATOR INTERACTIONS IN AGGRESSIVE FORMS OF T-ALL

Haigh, Jody Jonathan[1], Goossens, Steven[2,3], Van Loocke, Wouter[2], Piers, Sophie[2] and Van Vlierberghe, Pieter[2], [1]ACBD/Monash University, Melbourne, Australia, [2]Ghent University, Ghent, Belgium, [3]Monash University, Melbourne, Australia

We have demonstrated that the transcription factor Zeb2 is essential in multiple hematopoietic lineage differentiation decisions and in stem/progenitor migration using conditional loss of function approaches in the mouse. In particular, we have found that Zeb2 levels must be tightly controlled to ensure normal T-cell differentiation and function. Increased Rosa26-locus based expression of a tagged Zeb2 transgene (R26-Zeb2^{Tg}) either in the entire hematopoietic compartment (using Tie2-Cre) or in a cell-restricted manner (using Lck or CD4-Cre mouse lines) specifically transforms T-cells and leads to a disease that resembles human early thymic progenitor acute lymphocytic leukaemia (ETP-ALL). ETP-ALL is an aggressive form of T-ALL that is characterized by being refractory to chemotherapy. Initial characterization of this mouse model of ETP-ALL demonstrated that Zeb2 overexpression acts synergistically with p53 loss in enhancing T-ALL onset and involves enhanced IL7/IL7R signalling and increased leukaemia initiating capacity. Unbiased proteomics based analysis performed on both p53 null control as well as R26-Zeb2^{Tg}, p53 null T-ALL cell lines isolated from primary mouse tumours has identified several interesting Zeb2 interacting proteins including multiple components of chromatin modifying complexes. These results imply that Zeb2 may in part be transforming through alterations in both genetic and epigenetic programs. Our work reveals Zeb2 as a novel oncogene in the biology of immature/ETP-ALL and paves the way towards pre-clinical studies of novel compounds (including those targeting chromatin modulators) for the treatment of this aggressive subtype of human T-ALL using our Zeb2-driven mouse model and cell lines. Preliminary drug screening, ChIPseq and RNA-seq results will be presented concerning our novel Zeb2/chromatin modulator interaction data.

Funding Source: NH&MRC

F2232

A PHASE 1 CLINICAL TRIAL OF UC-961 (CIRMTUZUMAB), AN ANTI-ROR1 MONOCLONAL ANTIBODY, FOR TREATMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA: PLANNED SAFETY ANALYSIS

Jamieson, Catriona H.M.[1,2], Choi, Michael Y.[1], Widhopf, George[1], Castro, Januario[1], Li, Hongying[1], Juarez, Tiffany[1,2], Gorak, Susette[1], Pittman, Emily[1], Kidwell, Reilly[1], Gutierrez, Charlene[1], Rassenti, Laura[1], Messer, Karen[1], Charles, Prussak[1] and Kipps, Thomas[1], [1]Moores Cancer Center University of California San Diego, La Jolla, CA, U.S., [2]Division of Regenerative Medicine, University of California San Diego, La Jolla, CA, U.S.

ROR1 (Receptor tyrosine kinase-like Orphan Receptor 1) is an orphan-receptor tyrosine-kinase-like protein that is expressed during early embryogenesis, and plays a role in non-canonical Wnt signaling in skeletal, vascular, and neural organogenesis. ROR1 expression decreases during fetal development, and is not expressed by normal post-partum tissues. High levels of ROR1 protein are expressed in many cancers and cancer stem cells (CSC), including ovarian, pancreatic, lung, breast, and chronic lymphocytic leukemia (CLL). Cirmtuzumab is a first-in-class monoclonal antibody designed to bind the extracellular domain of ROR1. Preclinical studies confirmed activity against ROR1-expressing cancer cells and CSC, and lack of cross-reactivity with normal adult tissues. A first-in-human phase 1 study has been initiated to determine the safety, tolerability, and maximum tolerated dose (MTD) of cirmtuzumab for the treatment of patients (pts) with relapsed or refractory CLL. Dose escalation was performed using a '3+3' design with intra-patient dose escalation. 15 pts have received escalating doses of cirmtuzumab (range 15 mcg/kg to 4 mg/kg). Cirmtuzumab was well tolerated with the majority of adverse events (AEs) being grade 1 in severity. One pt had a grade 2 urinary tract infection associated with grade 2 neutropenia, thrombocytopenia, and confusion; these were assessed to be unlikely related to cirmtuzumab. All other AEs were grade 1 in severity, including anemia (6 pts), thrombocytopenia (4 pts), neutropenia (3 pts), restlessness (4 pts), bloating (3 pts), nausea (3 pts), and diarrhea (3 pts). Of the 11 pts evaluable for response, 2 experienced continued disease progression and 9 met criteria for stable disease. 2 additional pts came off study early. 1 due to disease progression after receiving a single dose; 1 pt discontinued the study after 3 doses with stable disease (a 20% decrease in target lesion dimensions by imaging) to pursue other treatment options. 2 patients have not yet reached the response assessment time point. In conclusion, pts with relapsed or refractory CLL have tolerated cirmtuzumab well. The ongoing phase 1 study will continue to evaluate higher doses to determine the MTD or biologically opti-

mal dose. Biomarker analysis, including ROR1 and other CSC marker expression, is ongoing.

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F2234

PRE-HEMATOPOIETIC PROGENITOR CELLS (CD34+ CD43+ CD45-) DERIVED FROM CML-iPSCs AS POWERFUL PLATFORM FOR ANALYSIS OF TKI-RESISTANT CML STEM CELLS

Miyauchi, Masashi[1], Arai, Shunya[2], Honda, Akira[2], Yamazaki, Sho[2], Kataoka, Keisuke[2], Yoshimi, Akihide[2], Taoka, Kazuki[2], Kumano, Keiki[2] and Kurokawa, Mineo [2], [1]University of Tokyo, Bunkyo-City, Japan, [2]University of Tokyo Hospital, Department of Hematology/Oncology, Tokyo, Japan

Since the emergence of tyrosine kinase inhibitor (TKI), imatinib, survival of patients with chronic myelogenous leukemia (CML) has been dramatically improved. However, not only imantib but also 2nd generation TKIs including nilotinib and dasatinib have not cured CML, mainly due to TKIs-resistant CML stem cells. CML stem cells represent an extremely minor population of CML cells and they have been quite difficult to analyze. To overcome this barrier, we established integration-free induced pluripotent stem cells (iPSCs) from bone marrow cells of two patients with CML in chronic phase and obtained CML pre-hematopoietic progenitor cells (pre-HPCs), immature hematopoietic-differentiated cells phenotypically defined by CD34+ CD45- among CD43+ hematopoietic-differentiated cells. In semisolid culture with methocult H4434 classic, pre-HPCs from CML-iPSCs gave rise to comparable numbers of myeloid and erythroid colonies to those from normal-iPSCs. In the liquid culture with stem cell factor, FLT-3 ligand, interleukin (IL)-3, IL-6 and thrombopoietin, pre-HPCs from CML-iPSCs exhibited increased cell proliferation compared with those from normal-iPSCs, which was canceled by imatinib. However, even in the presence of imatinib, pre-HPCs from CML-iPSCs did not undergo apoptosis and kept on growing, in marked contrast to differentiated hematopoietic cells (CD34- CD45+). These findings indicate that pre-HPCs from CML-iPSCs not only recapitulate CML disease phenotype but also show multipotent capacity and resistance against imatinib, principal features of CML stem cells. In conclusion, pre-HPCs from CML-iPSCs represent an useful and powerful platform for analysis of TKI-resistance among CML stem cells.

F2236

TARGETING A PLK1-CONTROLLED POLARITY CHECKPOINT IN GLIOBLASTOMA

Truffaux, Nathalene[1], Lerner, Robin G[1], Grossauer, Stefan[1], Kadkhodaei, Banafsheh[1], Meyers, Ian[2], Sidorov, Maxim[1], Koeck, Katharina[1], Hashizume, Rintaro[3], Ozawa, Tomoko[1], Phillips, Joanna J.[1], Berger, Mitchel S. Berger[1], Nicolaides, Theodore[4], James, David[4] and Petritsch, Claudia[5], [1]UCSF, San Francisco, CA, U.S., [2]University of California, San Francisco, San Francisco, CA, U.S., [3]Northwestern University, Chicago, CA, U.S., [4]UC San Francisco, San Francisco, CA, U.S., [5]University of California San Francisco, San Francisco, CA, U.S.

Stem-like glioma cells, frequently referred to as tumor-propagating cells or cancer stem cells are culprits for recurrence due to their intrinsic resistance to standard therapy and their ability to regrow the parental tumor in xenografts. Similar to normal neural stem cells CD133+ tumor-propagating cells undergo asymmetric, self-sustaining cell divisions (Gomez-Lopez S. et al, Cell Mol Life Sci, 2014, 71(4):575-97). Cancer-associated changes in cell division mode contribute to neoplastic transformation of glioma precursors (Sugiarto S. et al, Cancer Cell, 2011 20(3):328-40). While increased symmetric, self-renewing divisions of glioma precursors correlate with therapy sensitivity, asymmetric self-sustaining divisions potentially maintain the pool of tumor-propagating cells and contribute to therapy evasion. How pharmacological MAPK pathway inhibitors clinically evaluated for the treatment of GBM affect the division mode of tumor-propagating cells is unknown. We investigate CD133+ tumor-propagating cell responses to novel targeted therapies, in specifically, small molecule inhibitors of $BRAF^{V600E}$, a mutant kinase frequently found in pediatric malignant astrocytoma. Our investigations showed that CD133+ tumor-propagating cells have higher asymmetric divisions than progenitor-like glioma cells. CD133+ stem-like GBM subpopulation exhibit decreased sensitivity to the anti-proliferative effects of MAPK pathway inhibition and show extended G2/M phase. We find that the mitotic checkpoint kinase and polarity regulator Plk1 is more active in CD133+ tumor-propagating cells. Plk1 activity links asymmetric division and mitotic entry. In an orthotopic GBM xenograft model, combined MAPK-pathway and PLK1 inhibition showed increased anti-proliferative effects and cell death frequency towards CD133+ cells beyond that achieved by either inhibitor alone.

Ongoing work is investigating if Plk1 controls a polarity checkpoint, the integrity of which is especially important in the therapy–evasive compartment in GBM and that provides a rationale for combination therapy.

F2238

INHIBITION OF THE PROLIFERATION OF BRAIN TUMOR STEM CELLS BY TLX-INTERACTING PEPTIDE OF Lsd1

Hu, Rong[1], Sun, Xiang[1], Ma, Xin[1], Zhou, Li[1], Pei, Jianfeng[2], Swaminathan, Kunchithapadam[3] and **Yuan, Ping**[4], [1]The Chinese University of Hong Kong, Hong Kong, Hong Kong, [2]Peking University, Beijing, China, [3]National University of Singapore, Singapore, Singapore, [4]Li Ka Shing Institute of Health Sciences, the Chinese Universityof Hong Kong, Hong Kong, Hong Kong

High-grade gliomas, including glioblastoma, are aggressive primary brain tumors for which conventional treatment strategies such are mostly ineffective, as the recurrence of the tumor, due to the existence of brain tumor stem cells (BTSCs), is inevitable. Therefore, there is an urgent need to develop novel treatment methods. Tlx, an orphan nuclear receptor, is critical for the self-renewal of BTSCs. In this study, we found that Tlx recruits Lsd1, a FAD dependent lysine demethylase, to demethylate mono- and di-methylated H3K4 at the Pten and P21 promoters and repress their expression, so promoting BTSC proliferation. Through Amide Hydrogen/Deuterium Exchange and Mass Spectrometry (HDX-MS) assay, we identified four Lsd1 peptides that interact with Tlx and one of these peptides strongly inhibits BTSC proliferation by blocking the interaction between Tlx and Lsd1, leading to interesting therapeutic potential for high-grade brain tumors.

Funding Source: Nil.

TECHNOLOGIES FOR STEM CELL RESEARCH

F3002

ADVANCED DELIVERY TECHNOLOGIES FOR IMPROVED CRISPR-BASED GENOMIC EDITING IN STEM CELLS AND INDUCED PLURIPOTENT STEM CELLS FOR DISEASE MODEL GENERATION

Andronikou, Nektaria, Yu, Xin, Essex, Sean, Liang, Xiquan, Potter, Jason and de Mollerat du Jeu, Xavier, Thermo Fisher Scientific, Carlsbad, CA, U.S.

Stem cells, specifically induced pluripotent stem cells (iPSCs), hold promise for the future of regenerative medicine and therapeutic treatments for a myriad of diseases. More recently, manipulation of patient derived stem cells and progenitor lines has been achieved utilizing TALENs and CRISPRs for disease modeling. However, the lack of advanced technologies has been hindering the pace of research and discovery. Improved delivery of DNA, RNA or protein can help the progress of research and the understanding of disease pathways and mechanisms. Lipofectamine® 3000, an improved DNA delivery reagent, can achieve optimal transfection efficiency of various sizes of plasmid DNA with low toxicity in both embryonic stem cells (ESCs) and iPSCs, which have been traditionally hard to transfect. Alternative to DNA, transfection of mRNA requires that the cargo enters only the cell cytoplasm, not the nucleus, and therefore mitigates the risk of genomic integration and more importantly, greatly improves transfection efficiency. In a recent study, a newly developed reagent, Lipofectamine MessengerMAX™ showed more than 2-fold improvement in transfection efficiency with delivery of an mRNA cargo. Subsequently, delivery of Cas9-mRNA and gRNA complexes across multiple hard to transfect cell models, such as ESCs, neural stem cells and iPSCs had improved nuclease-mediated %indel rates when compared to a plasmid Cas9 approach. Most recently, we have shown that improved indel rates correlated with improved multiplex targeting and minimized off-target effects. A newly developed workflow, consisting of a Cas9-ribonucleoprotein and specifically developed transfection reagent, Lipofectamine CRISPRMAX™, can yield greater than 80% indel rates in Jurkat T-cells and iPSCs. The Cas9 RNP complex can act immediately upon cell entry, since transcription and translation are not required. Moreover, the complex is rapidly cleared from the cell, minimizing the chance for off-target cleavage events. These advancements in delivery can greatly improve downstream workflows by minimizing time and cost, enable more relevant and targeted stem cell manipulation, and ultimately augur the advancement of disease model analogs for phenotypic screens and drug development.

F3004

BIOLOGICAL CONSEQUENCE OF 3-DIMENSIONAL (3D) SPHERE FORMATION FOR EMBRYONIC AND SOMATIC STEM CELLS

Chang, Chia-Chi[1,2], Lee, Yu-Wei[2], Jiang, Shih-Sheng[3] and Yen, B. Linju[2,4], [1]The Graduate Institute of Life Sciences, National Defense Medical Center, Taipei, Taiwan, [2]Regenerative Medicine Research Group, Institute of Cellular&System Medicine, National Health Research Institutes (NHRI), Zhunan, Taiwan, [3]National Institute of Cancer Research, National Health Research Institutes (NHRI), Zhunan, Taiwan, [4]National Health Research Institutes, Zhunan Maoli County, Taiwan

3-dimensional (3D) culture for sphere formation is increasingly used as a method to better mimic physiological states. However, what the biological consequences of 3D sphere formation are for different cell types could be drastically differs for different cell type. For embryonic stem cells (ESCs) which are pluripotent stem cells, spher-

oid formation—termed embryoid bodies (EBs)—mimics the natural process of embryo development, and thus is known to result in loss of pluripotency/stemness with a concomitant occurrence of differentiation and lineage commitment. In contrast, spheroid formation in somatic cell types may allow for selection of higher 'stemness' possessing cells, i.e. selection of somatic stem cell (SSC) population as has been done with neural stem cells and mammary stem cells. Despite the biological differences in the starting cells, however, there still should be a "core process" governing the ability for 3D sphere formation, since there are also clear examples of cells which are not viable when cultured in 3D conditions. Surprisingly, at this time there is no clear understanding of convergent and divergent processes involved in 3D sphere formation of diverse cell types, including embryonic and adult stem cell types. We therefore performed transcriptome analysis on conventionally 2D-cultured and 3D spheroid ESCs and SSCs. As expected, pluripotency markers such as Oct4, Sox2, Nanog, & Klf4 were significantly down-regulated in ESCs after EB formation. Interestingly, these four markers were not significantly upregulated in somatic or cancer cells after spheroid formation. Convergent processes for both ESCs and SSCs include metabolic pathways involved in nucleotide metabolism, oxidative phosphorylation, and vitamin/cofactor metabolism. Other highly expressed pathways include MAPK signaling, cell cycle, and epithelial-mesenchymal transition. We are currently undertaking validation of these pathways. Further work is ongoing to clarify detailed and specific factors, including transcription factors and signaling molecules, involved in the ability for 2D-cultured cells to become 3D spheroids.

F3006

ACCURATE PREDICTION OF DEVELOPMENTAL CHEMICAL TOXICITY BASED ON GENE NETWORKS OF HUMAN EMBRYONIC STEM CELLS

Yamane, Junko[1], Aburatani, Sachiyo, Imanishi, Satoshi, Nishikawa, Hiromi, Nagano, Reiko, Kato, Tsuyoshi, Sone, Hideko, Ohsako, Seiichiroh, and **Fujibuchi, Wataru** [1]Kyoto University, Kyoto, Japan, [2]Advanced Industrial Science and Technology, Tokyo, Japan, [3]Tokyo Medical University, Tokyo, Japan, [4]Kanazawa University, Kanazawa, Japan, [5]National institute for Materials Science, Tsukuba, Japan, [6]Gunma University, Kiryu, Japan, [7]National Institute for Environmental Studies, Tsukuba, Japan, [8]The University of Tokyo, Tokyo, Japan, [9]CiRA, Kyoto University, Kyoto

One of the most important applications using iPS or ES cell technologies is toxicogenomic analysis as well as drug discovery. We have studied if gene expression data without using QSARs (Quantitative Structure-Activity Relationships) can predict toxicity effects on mouse and human ES cell system. We used a combination of machine learning (Support Vector Machines) and gene networks (Bayesian networks) generated from qRT-PCR data on selected 10 transcription factors for 20 diverse chemicals that were carefully selected by toxicology researchers based on literature. As a result, we first observed that mouse and human gene networks are totally different, therefore, we had to stop using mouse as human model by interspecific extrapolation. SVM predictions using only human ES cells yielded as high as 95.0%, 100.0%, and 95.0% accuracies for neurotoxins (NTs), genotoxic carcinogens (GCs), and non-genotoxic carcinogens (NGCs), respectively. As a counterpart method based on QSARs, we generated as many as 1,665 molecular descriptors using E-Dragon Website (http://www.vcclab.org/lab/edragon/) and predicted chemical toxicity categories with our SVM in the same scheme. Even the full range of 1 to 1,665 descriptors were tested, the highest accuracies for NTs, GCs, and NGCs were as low as 75.0%, 95.0%, and 80.0%, respectively, thus indicating that Bayesian network-based approaches outperform QSAR-based ones.

F3008

CHROMOSOMAL ABERRATIONS IN HUMAN ESC AND IPSC LINES OVER A TEN YEAR PERIOD FOUND BY KARYOTYPING AND COMPARATIVE GENOMIC HYBRIDIZATION

Hazelbauer, Stephanie Ann[1], Finger, Jared[2], Balaban, Esra[2] and Meisner, Lorraine Faxon[2], [1]Cell Line Genetics, Madison, WI, U.S., [2]Cell Line Genetics, Inc., Madison, WI, U.S.

Cytogenetic analysis (karyotyping and comparative genomic hybridization (CGH)) was performed on over 10,000 human embryonic stem cell (ESC) lines and induced pluripotent stem cell (iPS) lines during the years 2006 to 2016. 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. 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 balanced translocations. However, both ESC and iPS cell lines tend to develop abnormalities involving gains of the same chromosomes arms (12p, 17q, 1q and 20q). Full and partial gains of these chromosome arms have demonstrated a proliferative growth advantage that enables clones with additional copies of these regions to overtake a coexisting karyotypically normal cell line in relatively few passages. We will also show the importance

of using CGH in addition to karyotyping due to the prevalence of the subkaryotypic 20q duplication involving the BCL2L1 gene.

F3010

THE NOVEL HILIC/MS ASSAY OF 2-DEOXY-GLUCOSE-6-PHOSPHATE UPTAKE IN HUMAN IPSC-DERIVED SKELETAL MYOTUBES UNCOVERS NOVEL INDUCERS OF INSULIN RESISTANCE

Iovino, Salvatore, Bojic, Lazar, Babbs, Amanda, Rohm, Rory, Herath, Kithsiri, Shah, Vinit, Chouinard, My, Vogel, Jennifer, Brenner, Martin, Talukar, Saswata, Previs, Stephen and McLaren, David, Merck, Boston, MA, U.S.

Measuring the conversion of 2-deoxy-glucose (2DG) to 2-deoxy-glucose-6-phosphate (2DG6P) has been a viable way to determine glucose uptake in insulin-responsive cells such as adipocytes and skeletal muscle. Historically, this method has relied on measurements involving radioactive tracers. We have developed a rapid hydrophilic interaction chromatography (HILIC)-based method coupled with tandem mass spectrometry (MS) to measure non-radioactive 2DG6P in cells. We demonstrate that this method is useful for determining glucose uptake in multiple cell types such as mouse adipocytes, rat myotubes, mouse/human primary myotubes and human iPSC-derived myotubes. Moreover, we have used this method to determine the effects of novel inducers of insulin resistance in human iPSC differentiated into skeletal myotubes. To validate the relevance of this method in bridging potential translational gaps, we have studied the role of hepatokines implicated in the modulation of human metabolic processes. Cross-sectional studies in humans have demonstrated that one particular hepatokine (hepatokine-1) levels are positively correlated with insulin resistance, early atherosclerosis, fatty liver and negatively correlated with insulin sensitivity. We hypothesized that hepatokine-1 would also impair insulin-stimulated glucose uptake in skeletal muscle. Thus, using HILIC/MS we have determined the effects of hepatokine-1 on glucose uptake in human iPSC differentiated into myotubes. In control myotubes, insulin treatment robustly induced glucose uptake by 40% compared to untreated cells. Importantly, hepatokine-1 pre-treatment causes insulin resistance and decreases glucose uptake in a dose-response manner. In conclusion, we demonstrate that HILIC/MS can be used to measure glucose uptake in multiple cell lines, providing a simple, faster, non-radioactive and more accurate alternative to the traditional methodologies to quantify insulin effects on metabolic tissues, including iPSC-derived skeletal muscle.

F3012

MESENCHYMAL STEM CELLS DERIVED FROM HUMAN EMBRYONIC STEM CELLS REMAIN FUNCTIONALLY INTACT AFTER YM155 TREATMENT

Kim, Keun-Tae[1], Hong, Ki-Sung[2] and Cha, Hyuk-Jin[1], [1]Sogang University, Seoul, Korea, South, [2]Konkuk University, Seoul, Korea, South

A complete elimination of undifferentiated human pluripotent stem cells (hPSCs) is crucial for safe stem cell-based therapies in that a few residual hPSCs would develop teratoma formation when transplanted. Recently, YM155, a small-molecule to induce selective cell death on hPSCs was reported. However, the possible application of YM155 for stem cells therapy derived from hPSCs, in vivo has not been validated. Herein, we examined whether mesenchymal stem cell, derived from human embryonic stem cells (hESC-MSC) remained functionally intact after treated by YM155. hESCs-MSCs remained not only being survived under the condition of YM155, which could selectively achieve selective cell death in hESCs but also multipotent differentiation potential. Furthermore, paracrine secretion from hESC-MSC was minimally affected by YM155 treatment. Finally, hESC-MSC exhibited similar would-healing potential in vivo. Thereby, at the final procedure of MSCs derivation from hESCs, a single exposure of YM155 would be a useful approach to remove undifferentiated hESCs and assure functional characteristics of hESC-MSC for future cell therapy.

F3014

INTEGRATED CELLULAR IMAGING, ANALYSIS, AND PROCESSING TOOL FOR REGENERATIVE MEDICINE

Kwee, Edward[1,2], Powell, Kimerly ann[3] and Muschler, George F.[1], [1]Cleveland Clinic, Cleveland, OH, U.S., [2]Case Western Reserve University, Cleveland, OH, U.S., [3]Ohio State University, Columbus, OH, U.S.

Animal or human tissue is the source of cells for almost all cell-based assays, or autogenous/allograft cell therapies. The culture expanded cells from each tissue source represent the progeny of a heterogeneous population of tissue resident progenitors. Each progenitor gives rise to a clone of progeny, defined as a “colony founding unit” (CFU). The prevalence and biological performance of individual CFUs vary widely between clones. This provides an opportunity to make objectively informed choices about which CFU subtypes or clones should be included in a cell product or cell-based assay. We have developed a robust robotic platform that enables automated imaging, image analysis, and manipulation of cells. High precision motion control enables fast and accurate image acquisition and montaging. Automated motion controls

enable precise position of a pipette to aspirate and remove desired cells and transfer to another culture vessel for more specific analysis. Methods and software have been developed for image acquisition, montaging, tip management, surface detection, and resolving stage and imaging coordinates. Human cells were isolated from of discarded bone core samples from the proximal femur of patients undergoing hip arthroplasty procedures. Aspiration flow rates and heights off the culture surface were tested to determine parameters that enabled efficient cell removal from a harvest location, viable transfer of cells, and proliferation of cells after transfer. Cells were removed efficiently from the entire diameter of a pipette tip. After 24 hours in culture, transferred cells demonstrated adherence to the culture surface. After six days in culture, transferred cells showed signs of proliferation. We demonstrate the capability to image cells, efficiently remove cells, viably transfer cells to another culture surface, and show that these cells maintain their proliferative capabilities following transfer. Methods developed here can be used to inform cell picking as applied to other cell types used in cell therapy. These new set of tools will enable researchers to analyze heterogeneous populations of cells, perform clonal isolation and analysis more accurately, and provide better quality control and assurance in their cell handling processes.

F3016

SCALABLE EXPANSION OF HBM-MSC IN A FED-BATCH MICROCARRIER SUSPENSION BIOREACTOR

Lock, Lye Theng, Farrance, Iain K. and Rowley, Jon A., RoosterBio Inc., Frederick, MD, U.S.

Human bone marrow-derived Mesenchymal Stem Cells (hBM-MSCs) have been recognized as potential patient-specific drugstores, and will be a key raw material for future therapeutics, engineered tissues, and medical devices. Production technologies such as suspension bioreactors are robust, scalable platforms for generating hundreds of billions cells per manufacturing run to meet the demand for these applications. Here, we investigate the use of a suspension bioreactor, along with the use of concentrated bioreactor feed to replace nutrients and growth factors depleted from growth medium, for the scalable expansion of hBM-MSCs. The use of a media feed not only minimizes time required for media preparation and exchange, but also minimizes contamination risk associated with process manipulation. With the adaptation of cell culture from a 2D to 3D platform, we confirmed the maintenance of critical hMSC functional properties including angiogenic cytokine (FGF, HGF, IL-8, TIMP-1, TIMP-2, and VEGF) secretion, tri-lineage differentiation, and immunomodulatory potential. hBM-MSCs grown on microcarriers in bioreactors yielded comparable ($4.43x10^5$ vs. $4.25x10^5$ cells/ml) cell growth within 6 days of culture, with either half media exchange or a fed-batch process. Metabolite levels of lactate and ammonia were maintained below growth-suppression concentration of 2g/L and 2.5mM respectively with both feed regimens. In addition, hBM-MSCs in bioreactor cultures maintained their tri-lineage differentiation potential and displayed comparable angiogenic cytokine secretion levels and immunomodulatory activity to 2D cultures. Thus, microcarrier suspension culture of hMSCs, with a bioreactor feed in lieu of full or partial media exchanges, will scale hMSC culture, while streamlining the process, to provide significant time and cost savings for translational researchers in Regenerative Medicine and Tissue Engineering.

F3018

DEFINING RECURRENT DUPLICATIONS OF THE LONG ARM OF CHROMOSOME 1 IN HUMAN PLURIPOTENT STEM CELL LINES

McIntire, Erik[1], Taapken, Seth[1], Nisler, Benjamin[1], Leonhard, Kimberly[1], Larson, Anna Lisa[1], Velazquez, Gustavo[1] and Montgomery, Karen Dyer[1,2], [1]WiCell, Madison, WI, U.S., [2]Stem Cell and Regenerative Medicine Center, University of Wisconsin, Madison, WI, U.S.

Recurrent gains in specific chromosomes are acquired by human pluripotent stem cell (hPSC) lines during culture. We have found recurrent gains of chromosome 1 long (q) arm regions to be more prevalent than previously reported. In our large dataset of 1q duplications detected during routine testing of hPSC lines, there are 74 unique abnormalities. The extensive diversity of the 1q aberrations that we report here is unusual: gains of chromosome 1q take the form of trisomies, duplications, derivative chromosomes, isochromosomes, and insertions. The promiscuous nature of these changes is dissimilar to patterns in other chromosome abnormalities in hPSC. The abnormalities occur independent of culture systems (including suspension and adherent, feeder and feeder-free) and independent of passage number (3 to 142). We determined the smallest overlapping region of change to be 1q32, and defined genomic positions of the abnormalities using microarray technology. Using this data, we developed additional methods for detecting duplications in critical 1q regions. Use of fluorescence in situ hybridization (FISH) probes to screen for abnormalities in the target regions is a cost and time saving assay for routine assessment of hPSC for genomic stability.

F3020

MICROELECTRODE ARRAY: IN VITRO, FUNCTIONAL CHARACTERIZATION OF STEM CELL-DERIVED NEURONS

Parrish, Catherine, Nicolini, Anthony M, Arrowood, Colin A, Millard, Daniel C and Ross, James D, Axion BioSystems, Atlanta, GA, U.S.

The flexibility and accessibility of induced pluripotent stem cell technology has allowed complex human biology to be reproduced in vitro at previously unimaginable scales. Indeed, rapid advances in stem cell technology have lead to widespread adoption of in vitro models for neural "disease-in-a-dish" investigations and for screening applications in drug discovery and safety. However, to effectively characterize stem cell-derived neurons, and to extract meaningful and predictive information from these models, additional assays are needed to provide a functional phenotype. For these electro-active cells, measurements of electrophysiological activity across a networked population of cells provides a comprehensive view of function beyond standard characterization through genomic and biochemical profiling. Microelectrode array (MEA) technology offers such a solution by providing a label-free, non-invasive, bench-top platform to simply, rapidly, and accurately record functional activity from a population of cells cultured on an array of extracellular electrodes. Characterization of cellular activity can be achieved in minutes with advanced metrics available for in-depth analysis as needed. To demonstrate that application and utility of MEA assays provide a better understanding of stem cell-derived neuronal activity, here we will: present background on the Maestro multiwell MEA platform, discuss advantages of Axion's industry-leading MEA plate electrode density to ensure high-quality data, highlight ease of data analysis with integrated AxIS software, and illustrate that MEA data confirms neuronal functional activity through the use of ion channel blockers and compounds altering synaptic activity.

F3022

RECOMBINANT LAMININ-521 PRECOATED CULTUREWARE SUPPORT STEM CELL CULTURE AND DIFFERENTIATION

Saxena, Deepa[1], Nandivada, Hima[1], Montoya, Jorge[1], Albouy, Marion[2], Maruotti, Julien[2], Onteniente, Brigitte[2], Bergeron, Audrey B.[3], Gitschier, Hannah J.[3], Randle, David H.[3] and Flaherty, Paula[1], [1]Corning Incorporated, Life Sciences, Bedford, MA, U.S., [2]Phenocell, EVRY, France, [3]Corning Incorporated, Life Sciences, Kennebunk, ME, U.S.

Human pluripotent stem cells (hPSC) and neural stem cells (NSC) are valuable tools for application in regenerative medicine and drug development. Robust culture conditions are required for efficient large-scale expansion of these cells. Culture of hPSC and NSC requires coating of the culture vessel with extracellular matrix (ECM) protein, which can be a time consuming process. HPSC are typically cultured as clumps on complex protein substrates such as Matrigel®, synthetic substrates or recombinant proteins. Clump passaging can introduce variability and process complexity, not ideal for hPSC scale-up. NSCs are most commonly cultured on Laminin (animal-origin) with poly-L-ornithine. There is an increasing demand for higher yielding process that is free of animal-derived components. Recently, rLaminin-521 has been demonstrated to support single cell culture of hPSC. Here, we developed rLaminin-521 precoated ready-to-use cultureware and demonstrated expansion of human induced pluripotent stem cells (hiPSC) and hNSC. Human iPSC were cultured in mTeSR™1, NutriStem® XF/FF, and StemMACS™ iPS-Brew XF media for >10 passages using single-cell passaging without the ROCK inhibitor. The cells remained undifferentiated and expressed Oct-3/4, SSEA-4, TRA-1-81, Nanog and Sox2. After 10 passages the cells maintained normal karyotype and were successfully differentiated into the cells from 3 germ layers. Human NSC (H9 hESCs-derived) were cultured in a serum-free medium for three passages. During the culture, cells exhibited typical hNSC morphology and expressed hNSC-specific markers Nestin (>99%) and SOX2 (>98%) as demonstrated by flow cytometry. Furthermore, hNSC maintained their differentiation potential and successfully differentiated into neurons, olidogodendrocytes and astrocytes at the end of the three passages. The convenience and robustness of this precoated surface makes rLaminin-521 cultureware an ideal surface for culture, large-scale stem expansion and differentiation.

F3024

EVALUATION OF UNDIFFERENTIATED STATE OF HUMAN IPS CELLS BY NON-INVASIVE LC-MS/MS ANALYSIS APPROACH USING CELL CULTURE SUPERNATANT AS SAMPLES

Suzuki, Takashi[1], Gamo, Kentaro[2], Hatabayashi, Kunitada[2], Takahashi, Masatoshi[1], Kagawa, Kenichi[2], Hiramaru, Daisuke[1] and Ozaki, Shigenori[2], [1]Shimadzu Corporation, Kyoto, Japan, [2]Tokyo Electron Limited, Kobe, Japan

Pluripotent stem cells (PSCs) have an unlimited self-renewal capacity, and can differentiate into any cell type in the body. They consequently hold great promise as a source of cells for applications in regenerative medicine and drug discovery. For these applications, development of technology for the mass production of high quality pluripotent stem cells is essential. Quantitative-PCR and immunofluorescent staining are the standard methods to evaluate the undifferentiated state of PSCs. These methods are generally performed at the end of culture because

they are invasive to cells. We have aimed to establish a method for the evaluation of the undifferentiated state of PSCs without cell disruption. We focused on culture supernatant and have developed a simultaneous analysis method for 95 compounds of basal medium components and secreted metabolites using liquid chromatography-tandem mass spectrometry (LC-MS/MS). In this experiment, we used iPS cell line PFX#9 derived from umbilical cord bloods. Cells were maintained in either mTeSR1, TeSR-E8, Essential 8 medium on vitronectin. Appropriate cytokines were added to each medium at next day of seeding to induce three germ layers (endoderm, mesoderm, or ectoderm). Supernatants were collected every 24 hours from culture fluids. Biomarkers in the supernatants were screened for evaluation of the undifferentiated state of iPSCs. Comparative studies revealed that there were some characteristic compounds in each differentiated state of the cells. Multivariate analysis using the data acquired from each culture day was also performed to investigate potential ability of LC-MS/MS in culture supernatant analysis. As the result, LC-MS/MS analysis was able to distinguish undifferentiated state and differentiated state in the early stages of differentiation. Thus, our method has the potential to be an effective strategy to evaluate the undifferentiated status of PSCs without cell disruption.

F3026

OPTICALLY CONTROLLED PRECISION DELIVERY OF BIOCHEMICAL-LADEN MICROPARTICLES FOR TARGETED DEVELOPMENTAL SIGNALLING GRADIENTS AND CHEMOTACTIC CUES IN CELLULAR MICROENVIRONMENTS

Ware, Jamie, Kirkham, Glen, Allen, Stephanie, Shakesheff, Kevin and Buttery, Lee, University of Nottingham, Nottingham, U.K.

The microenvironment or niche in which cells reside, has a profound effect on development, differentiation and the maintenance of functional tissues. Therefore, to increase the therapeutic potential of tissue engineered products and models, a greater understanding of these microenvironments and recapitulating specific molecular and cellular signals and interactions is required. The formation of gradients of molecules is known to be a fundamental element in cell niche functionality from early embryonic development through to developed adult tissues. Currently, the capacity to fully investigate and replicate cellular microenvironments is limited by the lack of technologies which can manipulate molecular signalling gradients at a sufficiently small length scale, in 3D. We present a new technique for controlling dynamic molecular gradients in biomimetic platforms, with the ability to direct their chemical, spatial and temporal nature. Our approach uses low intensity laser light controlled using holographic optical tweezers to precisely position and manoeuver both cells and biochemical-laden microparticles for controlled delivery of biochemicals with unparalleled levels of tunability and accuracy at the sub-micron length scale. We demonstrate the sustained and targeted release of retinoic acid from polymeric microparticles to establish a controlled molecular gradient to stimulate expression of retinoic acid responsive elements particular zones within embryonic stem cells aggregates, replicating the morphogen based signalling that occurs in normal mouse embryonic development. Furthermore, we demonstrate the establishment of a platelet-derived growth factor gradient and localized chemotactic migration within a mouse primary osteoblast microenvironment. This approach of using laser light controlled by holographic optical tweezers enables precise and tuneable control over the geometry and the dynamics of molecular signalling at the scale of individual cells, and offers new opportunities for understanding the cellular microenvironments which are so important in the creation of functional tissue engineering analogues.

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F3028

UNDERSTANDING THE FUNCTIONAL ROLE OF RECEPTOR CLUSTERING IN EPH SIGNALING

Yang, Chun and Schaffer, David, University of California, Berkeley, Berkeley, CA, U.S.

Multivalent ligand interaction – the binding of multiple ligands on one entity to cognate receptors on another, leading to receptor clustering at the cell membrane – is an important process that is often required for signal initiation or potentiation. One important example is Eph signaling, which has vital roles in regulating tissue development and homeostasis and is implicated in multiple diseases. During the formation of a signaling complex, ephrin ligand presented from one cell surface binds to Eph receptors on a neighboring cell, which leads to receptor clustering and activation. Intriguingly, when ephrin bind to Eph in its monomeric form, it behaves as an antagonist that inhibits receptor oligomerization by separating and stabilizing individual ligand-bound-receptor complex. However, the fundamental mechanisms by which receptor-ligand complex formation impacts signal activation are currently unknown. Moreover, it is unclear whether clustering of receptors, even in the absence of a ligand, may be sufficient for signal activation. To this end, we conjugated an EphB4 binding peptide that binds to EphB4 with Kd of 70 nM to hyaluronic acid (HA) to generate a multivalent ligand, so that we can interrogate whether ephrin-B2 independent EphB4 clustering is possible, or even sufficient to induce neurogenesis. The addition of HA-peptide or HA-ephrin –B2 conjugates into neural progenitor cells induced distinct EphB4 cluster formation, indicated that the multivalent EphB4 binding peptide conjugate was capable of recruiting and stabilizing EphB4 cluster formation similar-

ly to the multivalent HA-ephrin-B2. Next, the expression of neuronal maker β-III-tubulin in NPCs was examined by immunostaining. Compared to the basal expression level in the negative control, the multivalent HA-peptide conjugate significantly promoted neurogenesis, in which 43.9 ± 3.7 % of the cells were identified to be β-III-tubulin positive as well as exhibited a neuronal morphology. This level is comparable to the one that was incubated with the HA-ephrin-B2. These preliminary results demonstrated that the synthetic multivalent complex could serve as an effective tool for mechanistic investigation of EphB4 clustering and the corresponding downstream differentiation.

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F3030

THE EFFICACY AND SAFETY OF COLLAGEN-I AND HYPOXIC CONDITIONS IN URINE-DERIVED STEM CELL EX VIVO CULTURE

Jung Yeon, Kim[1], Hyo-Jung, Lee[1], Na Hee, Yu[1], So Young, Chun[1] and Tae Gyun, Kwon[2], [1]Kyungpook national university, Daegu, Korea, South, [2]Kyungpook national university Hospital, Daegu, Korea

Upper urinary tract-derived urine stem cells (USCs) are considered a valuable mesenchymal stem cell source for autologous cell therapy. However, the reported culture condition for USCs is not appropriate for large-quantity production, because cells can show limited replicativity, senescence, and undesirable differentiation during cultivation. These drawbacks led us to reconstitute a culture condition that mimics the natural stem cell niche. We selected ECM protein and oxygen tension to optimize the ex vivo expansion of USCs, and compared cell adhesion, proliferation, gene expression, chromosomal stability, differentiation capacity, immunity and safety. Culture on ColI supported highly enhanced uUSC proliferation and retention of stem cell properties. In the oxygen tension analysis (with ColI), 5% O2 hypoxia showed a higher cell proliferation rate, a greater proportion of cells in the S phase of the cell cycle, and normal stem cell properties compared to those observed in cells cultured under 20% O2 normoxia. The established reconstituted condition (ColI/hypoxia, USCsrecon) was compared to the control condition (noECM/normoxia). The expanded 4th passage of USCsrecon showed highly increased cell proliferation and colony forming ability, maintained transcription factors, chromosomal stability, and multi-lineage differentiation capacity (neuron, osteoblast, and adipocyte) compared to that observed in the control. In addition, USCsrecon retained their immune-privileged potential by showing a lack of HLA-DR expression, as well as non-tumorigenicity with in vivo testing at week 8. Therefore, we think that our reconstituted condition allows for expanded uUSC cell preparations that are safe and useful for application in stem cell therapy.

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F3032

NATURALLY-OCCURRING DMSO-FREE CRYOPROTECTANTS TOWARD CLINICALLY EFFECTIVE SOLUTIONS: UNDERSTANDING THE EFFECTS OF CPA TYPE AND DELIVERY ON DIFFERENT CELL TYPES

Matosevic, Sandro, Pasley, Shannon, Torres, Fabiana and Zylberberg, Claudia, Akron Biotech, Boca Raton, FL, U.S.

As the search for clinically safer cryoprotectants (CPAs) that are devoid of DMSO progresses, so does our understanding of the biophysical cues that direct the cells to respond differently to the presence of different stabiliziers and cryoprotectants. This makes developing optimal cryopreservation media reliant on understanding the effects of these cryoprotectant candidates on individual cells' biophysical and mechanical properties. Work in our laboratories has focused on developing novel DMSO-free formulations based on combinations of natural biomolecules, amino acids, carbohydrates and antioxidants, tailored for a range of cell types: this includes mesenchymal stem cells, fibroblasts, red blood cells and NK cells as well as complex tissues, by optimizing the medium composition and the freezing procedure. Here we present recent work from our lab on developing formulations that address the shortcomings associated with traditional, largely DMSO-based cryoprotectants, and highlight factors such as freezing procedure, cryoprotectant loading and type on optimal recovery of cells post-thaw. Viability is measured alongside functionality to determine cryoprotectant performance and eliminate temporary, acute effects that mask longer term CPA performance. All of the components are naturally-occurring and safe to cells and suitable for direct clinical use. This works contributes to a deeper understanding of how different cell types respond to unconventional freezing procedures and CPAs and enables us to get closer to developing clinically effective, safer CPAs than the currently used standards.

TISSUE ENGINEERING

F3034

EVALUATION OF MULTIPLE SAFE GUARD SYSTEM FOR IMPROVED SAFETY PROFILE OF HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED CELLULAR THERAPEUTICS

Abujarour, Ramzey J.[1], Lan, Weijie[1], Lee, Tom Tong[1], Bonello, Greg[1], Meza, Miguel[1], Robinson, Megan[1], Clarke, Raedun Laurie[2], Huang, Xiaosong[1], Truong, Christopher[1], Lynn, Chris[1], Robbins, Dave[1], Rezner, Betsy[1], Abbot, Stewart[1], Shoemaker, Daniel[1] and Valamehr, Bahram[1], [1]Fate Therapeutics, Inc., San Diego, CA, U.S., [2]Fate Therapeutics, San Diego, CA, U.S.

Human induced pluripotent stem cell (hiPSC) technology has advanced into clinical trials, progressing toward fulfilling its potential as an unlimited source of genetically modified cellular therapeutics. To mitigate possible safety concerns related to cellular and gene therapies the use of conditional suicide genes has been proposed and currently under clinical investigation. Although these approaches significantly improve the safety profile of the treatment, they rely on random insertion of the genes of interest by viral vector systems and risk unpredictable effects such as insertional mutagenesis. A potentially safer and more predictable approach to deliver suicide genes into clinical-grade cells is through copy number controlled and safe harbor loci-specific targeting. We show targeting chemical-inducible Caspase 9 (iCasp9) suicide gene into the AAVS1 safe harbor locus in hiPSCs is a viable and attractive option to efficiently and specifically eliminate hiPSCs and cell progeny in vitro and derived teratomas in vivo. However, although rare, occasional resistance to iCasp9-inducible killing was observed in vitro and in vivo. Sequencing of the in vitro treatment-resistant clones derived from a single refractory line showed an inactivating point mutation in iCasp9 which was the consensus sequence for all tested clones. Furthermore, killing kinetics were improved and rate of resistance was lessened in vitro and in vivo by selecting hiPSC clones with biallelic iCasp9 insertions. To guard against the potential to develop resistance to a single inducible safety system, we explored the generation and selection of hiPSC clones containing multiple conditional suicide genes, including iCasp9 and herpes simplex virus thymidine kinase, each incorporated into a unique safe harbor locus. This type of high-resolution clonal selection was made possible by our single cell and feeder-free naïve hiPSC platform, previously reported to efficiently facilitate multiple genetic modifications at the single pluripotent cell level. The dual safe guard system was compared in vitro and in vivo to the single iCasp9 safety switch to evaluate safety and efficient elimination of engrafted cells. Our study describes novel findings toward designing optimal safety systems for integration into hiPSC-derived cellular therapies.

F3036

HUMAN MULTIPOTENT ADULT PROGENITOR CLASS CELLS PROMOTE BONE FORMATION IN VITRO AND IN VIVO VIA UPREGULATED ANGIOGENIC ACTIVITY

Bossert, Jeff and LoGuidice, Amanda, RTI Surgical, Alachua, FL, U.S.

The promotion of vascularization in a bone defect site is an important precursor to new bone formation and remodeling, and bone defects lacking adequate peripheral circulation frequently suffer from nonunion bone regeneration. The use of mesenchymal stem cell therapy for angiogenic and osteogenic applications has been extensively investigated; however the angiogenic potential of multipotent adult progenitor class cells (MAPCs) within osteogenic applications remains largely undiscovered. While both MAPCs and MSCs are multipotent mesodermal-derived cells that can differentiate into bone, MAPCs can also differentiate into endothelial cells and thrive under hypoxic conditions, ideal properties for bone wound environments. The objective of this study is to determine how the release of angiogenic proteins by MAPCs affects the bone remodeling process by examining the secretome of MAPCs exposed to bone in vitro, and through in vivo implantation of MAPCs in a bone defect site. An in vitro bone exposure study using human-derived MAPCs cultured indirectly with human demineralized bone matrix in a transwell system showed a two-fold increase in expression of Angiogenin and a ten-fold increase in vascular endothelial growth factor (VEGF) expression compared to MAPCs not exposed to bone after five days as measured in conditioned media using quantitative ELISA assays. When cultured under osteogenic conditions in media containing L-ascorbic acid, dexamethasone, and β-glycerophosphate, we found using quantitative colorimetric assays that MAPCs express high levels of alkaline phosphatase (ALP) and calcium mineralization, critical factors necessary for osteogenesis. When MAPCs were implanted into a bone defect in the fibula of athymic rats, a 3-fold increase in blood vessel ingrowth after 14 days was observed using staining with von Willebrand Factor (vWF) when compared to an empty defect. Evaluation of bone regeneration using μCT revealed that animals treated with MAPCs had a 1.5-fold increase in tissue mineral content and a higher degree of defect repair compared to animals not receiving MAPCs. Our results demonstrate that the properties of MAPCs make them a promising therapeutic for orthopedic applications, particularly in applications with limited vascularity.

F3038

ADIPOSE-DERIVED STROMAL CELLS FACILITATE EX VIVO ANGIOGENESIS AND ENDOTHELIAL CELL SURVIVAL WITH PERICYTE DIFFERENTIATION IN THE DECELLULARIZED RAT LUNGS

Doi, Ryoichiro[1], Tsuchiya, Tomoshi[1], Hatachi, Go[1], Nishimura, Satoshi[2,3], Matsuyama, Mutsumi[4], Nakazawa, Yuka[4], Yamasaki, Naoya[1], Matsumoto, Keitaro[1], Miyazaki, Takuro[1], Sengyoku, Hideyori[1], Ogi, Tomoo[5], Yukawa, Hiroshi[5], Mitsutake, Norisato[4] and Nagayasu, Takeshi[1], [1]Nagasaki University Graduate School of Biomedical Science, Nagasaki, Japan, [2]The University of Tokyo, Tokyo, Japan, [3]Jichi Medical University, Tochigi, Japan, [4]Nagasaki University, Nagasaki, Japan, [5]Nagoya University, Nagoya, Japan

Bioengineered lungs consisting of a decellularized lung scaffold repopulated with the patient's own cells could provide desperately needed donor organs in the future. However, bioengineered lungs are fragile because of the immature vascular structure. Mesenchymal stem/stromal cells (MSC) are multipotent cells that can differentiate into a variety of cell types. Among the MSCs, adipose-derived stem/stromal cells (ASCs) are an easily accessible-abundant cell source, which have equal differentiative potential compared to bone marrow-derived mesenchymal stem cells. With a view to overcoming the poor vascular performance of bioengineered lungs, the objective of the current study was to investigate the effects of allogeneic ASCs on engineering pulmonary vasculature in a decellularized lung scaffold. In the present study, we created three groups that differed in the composition of seeded cells and growth factors: rat lung microvessel endothelial cells (RLMVECs) alone (n=3), RLMVECs with ASCs (n=3), or a combination of RLMVECs, ASCs plus fibroblast growth factor 9 (FGF9) (n=3). The re-endothelialized scaffolds were maintained ex vivo under vascular flow for 8 to 16 days, and then, cell morphology, differentiation, location, viability and angiogenesis related gene expressions were examined. Thrombogenicity and vascular permeability of the bioengineered lungs were assessed in vivo transplantation models. We found that seeded ASCs contributed to endothelial cell survival and qPCR array revealed angiogenesis related genes were significantly increased in ASCs administrated groups. ASCs also exhibit a perivascular phenotype in the repopulated pulmonary vasculature. Cell tracking studies indicated that ASCs stabilized regenerated blood vessels in recellularized lungs. On the other hand, we could not detect differentiation of ASCs to endothelial cells and FGF9 addition did not show significant effects. In the transplanted bioengineered lungs, the administration of ASCs significantly reduced vascular permeability but not thrombogenicity. Our results indicate that ASCs stabilized regenerated pulmonary vessels and facilitated the alveolar capillary barrier function as perivascular cells. We conclude that ASCs might be an essential cell source for vascular regeneration in the bioengineered lungs.

F3040

ENGINEERED 3D MICROTISSUES TO PROMOTE MATURATION OF HUMAN PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES

Hookway, Tracy[1], Votaw, Nicole[1], Mendoza, Nik[1] and McDevitt, Todd[2], [1]Gladstone Institute, San Francisco, CA, U.S., [2]Gladstone Institutes, San Francisco, CA, U.S.

Human pluripotent stem cells (hPSCs) represent a potentially unlimited source of cardiomyocytes (CM) for the study of cardiovascular development and disease, as well as for use in regenerative cardiac therapies. However, current differentiation protocols of hPSCs generally yield CM exhibiting fetal-like phenotypes, with properties distinct from adult CM. In order to realize their full therapeutic potential, effective methods to mature hPSC-derived CM must be developed. In this study, we modulated CM maturation by generating engineered 3D cardiac microtissues and controlled their formation and culture conditions. CM differentiation from hPSCs was initiated by modulating Wnt signaling in 2D monolayer cultures. Cells were dissociated at various time points throughout differentiation and either seeded into microwells to form spheroidal or ring-shaped cardiac microtissues or mixed with cardiac fibroblasts at different ratios to form co-cultured tissues. After 24hrs of formation, 3D microtissues were maintained for an additional 4-21 days and a subset of microtissues was exposed to 10μM triiodothyronine (T_3), a thyroid hormone known to promote cardiac maturation during embryonic development. The formation of 3D cardiac tissues promoted up to 8-fold enrichment of the starting CM population and by day 7 of culture, nearly 100% of the tissues exhibited spontaneous beating, synchronous calcium transients, and expression of cardiac markers (Nkx2.5, α-actinin, connexin43). Cardiac microtissues could be electrically paced and exhibited ionotropy in response to calcium and isoproterenol. Culture of 3D microtissues promoted defined CM sarcomeric structures indicated by increased sarcomere length and length/width ratio, reflecting a more mature phenotype found in 3D constructs compared to 2D controls. CM microtissues treated with T_3 exhibited a >10-fold increase in expression of several cardiac genes involved in contractile function (acnt2, actc1, tnni3) and calcium handling machinery (serca2), compared to either untreated 3D or 2D controls. These results suggest that the combination of 3D tissue architecture and exposure to developmental hormones act synergistically to promote CM maturation, suggesting

that multiple soluble and biophysical cues are necessary for generating mature CM phenotypes.

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F3042

DUAL-REPORTER MOUSE INDUCED PLURIPOTENT STEM CELLS FOR VASCULAR CELL SHEET ENGINEERING

Kwong, George[1,2], Wong, Joyce Y.[2] and Kotton, Darrell N.[1,3], [1]Boston University Medical Campus, Center for Regenerative Medicine, Boston, MA, U.S., [2]Boston University, Department of Biomedical Engineering, Boston, MA, U.S., [3]Boston University School of Medicine, Boston, MA, U.S.

Current treatments for vascular occlusive disease (angioplasty/stenting and bypass surgery) have limitations, including restenosis and lack of suitable autologous grafts. Synthetic grafts and tissue-engineered blood vessels (TEBVs) have overcome some limitations; however, immunogenicity remains a problem when autologous cell sources are unavailable. Induced pluripotent stem cells (iPSCs) provide an alternative cell source, where non-immunogenic cells of multiple lineages, including endothelial cells (ECs) and vascular smooth muscle cells (vSMCs), can be derived from patient-specific iPSCs. Although many studies have developed protocols for differentiating iPSCs into SMCs, there is no good surface marker for isolating pure populations of vascular-specific SMCs. Smooth muscle actin (SMA) is a characteristic intracellular marker of SMCs; however, it is also expressed in cardiomyocytes and myofibroblasts. In addition, SMA does not distinguish between SMCs from different anatomical locations. To address these issues, we have generated and differentiated a novel mouse iPSC line with a dual-reporter (green fluorescent protein (GFP) for SMA and red fluorescent protein (DsRed) for neural/glial antigen 2 (NG2)) into both vascular cell types – ECs (using VEGF) and vSMCs (using PDGF-BB and TGF-β). ECs were isolated using co-expression of CD31/CD144, and vSMCs, which are characterized by co-expression of SMA/NG2, were isolated using our dual-reporter system. iPSC-derived SMCs expressed characteristic markers (SMA, SM22α, MHC, NG2), but with lower levels of expression compared to primary SMCs. Functionally, traction force microscopy was used to measure contractile forces of iPSC-derived vSMCs (SMA^{GFP}/$NG2^{DsRed}$), SMCs (SMA^{GFP}), and primary vascular and bronchial SMCs to characterize the functional phenotype of different SMCs. Ongoing studies with agonists and antagonists of SMC contractility will further elucidate the functional phenotype of different SMCs. With this study, we will gain a better understanding of SMC phenotype from different anatomical locations and how those phenotypes affect functional properties of TEBVs. This novel reporter system will also allow for generation of ECs and vSMCs, which can be used for in vitro models of vascular disease, such as pulmonary hypertension.

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F3044

DIRECT 3D PRINTING OF HUMAN STEM CELL-DERIVED CARDIOMYOCYTES AND EXTRACELLULAR MATRIX AS A NOVEL TISSUE MODEL

Liu, Justin[1], Ma, Xuanyi[2], Zhu, Wei[2] and Chen, Shaochen[2], [1]University of California, San Diego, La Jolla, CA, U.S., [2]University of California, San Diego, La Jolla, CA, U.S.

Cardiomyocyte alignment is paramount for cardiac pump function. Several techniques like micro-contact printing and photolithography have been used to guide sarcomeric alignment in two dimensions; however engineering physiologically relevant three-dimensional (3D) tissues that incorporate patient specific cell type and native extracellular matrix components remains a major challenge. In this work, we utilized a high-throughput microfabrication system designed in our lab called, Dynamic Optical Bioprinting (DOB), to print cardiomyocytes with photopolymerizable ECM-based materials in an aligned 3D configuration, similar to native left-ventricular myocardium. We have tailored the mechanical, physical, chemical, and biological properties of these photopolymerizable ECM-based materials to enhance cardiac adhesion, alignment, and spreading. Using this platform, induced pluripotent stem cell cardiomyocytes (iPS-CM) were successfully seeded within user-defined patterns, preferentially aligning to the designated geometry, and display a rod-like morphology. The anisotropic geometry induces sarcomeric alignment along the long-axis of the cardiomyocyte tissue construct resulting in regular coupled action potentials and increased contractility parallel to the patterned axis, as compared to an isotropic geometry. Beyond alignment, a 3D printed mechanical tester composed of photopolymerizable gelatin methacrylate (GelMA) and hyaluronic acid glycydyl methacrylate (HAGM) was designed to measure force production of 3D printed tissues. This 3D tissue construct can also be used as an in vitro diagnostic test for cardiac drug screening applications, as well as for future use in transplantation.

Funding Source: California Institute for Regenerative Medicine (Grant #: RT3-07899)

F3046

DISEASE-SPECIFIC INTEGRATED MICROPHYSIOLOGICAL HUMAN TISSUE MODELS

Onishi, Kento, UC Berkeley, Berkeley, CA, U.S., Español-Suñer, Regina, University of California, San Francisco, San Francisco, CA, U.S., Grimm, Andrew, UCSF, San Francisco, CA, U.S., Conklin, Bruce, Gladstone Institutes, San Francisco, CA, U.S., Willenbring, Holger, UCSF The Liver Center, San Francisco, CA, U.S. and Healy, Kevin E., University of California, Berkeley, CA, U.S.

The traditional paradigm of drug discovery has relied heavily on the use of animal models to inform and guide the development of therapeutics. Induced pluripotent stem cells (iPSCs) are an ideal source of cells for drug discovery and screening, as they are readily expandable, can recapitulate disease models by direct genetic modification or by derivation from patients, and can differentiate into physiologically relevant cell types. To increase throughput while lowering costs in drug discovery, we have developed microphysiological systems (MPS) to create functional modules of two organs that are often compromised by adverse drug effects: the heart and the liver. These MPS contain iPS-derived cardiac and liver cells and are designed to be facile to load, to feed, to collect real-time data, and to extract cells for end-point analysis. For the cardiac MPS, we have demonstrated robust, long-term culture of unidirectional beating cardiac microtissues, with drug IC_{50} values that more accurately reflect clinical data compared to simple 2D iPSC culture. For the liver MPS, using cues from developmental biology, we first optimized a co-culture system of iPS-derived endothelial cells, mesenchymal stromal cells, and hepatic endoderm to recapitulate the parenchymal and non-parenchymal populations of the liver. We demonstrated that these cells can be cultured long-term in our liver MPS and that they exhibit robust expression of liver markers and, importantly, functional cytochrome P450 (CYP) enzymes, specifically CYP3A4. To progress towards an integrative, human-on-a-chip model, we plan to combine our two MPS devices and show effects of drug metabolites from the liver MPS to the cardiac MPS. Taken together, we have developed an integrative, high-content platform that will serve to augment drug screening data generated from animal models and also to serve as a platform to investigate disease physiology in an isogenic setting.

F3048

AN ENGINEERED 3D MYOCARD MODEL BASED ON HUMAN IPS-DERIVED CARDIOMYOCYTES AND A PORCINE SMALL INTESTINAL SUBMUCOSA SCAFFOLD

Schuerlein, Sebastian[1], Kadari, Asifiqbal Mustak Ahmed[2], Ueda, Yuichiro[2], Walles, Heike[1,3], Edenhofer, Frank[4] and Hansmann, Jan[1,3], [1]Department Tissue Engineering and Regenerative Medicine, University Hospital, Wuerzburg, Wuerzburg, Germany, [2]Stem Cell and Regenerative Medicine Group, Institute of Anatomy and Cell Biology, University of Wuerzburg, Wuerzburg, Germany, [3]Translational Center Wuerzburg ‘Regenerative Therapies in Oncology and Musculoskeletal Disease, Wuerzburg, Germany, [4]University of Wuerzburg, Würzburg, Germany

Today, cardiovascular disorders are major causes of mortality worldwide. After myocardial infarction, pathological remodeling processes in the damaged heart can lead to cardiac insufficiency. Emerging innovative therapeutic concepts are based on the application of cells. However, a lack of suitable cell sources and robust functional integration into the host tissue are current shortcomings. Induced pluripotent stem (iPS) cells might serve as an unrestricted cell source of autologous, therapy-specific cardiac cells for future regenerative applications. We used the protocol of Kadari et al. for robust cardiac differentiation of human iPS cells. In particular cardiomyocyte differentiation proceed within 15 days with an efficiency up to 95 %, demonstrated by flow cytometry staining. hiPS-derived cardiomyocytes (iPS-CM) were functionally validated by α-actinin staining, transmission electron microscopy and electrophysiological analysis. In order to explore the possibility to derive iPS-CM-based cardiac 3D tissues, we seeded iPS-CM on a decellularized small intestinal submucosa scaffold. Tissue maturation was characterized by immunofluorescent staining and electrophysiological analyses. Furthermore, functionality was demonstrated by administration of drugs. Five days after seeding, the cardiac patch exhibited synchronous beating. Hematoxylin and eosin staining (HE) showed a physiological tissue architecture and a homogeneous cell distribution. Expression of cardiac-specific markers like cardiac Troponin T, cardiac Troponin C, alpha actinin and Connexin 43 confirmed cell identity. Obtained tissue patches responded to electrical stimulation (pacing) and beating rate was altered by isoproterenol and adrenalin. Our data demonstrates the possibility to establish a 3D myocardial tissue patch that can potentially serve as implant or in vitro test system.

F3050

HAIR FOLLICLE AND SEBACEOUS GLAND DE NOVO REGENERATION WITH CULTURED EPIDERMAL STEM CELLS AND SKPS

Wu, Yaojiong and Wang, Xiaoxiao, Tsinghua University, Shenzhen, China

Stem cell based organ regeneration is purported to enable the replacement of impaired organs in the foreseeable future. Here we demonstrated that a combination of culture expanded epidermal stem cell (Epi-SCs) derived from the epidermis and skin-derived precursors (SKPs) were capable of reconstituting functional hair follicles and sebaceous glands (SG). When Epi-SCs and SKPs were mixed in a hydrogel and implanted into an excisional wound in nude mice, the Epi-SCs formed de novo epidermis along with hair follicles, while SKPs contributed to dermal papilla in the neogenic hair follicles. Notably, a combination of culture expanded Epi-SCs and SKPs derived from adult human scalp were sufficient to generate hair follicles and hair. BMP4, but not Wnts, sustained the expression of alkaline phosphatase in SKPs in vitro and the hair follicle-inductive property in vivo when SKPs were engrafted with neonatal epidermal cells into excisional wounds. In addition, Epi-SCs were capable of differentiating into sebocytes and formed de novo SGs, which excreted lipids like normal SGs. Thus our results indicate that cultured Epi-SCs and SKPs are sufficient to generate de novo hair follicles and SGs, implying great potential to develop novel bioengineered skin substitutes with appendage genesis capacity.

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F3052

SINGLE-STEP SEAMLESS SDF CORRECTION OF F508DELCFTR IN INTEGRATION-FREE SENDAI VIRUS REPROGRAMMED CF-IPSCS

Suzuki, Shingo[1], Chosa, Keisuke[1,2], Barilla, Cristina[1,3], Hay, Sarah[1], Gruenert, Lukas[1], Kai, Hirofumi[2] and Gruenert, Dieter C.[1,4], [1]University of California, San Francisco, San Francisco, CA, U.S., [2]Kumamoto University, Kumamoto, Japan, [3]university of Pavia, Pavia, Italy, [4]University of Vermont School of Medicine, Burlington, VT, U.S.

Induced pluripotent stem cells (iPSCs) and effective gene modification via Homology Directed Repair (HDR) have been significant advances in the development of gene- and cell-based therapies for inherited diseases. Patient-specific iPSCs in which the disease-causing mutation has been corrected have the potential to functionally repair damaged tissues and organs. Cystic fibrosis (CF), the most common inherited disease in the Caucasian population, manifests with significant multiorgan damage. CF is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. Our previous studies demonstrated sequence-specific transcription activator-like effector endonucleases (TALENs) enhancement of single-step seamless gene correction of the F508delCFTR mutation by small/short DNA fragments (SDFs) in a retroviral-reprogrammed CF-iPSC. Because the CF-iPSCs are retrovirally reprogrammed, there is a likelihood of random integration of the viral vector gene in the genomic DNA limiting their therapeutic potential. To overcome this limitation, the studies here have employed integration-free Sendai virus reprogramming to generate iPSCs from a CF patient with a F508delCFTRF/508delCFTR genotype. One of the F508delCFTR was corrected via HDR enhancement with both TALENs used previously and several pairs of clustered randomly interspersed short palindromic repeat (CRISPR)/Cas9 nickases designed to cleave at specific sites adjacent to the F508del locus. These nickases were assayed for optimal targeting efficiency. The pair that most effectively introduced nicks in double strand DNA were co-transfected with the donor DNA in CF-iPSCs, and its correction efficiency was compared with that of TALENs. The corrected CF-iPSCs were detected with the allele-specific PCR and were isolated using a cyclic enrichment strategy followed by the limiting dilution. The single-step seamless SDF correction strategy in integration-free CF-iPSCs resulted in cells that retained pluripotent features.

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REGENERATION MECHANISMS

F3056

ADULT AXOLOTLS CAN REGENERATE COMPLEX NEURONAL DIVERSITY AND LOCAL CIRCUIT IN RESPONSE TO BRAIN INJURY

Amamoto, Ryoji[1], Huerta, Violeta[2], Takahashi, Emi[3], Dai, Guangping[4], Grant, Aaron[5], Fu, Zhanyan[2] and Arlotta, Paola[6], [1]Harvard University, Somerville, MA, U.S., [2]Broad Institute, Cambridge, MA, U.S., [3]Boston Children's Hospital, Boston, MA, U.S., [4]Massachusetts General Hospital, Boston, MA, U.S., [5]Beth Israel Deaconess Medical Center, Boston, MA, U.S., [6]Harvard University, Cambridge, MA, U.S.

The axolotl can regenerate multiple organs, including the spinal cord and the brain. It remains, however, unclear

whether complex neuronal diversity, intricate tissue architecture, and axonal connectivity can be regenerated; yet, this is critical for recovery of function and a central aim of cell replacement strategies in the mammalian central nervous system. Here, we demonstrate that, upon mechanical injury to the adult pallium, axolotls can precisely regenerate the original diversity of neurons. Notably, regenerated neurons acquire functional electrophysiological traits and respond appropriately to afferent inputs. Despite the striking precision of this regenerative process, we also uncovered previously unappreciated limitations by showing that newborn neurons organize within altered tissue architecture and fail to re-establish the long-distance axonal tracts and circuit physiology present before injury. The data provide a direct demonstration that even complex neuronal diversity can be regenerated in axolotls, but challenge prior assumptions of functional brain repair in regenerative species.

F3058

PRESERVED NEPHROGENESIS FOLLOWING PARTIAL NEPHRECTOMY IN EARLY NEONATES

Kirita, Yuhei[1], Kami, Daisuke[2], Ishida, Ryo[1], Adachi, Takaomi[1], Matoba, Satoaki[1], Kusaba, Tetsuro[1] and **Gojo, Satoshi**[2], [1]Kyoto Prefectural University of Medicine, Kyoto, Japan, [2]Kyoto Prefectural University of Medicine, Kyoto, Japan

Total nephron segments are never reconstituted after resection in adult kidney. However, whether the neonatal kidney can maintain the capacity for neonephrogenesis after resection is not elucidated yet. We performed partial resection of the kidney on neonatal rats on postnatal days 1 (P1x kidney) and 4 (P4x kidney) and examined the morphological change and its relevant factors. The P1x kidney bulged into the newly formed cortex from the edge of the wound with less inflammation and apoptotic cells, while regeneration failure and inflammation were prominent in the P4x kidney. Immunohistochemistry of the P1x kidney demonstrated preservation of the glomerulus number, cortex area, and collecting duct 28 days post resection, whereas these parameters in the P4x kidney were significantly decreased. Quantitative PCR of transcriptional factors related to nephrogenesis showed that Six2 expression was significantly higher in the kidney of postnatal day 1 than that of day 4. Immunofluorescence analysis showed that Six2 positive cells were scarcely found in the kidney of postnatal day4 and the bulk of them localized within the renal vesicle that is completely surrounded by the tubular basement membrane. In contrast, large number of Six2 positive cells in the kidney of postnatal day1 still existed at the peripheral layer and localized in both cap mesenchyme and renal vesicles. Time course analysis showed the Six2 mRNA expression was preserved in P1x kidney whereas rapidly lost in age matched control. Though, during normal development, Six2 positive cells in cap mesenchyme rapidly disappear after birth, these cells were well preserved in the tissue surrounding resected area even at 2days after resection in P1x kidney. In conclusion, our results indicate that the kidneys of early neonate rats retain the capability to accelerate nephrogenesis; however, this ability is lost soon after birth, which might be attributed to Six2 positive cells.

F3060

CHARACTERIZATION OF ENHANCER OF ZESTE HOMOLOGY 2 (EZH2) IN COMBAT BLAST RELATED HETEROTOPIC OSSIFICATION

Ji, Youngmi[1], Griffin, Daniel W[1,2], Patel, Vyomesh[1] and Nesti, Leon J[1,2], [1]USUHS, Bethesda, MD, U.S., [2]Walter Reed National Military Medical Center, Bethesda, MD, U.S.

Epigenetic control for gene expression is an important role in many biologic and disease conditions. Enhancer of Zeste Homology 2 (EZH2) is known for its role as a master epigenetic regulator and suppressor of gene expression. Its action is through trimethylation of histone 3 lysine 27, as a part of the polycomb repressive complex-2. EZH2 has been involved in stem cell maintenance, inflammation, hematopoiesis regulation, and in many cancers. Post-traumatic heterotopic ossification (HO), ectopic bone formation in the soft tissues, is a common complication in our wounded warrior population after combat related blast injuries. Repeated surgical observations have further linked areas of abundant fibrotic scarring within the wound to an abnormal healing process. It is known that increases in EZH2 levels correlate with increased fibrosis. Our laboratory has isolated and identified a population of mesenchymal progenitor cells (MPCs) within war-traumatized muscle tissues. Characterized MPCs were similar to bone marrow derived Mesenchymal Stem Cells (MSCs) in morphology, cell surface epitopes, and tri-lineage differentiation (e.g. osteoblasts, adipocytes, and chondrocytes). In this study, we hypothesized that EZH2 could function as an epigenetic controller through MPCs in the context of fibrosis and osteogenesis during this wound healing process after traumatic blast injury. By using QPCR analysis and immunohistochemistry, we found that the fibrosis markers (TGFB1, FN1, TIMP1, and COL1A1) were elevated at greater than 10-fold change in traumatized blast injured muscle tissues. One of the known osteogenic markers, RUNX2, also showed increased expression in traumatized blast injured muscle tissue at greater than 26-fold change. Immunohistochemistry demonstrated that EZH2 and RUNX2 expression was localized in the same area of the fibrotic tissue. This finding opens the interesting question of how MPCs contribute to HO development through this EZH2 epigenetic master in a fibrotic microenvironment after traumatic blast injuries. With further

understanding of how EZH2 plays role in a fibrotic microenvironment to induce ectopic bone, a possible inhibitor (e.g. DZNep, EPZ-6438, and GSK343) or inducer may be applied to prevent HO occurrence or disease progression in the future.

F3062

STEM CELL NICHE CONTROL OF HAIR REGENERATION

Rezza, Amelie, Wang, Zichen, Ma'ayan, Avi and **Rendl, Michael**, Icahn School of Medicine at Mount Sinai, New York, NY, U.S.

To coax adult tissue stem cells (SCs) towards specific cell fates for replacement therapy, we need to understand their regulation by signals from the SC niche. Adult hair follicle (HF) regeneration is an excellent model system for studying activation of adult tissue SCs by niche signals, since the spatial and temporal aspects of the physiological destruction, rest and re-growth during the normal hair cycle are well-defined. Specialized dermal papilla (DP) cells act as niche cells that instruct adult HF SCs to switch from a resting to an activated fate during the regeneration phase of the hair cycle. However, the specific activating niche signals are largely unknown due to the lack of genetic tools to systematically isolate, characterize DP niche cells and target them for gene ablation. Here we use triple transgenic reporter mice combined with immunofluorescence to isolate by multicolor cell sorting DP niche cells and bulge/germ HF SCs at all key hair cycle stages, and for comparisons also regular non-niche fibroblasts and all other HF and epidermal epithelial cells. We systematically define their molecular features at a transcriptome scale to tease out molecular signatures of enriched cell-type specific genes. We further establish for the first time inducible cre-mediated gene ablation in the adult HF SC niche and interrogate the functional role of PDGF signaling in the niche for SC activation during adult HF regeneration. This work provides essential new tools and insights for the HF SC niche, as it identifies the essential signal(s) from the DP niche that activate SCs during HF regeneration, a prerequisite for improving our ability of manipulating adult skin SCs for future hair regenerative therapies.

F3064

CELL SHAPE DETERMINE CELL FATE THROUGH EPIGENETI CHANGE

Zhang, Can, Chen, Xiao, Zhang, Erchen, Yang, Long and Ouyang, Hongwei, School of Basic Medical Science, Zhejiang University, Hangzhou, China

Adult mesenchymal stem cells, such as tendon stem/progenitor cells (TSPCs), are of great interest for cell-based tissue engineering strategies, but they tend to lose their phenotype and even function after being cultured in vitro. In recent years, epigenetic studies on stem cells have indicated that specific histone alterations and DNA methylation play essential roles in cell differentiation. However whether epigenetic modifications may play a role in the dedifferentiation process of cells upon ex vivo culture have not been investigated. In this study, we compared tendon tissue and different passages of TSPCs, found Histone deacetylation and DNA methylation were involved in the dedifferentiation process. Utilize of Scx-GFP reporter system (Scx is a key transcription factor of tendon), we found TSA (a pan inhibitor of HDACs) could only rescue Scx expression in early passages which indicated other incident must happen in the late passages. Gene expression, immunofluorescence, Western blot and histology analysis proved that TSA could efficiently promote tenogenesis of TSPCs by promoting the synthesis of tendon ECM and enhancing the expression of tendon-associated genes. Lastly, we found TSA can maintain phenotype of TSPCs in vitro through inhibiting HDACs and upregulating acetylation level of Histone. HDAC 1 and 3 target specific HDAC inhibitors produced a similar effect to that of TSA treatment on the expression of tendon-related markers. Hence, we conclude that phenotype change of cells from in vivo to in vitro is associated with epigenetic status and may involve a dynamic process. Suppression of HDACs by TSA in early passage could maintain phenotype of TSPCs so as to generate greater numbers of functional TSPCs for use as transplantation.

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ETHICS AND PUBLIC POLICY; HISTORY OF STEM CELL RESEARCH; SOCIETY ISSUES; EDUCATION AND OUTREACH

F3068

STEM CELL TOURISM AND THE CHALLENGE OF REGULATORY COSMOPOLITANISM

Foong, Patrick, Swinburne University, Melbourne, Australia

Stem cell tourism is a serious problem and unexplored territory. This is where acute patients, especially the very ill, who live in countries where stem-cell-based medical treatments are not available, travel to other countries to seek such therapies. There have been reports of baseless claims of cures, charlatans and adverse medical events including deaths. These treatments are at the experimental stage and most are unregulated. Some of the medical clinics offering these therapies are even supported by their local government, regulatory agencies and medical associations. Stem cell tourism is important to the economies of some developing countries, which may thus be re-

sistant to prohibitions by law. Different countries have different notions of 'right' and 'wrong' and state sovereignty dictates that no state can enforce its laws on another. In the international community, a nation cannot enforce its laws on other nations. It is a challenge if a regulatory approach of a country attempts to dictate local standards to another nation, that is, where it seeks to steam roller over local culture and difference. Stem cell tourism is a prudential calculation for the individual and, importantly, the state has a moral obligation to inform its citizens of the potential risks of receiving experimental and unregulated stem cell-based treatment in overseas. Also, the ISSCR guidelines might raise awareness effectively of these important issues in the international community. While stem cell tourism per se is a challenging area and appears to be irresolvable, local regulators still need to set up an effective national regulatory framework for regulating HESC research within the country. This presentation evaluates effective measures, legal as well as non-legal, to control and mitigate it.

F3070

ENHANCED ELECTRONIC CONSENT AND EDUCATIONAL MATERIALS FOR STEM CELL STUDIES MAY INCREASE PROTOCOL ADHERENCE

Kozlovich, Cory Edward[1], Patterson, Jennifer[1] and Jamieson, Catriona H.M.[1,2], [1]CIRM Alpha Stem Cell Clinic at UC San Diego Health, San Diego, CA, U.S., [2]Moores Cancer Center University of California San Diego, La Jolla, CA, U.S.

Clinical trial participants retain 30-48% of informed consent information, including procedures and major risks. Given the complexity of first in human stem cell interventions and limited information on the long term effects, investigators may wish to exceed the minimum elements of consent in order to ensure patient self-determination and to increase their safety. Recent FDA guidance recommends facilitating consent electronically to enhance such understanding of the trial risks and to increase retention and comprehension of information which may lead to an increase in protocol adherence. Enhanced electronic consent may include many types of media, and including multimedia has been shown to significantly increase understanding by 31%. Likewise, enhanced consent has been shown to significantly increase comprehension by 41%. To achieve these goals in such novel studies, the CIRM Alpha Stem Cell Clinic at UC San Diego Health is piloting this implementation. We present our process. Our site designed enhanced consent that is delivered via iPad tablets and uses free software. Validating electronic participant signatures is complicated by stringent federal regulations, so our method supplements the paper form (participants continue to sign paper). We include visual aids such as a calendar of events, videos and interactive graphics; navigation tools such as a table of contents and internal hyperlinks; distinct protocol sections; formatted text to highlight areas of frequent concern or confusion; annotation features and a glossary. Beyond an enhanced consent document, we offer interactive educational e-books covering basic clinical trial information and a stem cell primer, including content from ISSCR.We expect rapid deployment and positive response by sponsors, participants and investigators. A collection of 2 enhanced consents and 3 enhanced educational reference books were created in-house in 2 months. The enhancements were received by our IRB with encouragement and enthusiasm during a preliminary consultation. The first consent (CLL) was approved by our IRB within 5 business days of expedited submission. Pending results, other sites may wish to institute enhanced documents from our free model. Interest in deploying this technology has already been expressed by CIRM and other investigators at our site.

LATE BREAKING ABSTRACT

F4002

PROTEOMIC CHARACTERIZATION OF STEM CELL-DERIVED EXTRACELLULAR MATRICES

Ragelle, Héloïse, Larson, Benjamin L., Naba, Alexandra, Hynes, Richard O, Langer, Robert and Anderson, Daniel G, Massachusetts Institute of Technology, Cambridge, MA, U.S.

The extracellular matrix (ECM) is a complex three-dimensional network of proteins that provide stem cells with the appropriate environment and signals to regulate cell functions. It is hypothesized that the specific components and biophysical properties of the ECM coordinate intracellular signaling and downstream biological response through bidirectional interactions with the cells. In the context of stem cell biology, it is critical to decipher how individual components of the ECM regulate specific stem cell functions to better comprehend the role of the ECM. In addition, this understanding can provide further insight for the rational design of ECM mimicking substrates for tissue engineering. However, precise characterization of the ECM remains challenging because of the biochemical intractability of its components, which are composed predominantly of highly cross-linked, glycosylated and insoluble proteins. To address these challenges, we have applied a multidimensional proteomic approach that consists of a sequential digestion of the ECM proteins following by tandem mass spectrometry and bioinformatic analyses. By coupling this method to quantitative proteomics, we were able to characterize and compare the molecular composition of cell-derived ECM produced by different cell types, specifically bone marrow derived-human mesenchymal stem cells (hMSC), adipose-derived hMSC and human neonatal dermal fibroblasts and to evaluate ECM

variability across different donors. In parallel, we investigated the proliferation and mRNA transcriptome of the cells cultured on the different ECM and compared to the same cells cultured on tissue culture polystyrene (TCPS). We observe that while each ECM present tissue-specific proteins, they share a set of common proteins. Quantitative proteomic analysis of this common set of proteins reveals a specific matrisome signature for each type of ECM. The matrices lead to differential proliferation and gene expression across the cells as compared to TCPS culture, indicating that the ECM components influence each cell type in a unique manner. Further experiments are ongoing to understand the role of ECM components and properties in cell response. Our approach will inform the design of defined systems that recapitulate critical ECM effects for therapeutic applications.

F4004

HUMAN PLURIPOTENT STEM CELL DERIVED HUMAN CORTICAL NEURONS AS A NOVEL MODEL TO STUDY STROKE AND NEURODEGENERATION MECHANISMS

Fan, Jing[1], Xu, Jinchong[1], Lee, Yun-il[1], Rines, Jesse[1], Chen, Li[1], Zhu, Juehua[1,2], Kang, Hochul[1,3], Dawson, Ted[1] and Dawson, Valina[4], [1]Johns Hopkins University, School of Medicine, Baltimore, MD, U.S., [2]Jinling Hospital, Nanjing University School of Medicine, Nanjing, China, [3]Ajou University School of Medicine, Suwon, Korea, The Democratic People's Republic of, [4]Johns Hopkins University School of Medicine, Baltimore, MD, U.S.

Poly(ADP-ribose) polymerase-1 (PARP-1) has been shown to be a key regulator in neuronal death and survival in rodent studies. During toxic insults and ischemic stroke, excess PAR polymers are produced in the nucleus and then translocate to mitochondria to induce the release of apoptosis-inducing factor (AIF) and trigger neuronal death. However, whether PARP-1 plays a similar role in human neurotoxicity after stroke as well as how PAR polymers translocate out of nucleus remains unclear. One study suggested that histone H1.2 translocates from the cell nucleus to cytoplasm in response to DNA damage to induce cell death signaling. Furthermore, Iduna, a PAR-dependent E3 ubiquintin ligase, has been identified to protect neurons from PARP-1 dependent cell death (Parthanatos) by targeting PARsylated proteins for degradation. PARP-1 knockout and Iduna overexpression both protect neurons against ischemic insult in mice. Thus, we investigated whether human PARP1 interacts with Histone H1.2 to regulate the survival of human cortical neurons after NMDA and oxygen/glucose deprivation (OGD) challenge and whether Iduna regulates survival of human neuron through degradation of PARsylated H1.2. Human cortical neurons have been differentiated from human ES and iPS cell lines, where neurons represent more than 90% of the cultured population and exhibit complete cortical layer markers. Human cortical neurons challenged with toxic NMDA and OGD stimuli exhibit profound neuronal death, which is largely inhibited by a PARP-1 inhibitor, DPQ, but not with several other neuronal death pathway inhibitors. Lentiviruses carrying shRNAs or sgRNAs targeting histone H1.2 protect human cortical neurons against NMDA/OGD-induced neuronal death. Furthermore, H1.2 translocates from the nucleus to cytoplasm, and co-localizes with PAR polymer, in human cortical neurons after toxic NMDA or OGD stimulation, which can be reduced by overexpressing wild-type Iduna. Our data suggest that PARP1, H1.2 and Iduna together may play a critical role in regulating human stem cell-derived neuron survival. Based on this study, pharmaceutical agents targeting the PARP-1/H1.2/Iduna pathway can be tested in the human neuron neurotoxicity model to provide drug candidates that will promote survival of neurons in stroke and neurodegenerative disorders.

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F4006

EFFICIENT DERIVATION OF NEURAL PROGENITORS FROM ADULT BONE MARROW STROMAL CELLS BY TRANSIENT HYPOXIA TREATMENT

Shea, Graham, Tsui, Alex, Mung, Alan, Shum, Daisy and Chan, Ying Shing, The University of Hong Kong, Hong Kong, Hong Kong

Schwann cell transplantation promotes post-traumatic regeneration in the CNS and PNS. Neural progenitors enriched from adult bone marrow stromal cell (BMSCs) can be directed to differentiate into Schwann cells, representing a readily accessible source for autologous cell-based therapy. It is essential from a translational standpoint to achieve means of enriching for neural progenitors within these niches. In this current study, we demonstrate that transient culture of rat and human BMSCs in hypoxic conditions (1% for 24 hrs) significantly increases the numbers of neural progenitors. Following on, we identify that hypoxic treatment induced up-regulation of EGF receptor (EGFR) in BMSCs. Addition of EGFR inhibitor while cells are cultured in hypoxic condition largely abolished this increase in neural progenitor production. As a proof of principle that these neural progenitors can generate mature cells for transplantation purposes, neural progenitors generated from hypoxic treatment were able to differentiate into myelin-forming Schwann cells. Our findings provide a potential means of generating increased numbers of neural cell derivatives from the adult bone marrow, and

potentially other niches, for the purpose of nerve injury repair.

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F4008

MODELING HUMAN LUNG DEVELOPMENT AND DISEASE USING HUMAN PLURIPOTENT STEM CELLS

Huang, Sarah Xuelian[1], Ciancanelli, Michael[2], Toste de Carvalho, Ana[1], Zhang, Shen-Ying[2,3], Volpi, Stefano[4], Pellier, Isabelle[5], Notarangelo, Luigi[6], Casanova, Jean-Laurent[2,7] and Snoeck, Hans-Willem[1], [1]Columbia University Medical Center, New York, NY, U.S., [2]Rockefeller University, New York, NY, U.S., [3]INSERM UMR1163, Necker Hospital for Sick Children, Paris, France, [4]Harvard Medical School, Boston, MA, U.S., [5]Oncology Research Center Nantes-Angers, Angers, France, [6]Division of Immunology, Children's Hospital Boston, Boston, MA, U.S., [7]Howard Hughes Medical Institute (HHMI), New York, NY, U.S.

Directed differentiation of human pluripotent stem cells (hPSCs), either embryonic stem cells (ESCs) or induced PSCs (iPSCs), into specific organ cell types provides a unique platform for human disease modeling and the advancement of regenerative medicine. We have previously established, based on developmental paradigms, a highly efficient method for directed differentiation of hPSCs into lung and airway epithelial cells. Long-term differentiation in vivo and in vitro yielded cells expressing markers of basal, goblet, club, ciliated, type I and type II alveolar epithelial (AT I and II) cells and were particularly enriched in distal ATII cells capable of surfactant protein-B (SP-B) uptake and release. This technology may lead to the development of novel approaches for tissue or organ replacement therapy in the future, and has immediate applications in modeling human lung development and disease. First, we applied this technology to model genetic predisposition to severe influenza infection. We studied the intrinisc, non-hematopoietic immune response to influenza virus in hiPSC-derived lung epithelial cells from a patient with severe influenza caused by interferon regulatory factor 7 (IRF7) deficiency. Our results show that influenza virus-induced IFN-β production was impaired in patient's cells compared to healthy control lines. These findings indicate that human IRF7-dependent IFN-α/β amplification in pulmonary epithelial cells (intrinsic immunity) is important for protection against influenza virus in host defense. Second, since our generated cells are particularly enriched in distal ATII cells, we successfully modeled human distal lung development using a three-dimensional (3D) lung culture system. Embedded cells formed structures compatible with those from saccular/alveolar stage of developing lung. This platform provides an opportunity to study the important differences between human and mouse lung development.

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F4010

THERAPEUTIC EFFECTS OF INTRACEREBRALLY TRANSPLANTED HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS IN A RODENT MODEL OF SUBACUTE-STAGE ISCHEMIC STROKE

Jeong-Eun Noh[1], Chunggab Choi[1], Yongwoo Jeong[1], Seung-Hun Oh[2] and Jihwan Song[1]

ong, Jihwan, [1]CHA Stem Cell Institute, Department of Biomedical Science, CHA University, Gyeonggi-do, Korea, [2]Department of Neurology, CHA Bundang Medical Center, CHA University, Gyeonggi-do, Korea

Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) are candidate source of cells for restorative therapy in stroke as they have several advantages, including painless procurement procedure, abundant cell numbers, and lower risk of tissue contamination. Delivery route of administered stem cells can influence the migration and the final destination of transplanted cells. However, it remains unclear how the route of cell administration affects the grafting process, in terms of migration, distribution, and the numbers of cells in the target tissues, which will affect the therapeutic efficacy. In this study, we investigated the therapeutic effects of GMP-grade human umbilical cord-derived MSCs (hUC-MSCs) in a rodent model of middle cerebral artery occlusion (MCAo), according to transplantation routes. hUC-MSCs were injected either intravenously or intracerebrally at one week after MCAo, and the transplanted animals were examined up to 8 weeks using various behavioral tests. While intravenously transplanted animals showed little effect on functional recovery, intracerebrally transplanted ones exhibited significant behavioral improvements in rotarod, stepping and modified neurological severity score (mNSS) tests. We also found that hUC-MSCs transplanted directly into the brain at 1W post-MCAo can contribute to the reduction of infract volume, as well as glial scar formation area. Transplanted cells also contributed to the endogenous neurogenesis of the host brain, the number of proliferating neuroblasts significantly increases in the subventricular zone (SVZ). Taken together, these results provide strong evidence that intracerebrally transplanted hUC-MSCs at 1W post-MCAo are functional in vivo and may provide clinical implications for the treatment of subacute- or chronic-stage stroke patients using hUC-MCSs or MSCs in general.

F4012

CLINICAL STUDY OF INFLAMMATORY RESPONSE AFTER IMPLANTATION OF ALLOGENEIC MESENCHYMAL STEM CELLS IN HORSES

Alvarenga, Marina Landim[1], de Souza, Jaqueline[1], dos Santos, Vitor Hugo[1], Pfeifer, João Pedro[1], Viana, Gustavo[1], Rodrigues, Miriam[1], Amorim, Renée[1], de Carvalho, Marcio[2] and Alves, Ana Liz[1], [1]FMVZ- UNESP, Botucatu- SP, Botucatu, Brazil, [2]NeoGene FMB-UNESP, Botucatu, Brazil

The use of cell therapy for the treatment of musculoskeletal injuries in horses is increasing worldwide. Mesenchymal stem cells (MSCs) are easily isolated and expanded in vitro, which makes them promising targets for use in clinical trials. Typically, these cells are obtained from the own patient (autologous), however, the use of cells from a donor horse (allogeneic) creates the opportunity of immediate treatment after diagnosis. This study aimed to evaluate the local effects of allogeneic mesenchymal stem cells transplantation in horses. For such, three healthy horses were selected as donors and subjected to bone marrow aspiration for MSCs harvest and culture. The recipient animals were submitted to intra muscular implantation of allogenic stem cell or PBS application and divided into six groups (G1- implantation of allogenic MSC and biopsy after 24 hours; G2- implantation of allogeneic MSCs and biopsy after 7 days; G3- two applications of allogeneic MSCs with a 7 days interval between then and biopsy 7 days after the second application; G4- application of PBS and biopsy after 24 hours; G5- application of PBS in the and biopsy after 7 days; G6- two applications of PBS with 7 days interval between then and biopsy 7 days after the second application). A possible local inflammation reaction was analyzed through physical and ultrasound examination, thermography, histopathology and gene expression of IL 1beta and TNF-alpha. The results showed that the transplantation of allogeneic MSCs caused no significant clinical changes in the recipient animals. However, a second application of allogeneic MSCs from the same donor led to significant increase in gene expression of TNF-alpha, which can characterize a cellular immune response. Thus, we conclude that although the application of allogeneic MSCs is a clinically safe procedure, it must be held with discretion, particularly after multiple applications using the same donor. With these results we intend to contribute in future researches, besides support the treatment of musculoskeletal disorders in horses with allogeneic MSCs by creating a stem cell bank from healthy donors. Also we emphasize the importance of constant progress in studies related to immunogenicity of MSC in order to enable a safe clinical application, not only in veterinary medicine, but also in humans.

F4014

ECTOPIC EXPRESSION OF JMJC DOMAIN OF HISTONE H3K27 DEMETHYLASE ENHANCES DIFFERENTIATION OF PLURIPOTENT STEM CELLS

Akiyama, Tomohiko, Wakabayashi, Shunichi, Soma, Atsumi, Sato, Saeko, Nakatake, Yuhki, Oda, Mayumi, Murakami, Miyako, Sakota, Miki, Nohtomi-Chikazawa, Nana, Ko, Shigeru and Ko, Minoru, Keio University, Tokyo, Japan

Histone H3 lysine 27 trimethylation (H3K27me3) functions as a barrier against cell differentiation through the suppression of developmental gene expression in pluripotent stem cells (PSCs). In this study, we have generated human PSC lines in which genome-wide reduction of H3K27me3 can be reversibly induced by ectopic expression of the catalytic domain of histone demethylase (HDMc). We found that forced demethylation of H3K27me3 triggers the expression of mesendodermal genes even without changing the culture conditions of PSCs. Furthermore, transient expression of HDMc followed by the expression of lineage-defining transcription factors (TFs) enabled PSCs to directly activate the tissue-specific genes. We have also demonstrated that the introduction of HDMc combined with TFs results in rapid and efficient differentiation of PSCs into functional hepatic cells and skeletal muscle cells. These results suggest the usefulness of direct manipulation of epigenomes for generating desired cell types from PSCs for regenerative medicines.

F4016

CONTROLLED VECTORIZATION OF CORE PLURIPOTENCY TRANSCRIPTION FACTORS IN HUMAN CELLS BY A NEW CELL-PENETRATING PEPTIDE

Caulier, Benjamin[1,2], Berthoin, Lionel[3], Lenormand, jean-Luc[2], Dagher, marie-Claire[2], Polack, Benoit[2], Garbant, Frederic[1,2], Toussaint, Bertrand[2] and Laurin, David[1,2], [1]EFS Alpes-Auvergne, La Tronche, France, [2]TIMC-IMAG, La Tronche, France, [3]UCSF, San Francisco, CA, U.S.

The generation of induced Pluripotent Stem Cell (iPSC) from fibroblasts and the direct conversion of somatic cell fate hold a great promise for regenerative medicine and disease modelling. Route leading a cell to an iPSC or to another cell type has been originally obtained by the forced expression of Transcription Factors (TF) involved in the maintenance of the desired cell type. For example iPSC were generated from fibroblasts with only 4 TF, commonly known as the Yamanaka cocktail: Oct4, Sox2, Klf4 & c-Myc. From the discovery of these mechanisms, many teams have engineered cells by available tools such

has the use of retroviruses in order to express TF. However, these techniques use genetic materials. Development of alternative strategies for ectopic expression of TF has been conducted in order to be applied in a clinical environment. Direct protein delivery or small molecule treatment in culture could avoid the use of genetic material. In this context, our laboratory has developed a cell penetrating peptide (CPP) based on the Epstein-Barr virus ZEBRA transcription factor. The minimal amino acid region implicated in cellular uptake named MD_{11}, has been characterized and is able to translocate high molecular weight proteins in an endocytosis-independent mechanism, allowing the internalization of cargo proteins in fully biologically active form. We thus develop 6 proteins each fused to MD_{11} in N-terminus to the Yamanaka's factors as well as Nanog and Lin28a proteins known to increase reprogramming efficiency. This domain does not interfere with Oct4 association to its own DNA consensus sequence. Moreover, MD_{11}-Oct4 can be localized to the nucleus of in vitro treated cells in two hours. In a context of reprogramming experiments, the combination of repeated treatment leads to the transcriptional activation of target genes such as core pluripotency network. Overall results paved the way to a safe and clinically compliant vector. We are now working on inducing pluripotency from differentiated cells.

F4018

MATRIX METALLOPROTEINASES 2 AND 9 HAVE DISTINCT AND COMPLEMENTARY ROLES DURING EMBRYONIC HEMATOPOIETIC STEM CELL DEVELOPMENT

Cortes, Mauricio[1], Theodore, Lindsay N.[1], Natsuhara, Kelsey[2] and North, Trista E.[1], [1]BIDMC, Harvard Medical School, Boston, MA, U.S., [2]Harvard University, Cambridge, MA, U.S.

The extracellular matrix (ECM) is responsible for modulating cell-cell interactions, cell migration and growth factor signaling. Remodeling of the ECM is necessary for tissue maintenance and plays a critical role in disease. Despite the importance of the ECM in tissue homeostasis, its role during hematopoietic stem cell/progenitor cells (HSPCs) induction and self-renewal is poorly understood. To elucidate the contribution of the ECM in the process of definitive hematopoiesis, we utilized the zebrafish model due to strong conservation of the blood system and their amenability to chemical and genetic manipulation. Matrix metalloproteases (MMPs) are known to have an essential role in numerous developmental processes. A chemical screen of broad-spectrum MMP inhibitors identified MMP2 and MMP9 as potential modulators of definitive hematopoiesis. Inhibition of vascular-associated MMP2 caused abnormal expression of the HSPC markers runx1/cmyb by whole mount in situ hybridization (WISH) within the aorta-gonad-mesonephros (AGM) region, the site of HSPC emergence. FACS analysis of HSPCs in the trunk region revealed a significant accumulation of HSPCs in the AGM and a concomitant decrease of HSPCs in the caudal hematopoietic tissue (CHT), a secondary site of HSPC expansion. In addition, MMP2 inhibition resulted in reduced seeding of the thymus. Live imaging during the window of HSPC emergence using a novel transgenic reporter (Runx144:GFP) confirmed abnormal HSPC "budding" in the absence of MMP2 activity. Epistasis experiments using a fibronectin mutant indicated that fibronectin degradation by MMP2 was required for proper HSPC extravasation. In contrast, inhibition of the leukocyte-associated MMP9 resulted in abnormal CHT vascularization and aberrant accumulation of HSPCs within the CHT at the expense of thymic seeding. Interestingly, the MMP9-dependent CHT defect and HSPC aggregation phenotype could be rescued by stromal cell derived factor 1 (sdf1) knockdown, suggesting that MMP9 controls colonization of secondary sites of hematopoiesis by regulating SDF1 turnover. In sum, our findings indicate that MMP2 and MMP9 play distinct but complementary roles in HSPC mobilization within and between embryonic niches through remodeling of the ECM and regulation of chemokine activity, respectively.

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F4020

SPHINGOSINE-1-PHOSPHATE SIGNALLING REGULATES MOUSE PANCREATIC LINEAGE ALLOCATION AND SPECIFICATION

Serafimidis, Ioannis[1], Rodriguez-Aznar, Eva[2,3] and **Gavalas, Anthony**[2,3], [1]Biomedical Research Foundation of the Academy of Athens, Athens, Greece, [2]Paul Langerhans Institute Dresden of Helmholtz Center Munich, Dresden, Germany, [3]Deutsches Forschungszentrum Für Gesundheit Und Umwelt GmbH, Neuherberg, Germany

During development, progenitor expansion, lineage allocation and implementation of differentiation programs need to be tightly coordinated so that different cell types are generated in the correct numbers for appropriate tissue size and function. Pancreatic dysfunction results in some of the most debilitating and fatal diseases, including pancreatic cancer and diabetes. Signalling pathways and transcription factors regulating progenitor expansion and specification have been identified and Notch signalling has been implicated in lineage allocation but it remains unclear how lineage allocation and specification are coordinated. Using a combination of genetic approaches, organotypic cultures of embryonic pancreata and genomics we found that sphingosine-1-phosphate (S1p), signalling through the G protein coupled receptor (GPCR) S1p2, plays a key role in pancreas development linking these processes. First, it promotes progenitor survival as well as

acinar and endocrine specification by stabilising the Hippo pathway effector YAP. We found that YAP function is essential for endocrine specification thus linking a regulator of progenitor growth with specification. Additionally, endocrine cell specification relies on Gai subunits, revealing an unexpected specificity of selected GPCR intracellular signalling components. Finally, independently of YAP stabilization, S1p signalling attenuates Notch levels thus regulating lineage allocation. These findings identify S1p signalling as a novel key pathway coordinating cell survival, lineage allocation and specification and linking these processes by regulating YAP levels and Notch signalling. Understanding lineage allocation and specification in the pancreas will shed light in the origins of pancreatic diseases and may suggest novel therapeutic approaches.

F4022

THE GAMMA-SECRETASE INHIBITOR DAPT ENHANCES CARDIAC REPROGRAMMING BY INCREASING MEF2c BINDING TO CARDIAC GENE PROMOTERS

Abad, Maria[1], Hashimoto, Hisayuki[1], Zhou, Huanyu[1], Serrano, Manuel[2], Bassel-Duby, Rhonda[1] and N. Olson, Eric[1], [1]University of Texas Southwestern Medical Center, Dallas, TX, U.S., [2]Spanish National Cancer Research Center (CNIO), Madrid, Spain

Ischemic heart disease is the leading cause of mortality in developed countries. A fundamental but unsolved problem after myocardial infarction is the irreversible loss of cardiac tissue and its replacement by a fibrotic scar. Conversion of fibroblasts into functional cardiomyocytes represents a potential source of cells to restore cardiac function, but so far this technique remains inefficient, and little is known about the molecular mechanisms that govern this process. Here, we show that the gamma-secretase inhibitor DAPT, a classical Notch inhibitor, enhances the conversion of mouse fibroblasts into induced-cardiomyocytes mediated by Gata4, Hand2, Mef2c and Tbx5 (GHMT). DAPT synergizes with Akt to improve this process, achieving up to 70% conversion efficiency. Moreover, DAPT increases the quality of the conversion by substantially increasing the calcium flux and the number of spontaneous beating cells. A transcriptomic analysis by RNA-seq shows that DAPT treatment induces genetic programs related to muscle development, differentiation and excitation-contraction coupling. Mechanistically, DAPT treatment increases binding of the transcription factor MEF2c to the promoter region of the structural genes Myh6 and Actc1. In summary, we have demonstrated that the Notch pathway, through enhancement of MEF2c binding, plays an important role regulating the conversion of fibroblasts to cardiomyocytes. Our results provide new mechanistic insights on the reprogramming process and may bear important implications for cardiac regeneration therapies.

F4024

THE NOVEL DRUG J147 ENHANCES NEUROGENESIS AND IMPROVES STRUCTURAL AND FUNCTIONAL RECOVERY IN A MOUSE MODEL OF NEONATAL HYPOXIC-ISCHEMIC BRAIN INJURY

Kwak, Minhye, Lim, Sanghee and Mintz, Cyrus, Johns Hopkins University, Baltimore, MD, U.S.

Nearly one million newborns per year suffer neonatal hypoxic-ischemic brain injury (HI), which results in high rates of severe neurologic injury in survivors. Hypothermic neuroprotection is the only proven therapeutic alternative, but the benefit it provides is very limited. The primary pathology in neonatal HI is neuronal cell death, and thus enhancing neurogenesis to promote repair is a highly promising treatment strategy. J147 is a novel drug that increases endogenous production of brain derived growth factor, a trophic factor that enhances neural stem cell proliferation and differentiation. In this study we used a mouse model to test whether J147 can improve outcomes in neonatal HI by upregulating neurogenesis. Postnatal day 10 mice were subjected to the standard Vanucci model of neonatal hypoxic-ischemic injury and then treated with J147 at 10mg/kg/day for 2 weeks after injury. To assess the effects on neurogenesis we quantified expression of neural stem cell markers in the subgranular zone of the hippocampal dentate gyrus. We found that mice with HI treated with J147 showed a significant increase in cells positive for nestin, a marker for early uncommitted stem cells, acutely at one day after injury that is sustained at one week (80%, $p<0.001$ for 1 day and 265%, $p<0.0001$ for 1 week compared to control). At one week there is a significant increase in cells positive for T-box brain gene 2 (336%, $p<0.0001$ compared to control), which are rapidly proliferating intermediate neuronal precursors. Mice treated with J147 show reduced lesion size in the hippocampus by both histologic and magnetic resonance imaging measurements. Furthermore, there is a significant improvement in performance in dentate gyrus-dependent y-maze task spatial learning task (33%, $p<0.001$ compared to HI without treatment group). Taken together, these findings strongly suggest that J147 promotes neurologic recovery after neonatal HI via an upregulation of neurogenesis.

F4026

CHARACTERIZATION OF STEM CELLS ISOLATED FROM PLANTAR FASCIA OF RAT

Ko, Chun Hay[1], Siu, Wing Sum[1], Shiu, Hoi Ting[1], Leung, Ping Chung[1] and Lui, Pauline Po Yee[2], [1]The Chinese University of Hong Kong, Hong Kong, Hong Kong, [2]Hospital Authority, Hong Kong, Hong Kong

Plantar fasciitis is caused by degenerative irritation at the insertion of the plantar fascia on the medial process of

the calcaneal tuberosity. Identification of stem cell reside in the plantar fascia should result in the development of novel plantar fasciitis treatments. This study aimed to isolate plantar fascia derived stem cells (PFDSCs) of rats and compared their stem cell-related properties with bone marrow-derived stem cells (BMSCs). The effect of inflammatory cytokines IL-1β on the expression of connective tissue-related markers in PFDSCs was also examined. Our results showed that PFDSCs shared some common surface markers as BMSCs: CD44(+), CD90(+), CD106(+), CD31(-) and CD45(-), but they were CD71(-). Similar to BMSCs, PFDSCs expressed embryonic stem cell markers including sex determining region Y-box (Sox)2 and octamer-binding transcription factor 4, and Krüppel-like factor 4. They proliferated faster and formed more colonies compared with BMSCs. An increased amount of proteoglycan deposition was also observed in the PFDSC group. However, lower levels of adipogenic and osteogenic marker expression in PFDSCs were detected compared with BMSCs upon adipogenic and osteogenic induction. Except Scx and Tnc, the mRNA expression of Col1a1, Col3a1, Eln and Tnmd were similar in PFDSCs and BMSCs. PFDSCs therefore have poor multi-lineage differentiation potential and might account for poor healing outcomes after injury. Moreover, IL-1β suppressed the expression of connective tissue-related markers in PFDSCs. Inflammation after injury thus might negatively affect fasciocytic differentiation of PFDSCs. Our results imply a potential role of PFDSCs in the pathogenesis of plantar fasciitis.

F4028

THE EFFECT OF SURFACE COMPOSITION ON PLURIPOTENCY, NAÏVE STATE AND EFFICIENCY OF DIRECTED DIFFERENTIATION

Bamdad, Cynthia C, Carter, Mark G, Smagghe, Benoit J, Stewart, Andrew, Morse, Brian and Nash, Jac-Leen SS, Minerva Biotechnologies, Waltham, MA, U.S.

Several papers have been published recently on media that induce a naïve state in human stem cells. However, little attention has been paid to the surfaces that these cells are grown on and their effect on the naïve state. Many surfaces that are commonly used for stem cell culture are full of growth factors and integrins, which deliver biological signals that have been largely ignored. In addition to surfaces used for growth and maintenance of stem cells, most differentiation protocols begin with growth over Matrigel or similar for three (3) days before the start of induction. In the worst scenario, one would generate pristine naïve state stem cells but then corrupt them at the last minute by culturing them over a particular surface before inducing differentiation. We generated human naïve state stem cells by culturing the cells in serum-free minimal media plus a naturally occurring growth factor that is secreted by naïve stem cells but not primed, $NME7_{AB}$. No FGF, TGF-beta, biochemical inhibitors, LIF or any other growth factor or cytokine was added. Our stem cells were cultured on a surface coated with an anti-MUC1* monoclonal antibody; MUC1* is the $NME7_{AB}$ cognate receptor. Both $NME7_{AB}$ and bivalent anti-MUC1* dimerize the MUC1* growth factor receptor to promote growth and inhibit differentiation. That is to say that they both send the same biological signal to the cell. We then took our $NME7_{AB}$-induced naïve stem cells and transferred them onto Matrigel, vitronectin, fibronectin, laminin or MEFs, albeit using the same $NME7_{AB}$ media. The cells were then assayed at 24, 48 and 72 hours for expression of standard pluripotency genes, expression of naïve versus primed genes and X re-activation status. Results show that some of these surfaces significantly reduce expression of standard pluripotency markers as well as naïve markers within 24 hours. We then showed that the reduction in naïve markers negatively affects the efficiency of directed differentiation and the quality of the resultant cells. Specifically, we studied the efficiency of differentiating naïve stem cells to cardiomyocytes as a function of surface immobilization for 24 – 72 hours prior to beginning the differentiation protocol.

F4030

NOVEL MICROSATELLITE KNOCK-IN STRATEGIES TO MODEL MYOTONIC DYSTROPHY

Oliveira, Ruan Santos de and Swanson, Maurice S., University of Florida, Gainesville, FL, U.S.

Muscleblind-like (MBNL) proteins have been recently identified as RNA processing factors that regulate pluripotency and cell reprogramming. Their function in alternative splicing includes repressing the ES-cell-specific FOXP1 isoform, which is responsible for promoting the expression of core pluripotency genes. MBNL knockdown enhances reprogramming efficiency, increasing the expression of OCT4 and NANOG. In human diseases such as myotonic dystrophy (DM), MBNL sequestration leads to mis-splicing of developmentally regulated genes and favors an embryonic splicing profile in adult tissues. Therefore, DM modeling contributes to understand the molecular pathways critical for development and could potentially uncover new players in cellular differentiation and reprogramming.

Myotonic dystrophy type 1 (DM1) is caused by a CTG expansion (CTG^{exp}) in the 3' untranslated region (3' UTR) of the DMPK gene. Transcripts from the expanded DMPK allele accumulate in nuclear inclusions known as RNA foci and sequester multiple RNA-binding proteins including MBNL. The knock-in of large CTG expansions into the 3' UTR of the endogenous mouse Dmpk gene is a promising modeling strategy since it would lead to expression of toxic RNA molecules with a spatiotemporal distribution mirroring that observed in DM1 patients. Until recently, development of such a model has been hindered

by technical limitations, such as the instability of targeting vectors carrying large expansions in most bacterial strains and the small numbers of repeats capable of being introduced into mouse embryonic stem cells. Using a bacteria-free cloning approach followed by CRISPR/Cas genome engineering, we generated a CTG^{224} Dmpk knock-in, the largest microsatellite knock-in developed to date. This technology establishes a novel modeling strategy for microsatellite expansion diseases of any repeat size, and provides a platform to investigate additional factors potentially involved in the developmental changes underlying DM pathogenesis.

F4032

ICELL8TM, A VERSATILE SINGLE CELL ANALYSIS SYSTEM TO PROCESS THOU.S.NDS OF INDIVIDUAL CELLS

Mann, Ishminder, Schaal, Thomas, Chan, Leo, Swaminathan, Karthikeyan, Anandakrishnan, Sangeetha, Citarella, Mathew, Espinoza, Patricio, Ho, Mai, Hubschle, Hermann, Husain, Syed, Lin, Philip, Mir, Alain, Shapiro, Harris, Wei, Sherry, Dunne, Jude and Srinivasan, Maithreyan, WaferGen Biosystems, Fremont, CA, U.S.

A big challenge in the field of single-cell genomics is precise isolation of single cells. As a consequence, WaferGen BioSystems designedd the ICELL8™Single-Cell System, a simple and accurate single-cell isolation and analysis platform to simultaneously process 1000s of cells from up to 8 samples for RNA-seq applications. We have dispensed over 50 different cell types of varying size including, planarian, mosquito, human, mouse embryonic stem cells, bone marrow and hematopoietic stem cells. The system consists of: 1.MultiSample NanoDispenser - a robotic liquid handler enabling rapid, low-injury deposition of up to 1,800 live single cells into a single 5184 nanowell SmartChip, 2.Imaging Station - microscope-based automated image acquisition of the dispensed single cells. 3.CellSelect™ - imaging software that accurately identifies single cell containing wells (or wells of researcher's choice) and selectively programs deposition of cDNA synthesis reagents in either auto called or user selected specific wells of the SmartChip. Here we used ICELL8™ to dispense from a mixture of human cells that express Red Fluorescent Protein (RFP) and mouse cells to evaluate the fidelity of identifying single-cell containing wells. First, we automatically selected cells using CellSelect™ software. As not all human cells express RFP, we picked 100 wells based on RFP expression and 300 wells, where we expect a 20:80 ratio of human and mouse cells, based on lack of RFP. Single cells were processed for RNA-seq and the resulting 3' end sequences from individual cells aligned to the human and mouse genomes. Genome alignment resulted in ~98% assignment of the cells as either human or mouse. The alignment data indicates CellSelect™identifies single cell containing wells with a high degree of accuracy i.e. that 2 mixed genome single cells were not co-located in a single well. Furthermore, in the mix of cells lacking RFP expression, the observed ratio of human to mouse cells was 19:81, matching the expected 20:80 ratio with great accuracy. In summary, the ICELL8™system permits accurate single cell detection of desired markers from up to 1,800 single cells in a SmartChip. The single-cell occupancy rate of the system is ~98%.

F4034

DEFAULT PATTERNING OF HUMAN PLURIPOTENT STEM CELL-DERIVED NEURONS RESULTS IN PAN--CORTICAL AND SUBPALLIAL GENE EXPRESSION PATTERNS

Floruta, Crina, University of New Mexico Health Sciences Center, Albuquerque, NM, U.S.

In vitro differentiation of human pluripotent stem cells (hPSCs) offers a unique system to study aspects of development and disease processes of the nervous system and to generate specific cell types for use in cell replacement therapies. Previous studies have reported that hPSCs generate dorsal forebrain, cortical-like neurons with layer-specific markers generated in the proper temporal profile. However, most reports rely on relatively few select markers to illustrate this important developmental potential of hPSCs, without highlighting the diversity of phenotypes generated. Here we used global transcriptome analysis of gene expression at critical developmental time points during default patterning of hPSC-derived neurons (hPSNs). To determine the temporal and regional similarities between in vitro generated hPSNs and in vivo human brain development we used the recently created Transition Mapping (TMAP), CoNTExT, and WGCNA scripts in R Bioconductor. Temporally, we found hPSNs display significant expression of many functional markers (e.g. GRIA2 and SNAP25) and day 50 hPSNs resemble 13-19 post-conception week fetal brains. In addition, our data largely agree with previous reports suggesting pan-cortical gene expression profiles in SFEB-specified hPSNs, with strong expression of PAX6, SP8, EMX2, and NR2F1 simultaneously. However, SFEB-derived cultures also displayed significant subpallial transcriptional profiles, consistent with the presence of markers for GABAergic neurons (DLX1/2/5/6, ASCL1, multiple GABA receptor subunits). Using in vivo human transcriptome data, we created novel WGCNA modules comprised of genes enriched in human ganglionic eminence as well as striatal transcriptional profiles and found significant overlap with in vitro hPSNs. Interestingly, hPSN profiles did not resemble MGE progenitors but showed significant overlap with expression profiles found in CGE/LGE progenitor domains. Furthermore, hPSNs expressed several markers of mature interneurons (SST, NPY, CALB2), but lacked definitive markers of projection neurons (PPP1R1B). To-

gether, these data suggest that SFEB-mediated default differentiation leads to the production of both cortical glutamatergic neurons and subpallial GABAergic interneurons from progenitors expressing CGE/LGE-specific markers.

F4036

INTEGRATION OF MOLECULAR NETWORK GOVERNED BY SMADS TARGETED GENES IN HAIR FOLLICLE STEM CELLS REGULATION.

Kobielak, Krzysztof[1,2], Wang, Xiaoyang[1], Wang, Guangfang[1], Plikus, Maksim[1], Chen, Yi-Bu[3], Yun, Kyuson[4], Israel, Mark[5] and Kandyba, Eve[6], [1]University of California Irvine, Stem Cell Research Center, Irvine, CA, U.S., [2]University of Warsaw, Warsaw, Poland, [3]University of Southern California, Los Angeles, CA, U.S., [4]The Jackson Laboratory, Bar Harbor, ME, U.S., [5]Dartmouth-Hitchcock Medical Center, Lebanon, NH, U.S., [6]University of California San Francisco, San Francisco, CA, U.S.

Hair follicles facilitate the study of stem cells (SCs) behavior because SCs in progressive activation stages are ordered within the follicle architecture and they are capable of cyclic regeneration. Previously, we revealed how the underlying molecular mechanism of Bone Morphogenetic Protein (BMP) signaling governs the homeostasis of hair follicle stem cells (hfSCs) in vivo. Here, we focused further on identifying the key interactions of specific canonical BMP-Smad proteins in hfSC regulation using our recently developed inducible constitutively active Bmpr1a transgenic system. This allowed us to sort hfSCs and perform Chip-Seq analysis in vivo during the 1st and the 2nd hair cycle. We analyzed the peak calls for the two in vivo samples and received the predicted target lists for Smads for both p22 and p62 telogen hfSCs. Our results showed that approximately 95% of the peaks overlap in hfSCs during 1st and 2nd telogen.Comparison of our previously published global genes expression where BMP signaling has been inhibited in hfSCs to all overlapped in vivo Chip-Seq data between p22 v.s p62 revealed that approximately 72% of genes which previously has been 2 fold up or down regulated after BMP ablation in hfSCs were found directly regulated by BMP/Smads pathway. Previously, we identified Id2 gene as a target of BMP signaling in hfSCs with transcriptional down-regulation following targeted BMP inhibition. Here, we confirmed that Id2 gene has been consistently present in our recent Chip-Seq analysis both at p22 and p62, therefore, we decided to further test the functional role of the BMP effector, Id2 in hfSCs using an in vivo Id2 Gain of Function approach. Our data demonstrated that Id2 overexpression in hfSCs results in prolonged telogen in 1st hair cycle and a delay in anagen activation, maintaining stem cells quiescence. Thus, we confirmed the functional role of Id2 gene as a direct target and effector of BMP pathway in hfSCs in vivo.

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F4038

STEM CELL INTERVENTIONS FOR PEOPLE WITH CEREBRAL PALSY: SYSTEMATIC REVIEW WITH META-ANALYSIS

Novak, Iona[1], Walker, Karen[1,2], Hunt, Rod[3,4], Wallace, Euan[5], Fahey, Michael[6] and Badawi, Nadia[1,2], [1]Cerebral Palsy Alliance Research Institute, The University of Sydney, Sydney, Australia, [2]Grace Centre for Newborn Care, Children's Hospital at Westmead, The University of Sydney, Sydney, Australia, [3]The Royal Children's Hospital, Melbourne, Australia, [4]Murdoch Childrens Research Institute, Melbourne, Australia, [5]The Ritchie Centre, Melbourne, Australia, [6]Monash University, Melbourne, Australia

Cerebral palsy is the most common physical disability in childhood and the evidence for stem cells as a potential intervention is emerging. The mechanisms by which stem cells might treat cerebral palsy are proposed to include, regenerative, anti-inflammatory and trophic mechanisms. Our objective was to determine the efficacy and safety of stem cells for improving motor and cognitive function of people with cerebral palsy. We conducted a systematic review of the literature. Searches were conducted in October 2015 in CENTRAL, DARE, MEDLINE and Cochrane Library. Randomised controlled trials and controlled clinical trials of stem cells for cerebral palsy were included. Two authors independently decided upon included trials, extracted data, quality and risk of bias. The primary outcome was gross motor function, since cerebral palsy is a physical disability. Secondary outcomes were cognitive function and adverse events (AEs). Effects were expressed as standardized mean differences (SMD) with 95% confidence intervals (CI), using a random-effects model. Five trials comprising of 328 participants met inclusion criteria. Four cell types were studied: olfactory ensheathing (OECs); neural (NSCs); neural progenitors (NPCs); and allogeneic umbilical cord blood (UCBs). Transplantation procedures differed from central nervous system neurosurgical transplantation to intravenous/arterial infusion. Participants were followed short-term for only 6-months. Evidence of variable quality indicated a small statistically significant intervention effect from stem cells on gross motor skills (SMD 1.27; 95%CI 0.22, 2.33), with UBCs most effective. There was insufficient and heterogeneous data to compare cognitive effects. Serious AEs were rare (n=4/135 [3%] stem cells; n=3/139 [2%] controls). Stem cells appeared to induce short-term improvements in motor skills. Different types of stem cell interventions were compared meaning the data were heterogeneous and

is a study limitation. Further randomised controlled trials are warranted using rigorous methodologies. We have summarized cerebral palsy stem cell research progress to date and made recommendations for the field going forward.

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F4040

THE MECHANISM OF PROMOTING NEUROGENESIS BY SOME NEUROLOGICAL MARKET DRUGS AND THEIR POTENTIAL CLINICAL APPLICATIONS

Lei, Ying[1], Zhang, Yue[1], Li, Gang[2] and Zhao, Jian[3], [1]Shanghai East Hospital, Shanghai, China, [2]Department of Neurology, East Hospital, Tongji University School of Medicine, Shanghai, China, [3]Stem cell GMP Lab, Shanghai East Hospital, School of Medicine, Tongji University, Shanghai, China

Neurogenesis is the process of producing new neurons from neural stem cells (NSCs), comprising a series of sequential developmental events. Neurogenesis is critical for learning and memory, and plays essential roles in recovery from brain damage, which is regulated by a variety of extracellular factors and intracellular pathways. In our preliminary work, we screened a series of clinical drugs of neurological diseases with a cellular assay systematically evaluating neurogenesis. Interestingly, several drugs were identified to promote neurogenesis in the exploratory experiments. Therefore, we tried to investigate the regulatory mechanism of neurogenesis by these clinical drugs through a variety of in vitro and in vivo experiments, taking advantage of the established assays and systems in the previous work. Furthermore, we also explored their clinical applications on some neurodegenerative diseases with relevant mouse models. This study may not only broaden our sight on the regulatory mechanism of neurogenesis, but also suggest novel therapeutic strategies for neurodegenerative disease.

Funding Source: Key Program of Shanghai Zhangjiang National Innovtaion Demonstration Zone Development Fund

F4042

Smek1/2 BALANCE KEY SIGNALS DIRECTING NEURAL STEM CELL FATE SWITCH.

Chang, Wen-Hsuan[1], Choi, SiHo[2], Lyu, Jungmook[3], Cai, Mingyang[1], Moon, Byoungsan[1] and Lu, Wange[1], [1]University of Southern California, Alhambra, CA, U.S., [2]Dongnam Institute of Radiological and Medical Sciences (DIRAMS), Busan, Korea, [3]The Catholic University of Korea, Seoul, Korea

During cortical development, neural stem cells receive various intrinsic or extrinsic cues spatio-temporally. However, the balances between various signals, and handfuls of the detail regulatory mechanism are still in dark. Here, we show that Smek proteins, which negatively regulate Notch signaling in neural stem cells, mediate non-canonical Wnt signaling through the Ryk receptor to drive mammalian cortical neurogenesis. Smek1/2 bind cleaved Ryk intracellular domain in the cytoplasm and enable its import into the nucleus. In the nucleus, Ryk intercellular domain acts as a cofactor for Smek and is required for Smek binding of regulatory sequences and activation of several interneuron-specific transcription factors. Smek1/2 double knockout mice display pronounced defects in the production of cortical interneurons. Our findings therefore identify Smek proteins as key neural cell fate switches, acting as essential mediators of non-canonical Wnt signaling through Ryk and inhibitors of Notch signaling.

F4044

INCREASED SENSITIVITY TO BUSULFAN IN ARTEMIS-DEFICIENT MICE PERMITS LOW-DOSE CONDITIONING.

Roy, Sushmita, Sanford, Ukina, Khan, Sara, Stillion, Misako, Punwani, Divya, Puck, Jennifer and Cowan, Morton J, University of California San Francisco, San Francisco, CA, U.S.

Currently, hematopoietic stem cell transplantation (HCT) is the only effective cure of $T^-B^-NK^+$ SCID, but without conditioning with alkylating agents the majority of children with $T^-B^-NK^+$ SCID fail to reconstitute B cell immunity even with an HLA matched sibling donor. Defects in DCLRE1C, encoding the DNA repair protein Artemis, cause $T^-B^-NK^+$ SCID (ART-SCID), in which patients are particularly susceptible to high doses of alkylating agents and ionizing radiation. ART-SCID patients conditioned with alkylating agents have poor survival and late toxicity. To evaluate potential conditioning approaches for gene corrective autologous HCT we used non-leaky $Art^{-/-}$ mice to evaluate non-alkylating agents in conjunction with very low doses of busulfan (Bu) to open marrow niches. Recipients were C57Bl/6 CD45.2 $Art^{-/-}$ or wild type (WT), and donors were congenic WT CD45.1 mice. The pre-conditioned recipient mice were transplanted with WT CD45.1

lin^{-}cKit^{+}Sca1^{+} (LSK) sorted cells. Sham-treated Art$^{-/-}$ or WT control mice received DMSO+H_2O or PBS injections. First, we evaluated possible synergy of anti-ckit (ACK2) and AMD3100 with non-myeloablative Bu doses. ACK2 pre-treatment resulted in significant multilineage engraftment compared to sham-treated controls, while AMD3100 had no added impact on chimerism, and there was no synergy between AMD3100 or ACK2 with 10mg/kg (25% of myeloablative dose) Bu in Art$^{-/-}$ recipients. Bconditioned Art$^{-/-}$ recipients showed a significant increase in overall donor chimerism compared to sham treated Art$^{-/-}$ recipients even at the lowest Bu dose (5mg/kg). Interestingly, 10mg/kg of Bu resulted in significant T, B and neutrophil engraftment in all 6 Art$^{-/-}$ but in only 1 of 6 WT recipients, suggesting that Artemis deficiency confers increased sensitivity to this alkylating agent. Also, high donor B cell and neutrophil chimerism were observed in the 10mg/kg Bu-conditioned Art$^{-/-}$ recipients compared to the one engrafted WT recipient. Positive anti-NP IgG responses were also noted in Bu-treated Art$^{-/-}$ mice. These results show that 1) very low-dose busulfan (5mg/kg) by itself provides effective conditioning in Art$^{-/-}$ mice, resulting in T and B cell reconstitution; and 2) Art$^{-/-}$ recipients are more sensitive than WT to this alkylating agent.

F4046

IN VITRO INVESTIGATION INTO THE PROLIFERATIVE ADVANTAGE OF RET M918T MUTATIONS IN SPERMATOGONIAL STEM CELLS.

Whelan, Eoin Christopher, Stacey, Michael and Osgood, Christopher, Old Dominion University, Norfolk, VA, U.S.

As the average age of parenthood increases, the effect of paternal age on the incidence of genetic disease and child health becomes ever more important. A number of autosomal dominant disorders exhibit an exponential paternal age effect. Several studies have linked this to premeiotic expansion of spermatogonial stem cells (SSCs). These mutations provide a selective advantage to the stem cells within the niche but a deleterious effect in offspring. In order to test this hypothesis, we selected the mutation in the RET (*RE*arranged during *T*ransfection) gene that causes multiple endocrine neoplasia type 2B (M918T) as this disease is caused exclusively by this single substitution, shows a strong paternal age effect and occurs in the canonical signalling pathway involved in SSC identity. RET is the receptor for GDNF (Glial Derived Neurotropic Factor), which is required for SSC self-renewal.SSCs were created using induced pluripotent stem cells derived from BJ fibroblasts and differentiating them with SSC media including the growth factors GDNF, bFGF and GFRa1 over a period of 10 days. SSC identity compared with the iPSCs was confirmed by immunohistochemistry with the SSCs staining positive for CD9, PLZF and DDX4 and negative for the pluripotency marker Tra-1-81. Wildtype and mutant SSCs were generated by transfection with a pcDNA3.1 plasmid containing the normal RET gene and the gene containing the M918T mutation, respectively. Plasmid integration was achieved through treatment with gentamicin and a restriction-enzyme assay for the mutation was developed.Mutant SSCs showed increased proliferation in culture. This effect was magnified by decreasing GDNF concentration in culture and reduced when the GDNF concentration was increased, saturating the receptors. This research demonstrated experimental evidence for the positive selection effect of the M918T mutation in RET and validating the hypothesis that this selection effect is to blame for the marked paternal age effect of multiple endocrine neoplasia 2B.

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F4048

ROLE OF RUNX1 IN REMDODELLING THE CELLULAR LIPID METABOLISM OF HAIR FOLLICLE STEM CELLS

Jain, Prachi[1], Nattakom, Mary[1], Wang, Donghao[2], Brenna, Tom[2] and Tumbar, Tudorita[1], [1]Cornell Univ, Ithaca, NY, U.S., [2]Cornell, Ithaca, NY, U.S.

We uncovered the transcription factor Runx1 as an important hair follicle stem cell (HFSC) regulator during the transition to early progenitors in adult skin homeostasis. In the absence of Runx1 these cells fail to be activated from quiescence in a timely manner, and this delays hair follicle growth. Moreover, we found Runx1 was required for mouse and human skin and oral epithelial carcinogenesis. However, a comprehensible model for Runx1 mechanisms of action in these epithelial SCs and cancers has not emerged. Upon physiological high level of RUNX1 global gene expression changes were induced, interestingly many of these genes were related to lipid metabolism. We hypothesize that changes in lipid metabolism help the HFSC to transition towards early progenitor cell fate that is more prone to subsequent rapid proliferation and differentiation. Similarly, in cancer cells changes in lipid metabolism might help to synthesize fatty acid for membrane of proliferating cells and act as fuel to meet energy demand. Consistent with the known role of Runx1-putative targets in lipid metabolism, we find that human skin and oral carcinomas shows elevated expression of SCD1, the enzyme responsible for adding double bonds at the 9th position of saturated fatty acids. Likewise, our gene expression and lipidome analysis revealed significant changes in the concentration of mono-unsaturated fatty acids in Runx1 mutant cells, which correlated with dis-regulation of SCD1. Further probing our lipidome data to examine the cellular attributes we found that fluidity of the plasma membrane can be regulated by altering the monounsaturated fatty acid and by varying expression of

RUNX1. Finally, we found some RUNX1 target lipid genes such as SCD1 and SOAT1 (enzyme catalyzing cholesterol-ester synthesis) are necessary for the proliferation of keratinocytes. Our results suggest that Runx1 remodels the cellular lipid metabolism, thus providing the required conditions for rapid activation and cell growth of normal HFSCs and of cancer cells.

Funding Source: NYSTEM

F4050

NETRIN-1 AS A PIVOTAL ANGIOGENIC MOLECULE PRODUCED BY HUMAN WHARTON JELLY MESENCHYMAL STEM CELLS (WJ-MSC)

Prieto, Catalina, Arros Villanueva, Andrea and **Palma, Verónica A.**, University of Chile, Santiago, Chile

The Netrin ligands belong to the laminin protein superfamily. They promote/inhibit angiogenesis, depending on the physiological context, through different intracellular signaling pathways, such as the RhoA/ROCK pathway, via their receptors belonging to both the DCC/NEO1 and UNC families. WJ-MSC are known to produce classic angiogenic factors (VEGF, bFGF, etc.), as well as Netrins. However, the putative role of Netrins has not yet been explored. We aimed to elucidate the role of Netrins secreted during angiogenesis by WJ-MSC. More specifically, we examined their function, *in vivo* and *in vitro*, in relation to their putative receptors in human umbilical vein endothelial cells (HUVEC). We evaluated Netrin-1 and 4 production in WJ-MSC, the canonical and non-canonical receptor activation in HUVEC cultures through qPCR, WB, FACS, and immunohistochemistry, and the putative downstream signaling by phosphorylated ERM (p~ERM) (index of activated RhoA/ROCK signaling). These analyses revealed that HUVEC express both classical (UNC5a, UNC5b, UNC5c, Neogenin-1) and non-classical (integrin β1, integrin β4, integrin α6) Netrin receptors. In evaluating the angiogenic capacity of conditioned medium, obtained from WJ-MSC, via tubule formation assays and CAM assays, we found that both endothelial migration and tubule formation are controlled by Netrin-1. We also investigated the specific contribution of Netrins, secreted by WJ-MSC, by implementing a pharmacological loss of function, and the involvement of the RhoA/ROCK activity using the C3 exoenzyme (Clostridium botulinum). Overall, we demonstrate that WJ-MSC conditioned medium promotes angiogenesis in vivo and in vitro and that this effect is partially mediated by Netrin-1 possibly dependent on the RhoA/ROCK signaling pathway. The present findings show that the healing effect of WJ-MSCs is presumably because they act as trophic mediators that help to create a pro-angiogenic and reparative microenvironment in the wound bed. This work has deep implications in modeling processes such as that of wound healing and may be useful in optimizing therapeutic approaches that address vascular function.

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F4052

A BIOINFORMATIC AND NANOTECHNOLOGY PERSPECTIVE ON CARDIOMYOCYTE CONTRACTILITY IN ETHNICALLY AND EPIGENETICALLY DIVERSE IPSC LINES

Tomov, Martin[1], Olmsted, Zachary[1], Dogan, Haluk[2], Otu, Hasan H[2], Buck, Michael[3], Cibelli, Jose[4,5] and **Paluh, Janet**[1], [1]SUNY Polytechnic Institutue Colleges of Nanoscale Science & Engineering, Albany, NY, U.S., [2]University of Nebraska-Lincoln, Lincoln, NE, U.S., [3]SUNY Buffalo, Buffalo, NY, U.S., [4]Michigan State University, East Lansing, MI, U.S., [5]LARCEL, Laboratorio Andaluz de Reprogramacion Celular, BIONAND, Andalucia, Spain

Stem cell based cardiomyocyte therapies have potential to play a critical role in recovery from myocardial infarctions directed at improving short or long-term outcomes of heart disease. Multiple adult, embryonic, and induced strategies for cardiomyocyte generation are being explored in the scientific and biomedical communities and clinical trials are revealing the importance of understanding the underlying transcriptomic functional state of cardiomyocytes. By use of expanded transcriptomics, epigenomics, and bioinformatics studies, critical staging of cells for generating cardiomyocytes as well as improved understanding of pathways critical for developmentally appropriate functional tissues will be revealed. Previously we derived replicate iPSC lines of African American, Hispanic-Latino and Asian self-designated ethnically diverse (ED) origins with normal karyotype, qRT-PCR verified pluripotency biomarkers, teratoma formation and in vitro tri-lineage commitment. Here, bioinformatics of generated high quality ED-iPSC RNA-Seq and ChIP-seq pluripotency data sets were compared and multi-lineage differentiation validated to multiple neural, pancreatic, smooth muscle and cardiomyocyte cell types. Our analysis revealed functional differences within ED-iPSC replicate lines and across ethnicities solely in generation of beating cardiomyocytes. Towards advancing cell therapeutic goals for cardiac health that use iPSC derived cardiomyocytes we applied bioinformatics analysis of iPSC transcriptome data to compare Asian and Hispanic-Latino ED-iPSC replicate paired lines that contained a beating and non-beating partner. Our analysis identified genes that may serve as potential predictors of beating outcomes across ethnicities and the relevant GO pathways in which they participate. We provide detailed analysis of ethnically distinct, shared or inversely regulated genes associated with beating or non-beating outcomes. We discuss our findings in relation to other cardiac transcrip-

tome studies and disease pathways. This study enhances our ability to correlate the iPSC transcriptome state NYSTEM C026186.

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F4054

DEVELOPING THERAPEUTIC CANDIDATE FOR CANAVAN DISEASE USING GENETICALLY-MODIFIED PATIENT IPSCS

Chao, Jianfei, LI, Wendong, Tian, E, Ye, Peng, Cui, Qi, Sun, Guihua, Riggs, Arthur, Matalon, Reuben and Shi, Yanhong, City of Hope National Med Center Beckman Research Institute, Duarte, CA, U.S.

Canavan disease is a devastating neurological disease that lacks effective therapy. There is neither cure nor a standard course of treatment for this disease. In this study, we develop patient-specific iPSCs as an autologous donor source for cell therapy. We generate Canavan disease iPSCs from patient dermal fibroblasts, introduce the wild type aspartoacylase (ASPA) gene, the mutations of which cause the disease, and demonstrate that these genetically-modified iPSCs maintained human embryonic stem cells (ESC)-like features. We then differentiate the genetically modified ASPA iPSCs into neural precursors and show that the genetically modified neural precursors (ASPA neural precursors) had significantly increased ASPA enzymatic activity. After transplanting into a Canavan disease mouse model, the ASPA neural precursors were able to survive and rescue major pathological features of Canavan disease, including the deficiency of ASPA enzymatic activity, high NAA levels, extensive vacuolation and defective myelination in brains of the transplanted mice. These cells were also able to restore the body weight and motor function of Canavan disease mice. These genetically modified patient iPSC-derived neural precursors could lead toward preclinical development of an iPSC-based therapy for the treatment of Canavan disease.

F4056

THE USE OF DEMINERALIZED BONE MATRIX (DBM) BASED IMPLANTABLE AND BIOMIMETIC MICROCARRIER FOR LARGE SCALE STEM CELL EXPANSION AND ONE-STEP TISSUE ENGINEERED BONE GRAFT CONSTRUCTION

Zhang, Zhiyong, Shanghai Jiao Tong University, Shanghai, China

Tissue engineered bone grafts (TEBG) using mesenchymal stem cells (MSCs) demonstrate great potential for large bone defect treatment. However, the current MSC expansion technique and the multiple-step TEBG construction strategy are harassed with many problems such as labor-intensive procedures and repeated trypsinization, limiting the clinical application of bone tissue engineering (BTE) technology. Microcarriers may present a promising approach to address these problems, but currently available microcarriers are either non-implantable or insufficient of biomimetic potential to promote effective stem cell proliferation and function. In current study, we used demineralized bone matrix(DBM) to develop a biomimetic and implantable microcarrier (DBM-MC), which could preserve the essential biochemical composition and surface topography of nature bone tissue and provide the biomimetic matrix microenvironment for cell adhesion, proliferation and differentiation. Furthermore, we established an DBM-MC based integrated TEBG fabrication strategy to seamlessly integrate the multiple procedures including cell seeding, expansion, and osteogenic priming into a single-step procedure under a dynamic culture condition. When benchmarked with Cytodex 3, a widely used microcarrier, the DBM-MC shared similar density, and supported efficient cell adhesion and fast cell proliferation with the MSC characteristics well maintained. However, when expanded in vitro and implanted in vivo ectopically, the MSC mediated DBM-MC constructs achieved much more new bone formation with well vascularization throughout the dense bone tissue; whereas, MSC mediated Cytodex 3 constructs experienced limited bone formation with scarce vascular network and empty necrotic cavities in the core region. Moreover, the μTEBG constructs generated via this integrated system was used for critical sized cranial defect treatment and achieved successful defect bridging at 3 months with two folds more bone regeneration. The biomimetic and implantable DBM-MC based integrated system can provide an enclosed, large-scale, reduced trypsinization, semi-automatic and single-step fabrication process to generate μTEBGs with outstanding osteogenic and angiogenic efficacy, demonstrating great potential for clinical application.

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F4057

DEVELOPING A 3-DIMENSIONAL HUMAN iPSC DERIVED MODEL OF BRONCHOPULMONARY DYSPLASIA

Sucre, Jennifer[1], Wilkinson, Dan Charles[1], Vijayaraj, Preethi[1], Paul, Manash K.[1], Dunn, Bruce[1] and Gomperts, Brigitte[2], [1]Univeristy of California, Los Angeles, Los Angeles, CA, U.S., [2]David Geffen School of Medicine at UCLA, Los Angeles, CA, U.S.

Bronchopulmonary dysplasia (BPD) is the leading cause of morbidity in premature infants. While there are exper-

imental animal models that approximate BPD with environmental exposures, there is currently no comparative human model of BPD. Cells differentiated from human induced pluripotent stem cells (iPSCs) have been shown to be similar to fetal cells and therefore provide an ideal platform to study developmental diseases such as BPD. Our overall goal is to create a disease model of BPD in order to investigate the cellular pathways involved in the disease pathophysiology. iPSC-derived mesenchymal cells (iMC) and 20-week gestation human fetal lung fibroblasts (FLF) were cultured on alginate beads to form 3D alveolar-like lung organoids. The organoids were exposed to a hypoxia-hyperoxia model of BPD. Comparisons in gene expression patterns, histology, and proliferation were made using quantitative real-time PCR (qPCR), immunofluorescence, and H&E staining. Tissue from BPD patients was used to validate the model and its pathophysiology. In our 3D model of BPD, when iMCs and FLFs were exposed to alternating hypoxia and hyperoxia, there was an increase in cellular proliferation and increased α-SMA expression by immunostaining when compared with normoxic controls. qPCR analysis showed increased expression of 10 genes including α-SMA, TGFβ1, collagen I, and elastin, which is consistent with known expression patterns in BPD. Exposure of 3D lung organoids to hypoxia-hyperoxia caused increased expression of NOTCH1, NOTCH3, and other downstream effectors in the Notch pathway, including HES1 and HEY1. Treatment of the mesenchymal organoids with a Notch inhibitor prior to exposure to hypoxia-hyperoxia prevented the development of the BPD phenotype. Examination of autopsy specimens from infants who died from BPD demonstrated activation of the Notch pathway in pulmonary fibroblasts similar to that seen in the organoid model. We have developed a human 3D model of BPD that recapitulates the fibrotic component of BPD by histopathology and gene expression. Using this model, we have identified the Notch pathway as one of the drivers of BPD pathophysiology. With further development of this high throughput model, we hope to broaden our understanding of BPD and to use this model to identify novel therapies.

Funding Source: Broad Stem Cell Research Center, UCLA

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